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DIEISON ANDRÉ MOI

Human-induced pressures driving biodiversity and functioning of freshwater ecosystems

Maringá-PR
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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ecologia e Limnologia.
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Orientador: Dra. Claudia Costa Bonecker
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Pressões induzidas pelo homem impulsionando a biodiversidade e o funcionamento dos ecossistemas de água doce

RESUMO

Muitos estudos demonstraram que as pressões induzidas pelo homem estão causando a perda de espécies em muitos grupos tróficos, com potenciais efeitos negativos na capacidade dos ecossistemas de manter funções e prestar serviços para o bem-estar humano. No entanto, ainda faltam evidências consistentes para esta previsão nos ecossistemas de água doce. Reportou-se os resultados de quatro estudos que investigaram os efeitos da perturbação induzida pelo homem sobre (i) a biodiversidade, (ii) o funcionamento dos ecossistemas e (iii) as relações entre biodiversidade e funcionamento dos ecossistemas. Estes estudos foram conduzidos em diferentes ecossistemas de água doce e abrangeram múltiplas escalas espaciais e temporais. Utilizou-se um conjunto de dados de 12 anos de um lago raso exibindo mudanças entre três estados alternativos (claro, túrbido e sombreado). Investigou-se como a biodiversidade (taxonômica e funcional) de peixes e zooplâncton, a multifuncionalidade do ecossistema (provisão de múltiplas funções ecossistêmicas simultaneamente) e suas relações foram afetadas por mudanças entre estados alternativos. A biodiversidade de peixes e zooplâncton e a multifuncionalidade aumentaram durante o estado de águas claras, mas diminuíram durante os estados turvos e sombreados. A relação entre biodiversidade e multifuncionalidade foi fortemente positiva durante o estado de águas claras, mas enfraqueceu após a mudança do lago para estados turvos e sombreados. Empregou-se um conjunto de dados de 72 lagos de quatro grandes planícies do Brasil para examinar como o aumento da pressão humana (*pegada humana*) afetou a relação entre a biodiversidade (taxonômica e funcional) de sete grupos de organismos aquáticos e a multifuncionalidade. A biodiversidade da maioria dos grupos de organismos foi positivamente associada à multifuncionalidade. Entretanto, o aumento da pressão humana enfraqueceu estas relações, e para alguns grupos estas relações se tornaram negativas. Utilizou-se também um conjunto de dados de 61 córregos de dois biomas neotropicais (floresta amazônica, pastagens uruguaias) para investigar como o aumento da cobertura do uso do solo humano (agricultura, pastagem, urbanização e florestamento) afetou a biodiversidade (taxonômica e funcional) de peixes, artrópodes e macrófitas, e as consequências disso para a produção de biomassa animal. Em ambos os biomas, a biodiversidade dos conjuntos de animais e plantas diminuiu com o aumento da cobertura de uso do solo. Os usos do solo reduziram a biomassa animal através de caminhos diretos e indiretos mediados por declínios na biodiversidade. Por fim, investigou-se como a crescente pressão humana afeta a diversidade dos peixes e as consequências disso para o fluxo de energia nas teias de alimento de peixes durante 17 anos em um rio subtropical (rio Uruguay). A pressão humana foi associada a declínios temporais na diversidade e no fluxo de energia em todos os compartimentos tróficos de peixes, e a relação entre diversidade e fluxo de energia enfraqueceu com o tempo. Coletivamente, estes estudos demonstram, de maneira consistente, que as perturbações induzidas pelo homem reduzem a biodiversidade de vários grupos de organismos em diferentes tipos de ecossistemas aquáticos. O declínio da biodiversidade, por sua vez, reduz a capacidade desses ecossistemas de sustentar múltiplas funções.

Palavras-chave: Biodiversidade. Funcionamento ecossistêmico. Rio. Riacho. Lago. Teia alimentar. Pressão humana.

Human-induced pressures driving biodiversity and functioning o freshwater ecosystems

ABSTRACT

Many studies have shown that human-induced pressures are causing species loss across many trophic groups, with potential negative effects ability of ecosystems to maintain functions and provide services to human well-being. However, consistent evidences for this prediction is still lacking in freshwater ecosystems. We report the results of four studies that investigated the effects of human-induced disturbance on (i) biodiversity, (ii) ecosystem functioning, and (iii) biodiversity-ecosystem functioning relationships. These studies were conducted in different freshwater ecosystems and encompassed multiple spatial and temporal scales. In the first study, we used a 12-year data set from a shallow lake displaying shifts between three alternative states (clear, turbid and shaded). We investigated how the biodiversity (taxonomic and functional) of fish and zooplankton, ecosystem multifunctionality (provision of multiple ecosystems functions simultaneously) and their relationships were affected by shifts between alternative states. Biodiversity of fish and zooplankton and multifunctionality enhanced during clear-water state, but decreased during turbid and shaded states. The relationship between biodiversity and multifunctionality was strongly positive during the clear state, but weakened after the lake shifted to turbid and shaded states. We used a dataset of 72 lakes from four large Brazil wetlands to examine how increased human pressure (human footprint) affected the relationship between the biodiversity (taxonomic and functional) of seven groups of aquatic organisms and multifunctionality. The biodiversity of most organismal groups was positively associated with multifunctionality. However, increased human pressure has weakened these relationships, and for some groups these relationships have become negative. We employed a dataset of 61 streams from two Neotropical biomes (Amazonian rainforest, Uruguayan grasslands) to investigated how increased cover human land-uses (agriculture, pasture, urbanization and afforestation) affected the biodiversity (taxonomic and functional) of fish, arthropods and macrophytes, and the consequences of this for animal biomass production. In both biomes, the biodiversity of animal and plant assemblages decreased with increasing cover of land-uses. Land-uses reduced animal biomass through direct and indirect pathways mediated by declines in biodiversity. We investigate how increasing human pressure affects fish diversity and the consequences of this for energy flux in fish food webs over 17 years in a subtropical river (Uruguay River). Human pressure was associated with temporal declines in diversity and energy flux in all fish trophic compartments, and the relationship between diversity and energy flux weakened over time. Collectively, these studies consistently demonstrate that human-induced disturbances reduce the biodiversity of various groups of organisms in different types of aquatic ecosystems. The decline in biodiversity, in turn, reduces the ability of these ecosystems to sustain multiple functions.

***Keywords*:** Biodiversity. Ecosystem functioning. River. Stream. Lake. Food web. Human pressure.

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SUMMARY

1 INTRODUCTION

Human-induced pressures, including habitat conversion and degradation, and regime shifts threaten biodiversity worldwide (Sala et al. 2000; Scheffer et al. 2001; Romero et al. 2020). In the past two decades, global assessments have revealed marked biodiversity losses with increasing human-induced pressures on natural ecosystems (Newbold et al. 2015; Gossner et al. 2016). Extirpation of species in aquatic and terrestrial ecosystems (Pereira et al. 2010; Dirzo et al. 2014) suggests that planet Earth may be facing a sixth wave of mass extinction (Cowie et al. 2022). Compared to the mass extinctions of prehistoric periods, current rates of species loss are more accelerated and are especially driven by human activities (Barnosky et al. 2011). The human population is growing rapidly, – now eight billion people inhabit planet Earth, which is projected to increase to 10 billion by 2050 (United Nations 2018; Adam 2021). Consequently, human-induced pressures on natural ecosystems are expected to increase in the coming years. This scenario has raised questions about the risks that biodiversity decline may have for ecosystem functioning, and the consequences of this for the services that ecosystems provide to the human well-being (Cardinale et al. 2012; Hooper et al. 2012).

Empirical evidence reveals positive effects of biodiversity on ecosystem processes, including productivity and nutrient cycling (Isbell et al. 2015; Moi et al. 2021). There is also evidence of positive biodiversity effects on energy flux through food webs (Barnes et al. 2014). Considering that species are ecologically unique and can play complementary roles in natural systems, thus varying in their contributions to different functions (Cardinale et al. 2012), it has been proposed that the effect of biodiversity on ecosystem functioning is stronger when multiple functions are considered simultaneously (i.e., 'ecosystem multifunctionality'; Hector and Bagchi 2007). In this vein, it is increasingly recognized that biodiversity is fundamental for ecosystems to maintain their functioning. This recognition has led to the prediction that as biodiversity declines, the ability of ecosystems to sustain multiple functions is impaired (Tilman et al. 2014; Soliveres et al. 2016).

Despite recent advances in understanding the effects of biodiversity on ecosystem functioning, multiple gaps still remain. First, most of the understanding of the importance of biodiversity for ecosystem functioning comes from studies focusing on single trophic levels, using simplified food webs (Tilman et al. 2001; Hooper et al. 2005). As a consequence, it is difficult to understand the functioning of natural ecosystems that are multitrophic systems, –

that is, they comprise multiple groups of organisms of varying trophic levels interacting through mutualistic and antagonistic networks. Such interactions, in turn, modulate the performance of ecosystem functions such as energy flux, productivity and nutrient cycling (Wang et al. 2018). Therefore, understanding the importance of biodiversity for the functioning of natural ecosystems requires the use of multitrophic perspectives (Eisenhauer et al. 2019).

Second, most studies investigating the effects of biodiversity on ecosystem functioning are from terrestrial and marine realms (Lefcheck et al. 2015; Isbell et al. 2015). There are proportionally few studies investigating the biodiversity-ecosystem functioning relationships in freshwater systems, generating an urgent need for more studies on this subject in this realm (Daam et al. 2019). Similarly, current evidence supporting anthropogenic impacts on biodiversity-ecosystem functioning relationships are scarce and come mostly from experimental manipulations (Allan et al. 2015; Jing et al. 2015). Consequently, there is an urgent need for more studies to be conducted using data from natural ecosystems. It is possible that the relationship between biodiversity and ecosystem functioning is under-estimate in natural ecosystems, especially in freshwater systems. This is a hot topic in ecology, since freshwater systems is highly productive ecosystems and supports a wide variety of unique life forms and provides numerous services to human society (Ripl 2003; Heino et al. 2021). Despite their importance, these ecosystems are increasingly degraded by human-induced pressures (Folk et al. 2004; Vörösmarty et al. 2010; Reid et al. 2019).

This thesis compiles four studies that independently evaluated the impact of human-induced disturbance on (i) biodiversity, (ii) ecosystem functioning, and (iii) biodiversity-ecosystem functioning relationship across different freshwater ecosystems, employing multiple spatial and temporal scales. In the first study, we used 12 years of data from a shallow lake, which during this period shifted between three alternative states: clear, turbid and shaded. Such changes were driven by external factors (e.g., nutrient loading), which cause changes in primary producer communities. This study assessed how biodiversity (taxonomic and functional) of fish and zooplankton, the ecosystem multifunctionality (including five ecosystem variables), and the relationship between biodiversity and multifunctionality responded for each of the three alternative states. In the second study, we used an extensive dataset of 72 lakes distributed across the four largest Brazilian wetlands (Amazon, Araguaia, Pantanal, and Paraná). This study examined how increased human pressure (estimated using *human footprint* index; Venter et al. 2016) affected the relationship between the biodiversity

(taxonomic and functional) of multiple groups of aquatic organisms and ecosystem multifunctionality (including 11 ecosystem variables). In the third study, we used an extensive dataset of 61 streams distributed across the two neotropical biomes (*Amazonian rainforest*, *Uruguayan grasslands*). This study evaluated how the increased in cover of four human land-uses (agriculture, pasture, urbanization and afforestation) affected the biodiversity (taxonomic and functional) of fish, arthropods and macrophytes, and the consequences of this for the animal biomass production of these streams. In the fourth study, we used 17 years of data from a Neotropical River (Uruguay River) to investigate how increased human pressure (*human footprint*) impacted fish biodiversity (species richness and abundance), and the consequences of this for energy flux in fish food webs. This project represents the most comprehensive assessment, both temporal and spatial, of human impacts on the biodiversity and functioning of freshwater ecosystems, making this thesis a pioneer.

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2 REGIME SHIFTS IN A SHALLO LAKE OVER 12 YEARS: CONSEQUENCES FOR TAXONOMIC AND FUNCTIONAL DIVERSITY, AND ECOSYSTEM MULTIFUNCTIONALITY

ABSTRACT

1. Under increasing nutrient loading, shallow lakes may shift from a state of clear water dominated by submerged macrophytes to a turbid state dominated by phytoplankton or a shaded state dominated by floating macrophytes. How such regime shifts mediate the relationship between taxonomic and functional diversity and lake multifunctionality is poorly understood.
2. We employed a detailed database describing a shallow lake over a 12-year period during which the lake has displayed all the three states (clear, turbid, and shaded) to investigate how species richness, functional diversity of fish and zooplankton, ecosystem multifunctionality, and five individual ecosystem functions (nitrogen and phosphorus concentrations, standing fish biomass, algae production, and light availability) differ among states. We also evaluated how the relationship between biodiversity (species richness and functional diversity) and multifunctionality is affected by regime shifts.
3. We showed that species richness and the functional diversity of fish and zooplankton were highest during the clear state. The clear state also maintained the highest values of multifunctionality as well as standing fish biomass production, algae biomass, and light availability, whereas the turbid and shaded states had higher nutrient concentrations. Functional diversity was the best predictor of multifunctionality. The relationship between functional diversity and multifunctionality was strongly positive during the clear state, but such relationship became flatter after the shift to the turbid or shaded state.
4. Our findings illustrate that focusing on functional traits may provide a more mechanistic understanding of how regime shifts affect biodiversity and the consequences for ecosystem functioning. Regime shifts towards a turbid or shaded state negatively affect the taxonomic and functional diversity of fish and zooplankton, which in turn impairs the multifunctionality of shallow lakes.

Keywords: Alternative states; ecosystem multifunctionality; fish; functional diversity; shallow lakes; zooplankton.

2.1 Introduction

Shallow lakes are among the most common freshwater ecosystems on Earth (Verpoorter et al., 2014), and in a pristine state they are characterized by clear water and dominance of submerged macrophytes. However, increasing nutrient loading may shift shallow lakes to a turbid state dominated by phytoplankton or a shaded state dominated by small floating macrophytes (Moss, 1990; Scheffer & van Nes, 2007). Previous research has focused on factors that trigger the shift among the states or mechanisms that stabilize the different states (for review see Hilt et al., 2017), but the impacts of regime shift on the taxonomic and functional diversity of shallow lakes remain poorly understood. Moreover, whereas previous work has shown that regime shifts can impact a wide variety of individual ecosystem functions (such as primary production or nutrient concentrations; Hilt et al., 2017), very little is known about their effect on ecosystem multifunctionality. Multifunctionality is the ability of ecosystems to simultaneously support a multitude of ecological functions and as such has become a central topic of contemporary ecology and ecosystem management (Hector & Bagchi, 2007).

During the clear, turbid, and shaded states, shallow lakes exhibit different functioning and different biodiversity patterns (Moss, 1990; Scheffer et al., 1993). Ecosystems in the clear state support a high taxonomic richness of fish and zooplankton (Jeppesen et al., 1998), in part because submerged macrophytes provide effective shelters (Carpenter & Lodge, 1986; Blindow et al., 2014). During the clear state, shallow lakes have high habitat heterogeneity, favoring the development of more complex food webs with high proportions of apex piscivorous predators (Jeppesen et al., 2000; Moi et al., 2021a). Conversely, lakes in the turbid and shaded states have low taxonomic richness of fish and zooplankton (Jeppesen et al., 1999). The low habitat heterogeneity during turbid and shaded states results in a simplified food web with a low proportion or absence of apex predators and dominance of benthic and planktivorous fish and small-sized zooplankton (Mormul et al., 2012; Moi et al., 2021a).

It can be hypothesized that more complex food webs in the clear state (Jeppesen et al., 1999; Moi et al., 2020a) have a higher functional diversity of fish and zooplankton than lakes in the turbid and shaded states. Although this prediction has not yet been directly tested, the clear state sustains a richer combination of unique sets of functional traits, including fish (e.g., piscivores) and zooplankton (e.g., large-sized filter-feeders) compared to the turbid and shaded states (Moss, 1990; Jeppesen et al., 1998; Scheffer & van Nes, 2007). Ecosystem

multifunctionality is also expected to be higher in the clear state since higher taxonomic and functional diversity is required to sustain a greater range of ecosystem functions (Hector & Bagchi, 2007; Bagousse-Pinguet et al., 2019). Furthermore, the clear state often supports a great diversity of organismal traits that underlie ecosystem functioning. For example, large-sized apex predators and filter-feeders control multiple ecosystem functions such as fluxes of nutrients, primary production, and standing biomass (Moi et al., 2021b). The great diversity of traits related to feeding modes and habitat use during the clear state may enhance the overall resource utilization by communities, in turn increasing their ability to maintain ecosystem multifunctionality (Gross et al., 2017).

Ecological theory predicts that biodiversity (taxonomic and functional) may enhance ecosystem multifunctionality through two general mechanisms: (i) complementarity and (ii) selection effect (Loreau & Hector, 2001). Complementarity enhances ecosystem multifunctionality via niche partitioning and facilitative interactions among species, or through the overall increase in resources utilization induced by species with contrasting functional traits (Bagousse-Pingueta et al., 2019). By contrast, the selection effect enhances ecosystem multifunctionality through the greater statistical probability of highly productive species being more common in more diverse ecosystems (Loreau & Hector, 2001). Recent evidence suggested that single ecosystem functions, such as nutrient retention and primary productivity, were higher in the clear state than in the turbid and shaded states (Hilt et al., 2017; Su et al., 2019; Janssen et al., 2020). However, these studies did not link their findings to taxonomic and functional diversity and did not consider multiple ecosystem functions simultaneously (i.e., multifunctionality).

Here, we used a detailed database holding data for 12 years from a shallow lake, which during this period has displayed regime shifts among three alternative states (clear, turbid, and shaded). We compared the taxonomic and functional diversity of fish and zooplankton among the three states and determined their consequences for ecosystem multifunctionality. We then quantified an ecosystem multifunctionality index using a set of five ecosystem variables, including nutrient concentrations (in situ measurements of N and P water concentrations), algae production (biomass of edible algae), underwater light availability, and standing animal biomass (biomass of fish community). Together, these variables provide proxies for primary production, photosynthetically active radiation, nutrient availability, and standing biomass, which are important determinants of ecosystem functioning in shallow lakes (Moi et al., 2021b; Austin et al., 2021). We predicted that (i) the taxonomic and functional diversity of fish and

zooplankton would be lower in the shaded and turbid states than in the clear state and that (ii) ecosystem multifunctionality would be lower in the shaded and turbid states despite differences between single ecosystem functions (Hilt et al., 2017). As biodiversity-multifunctionality relationship tends to weaken with biodiversity loss (Cardinale et al., 2012), we also predicted that (iii) when the taxonomic and functional diversities of fish and zooplankton decrease in the turbid and shaded state, the relationship between multifunctionality and biodiversity would weaken as well.

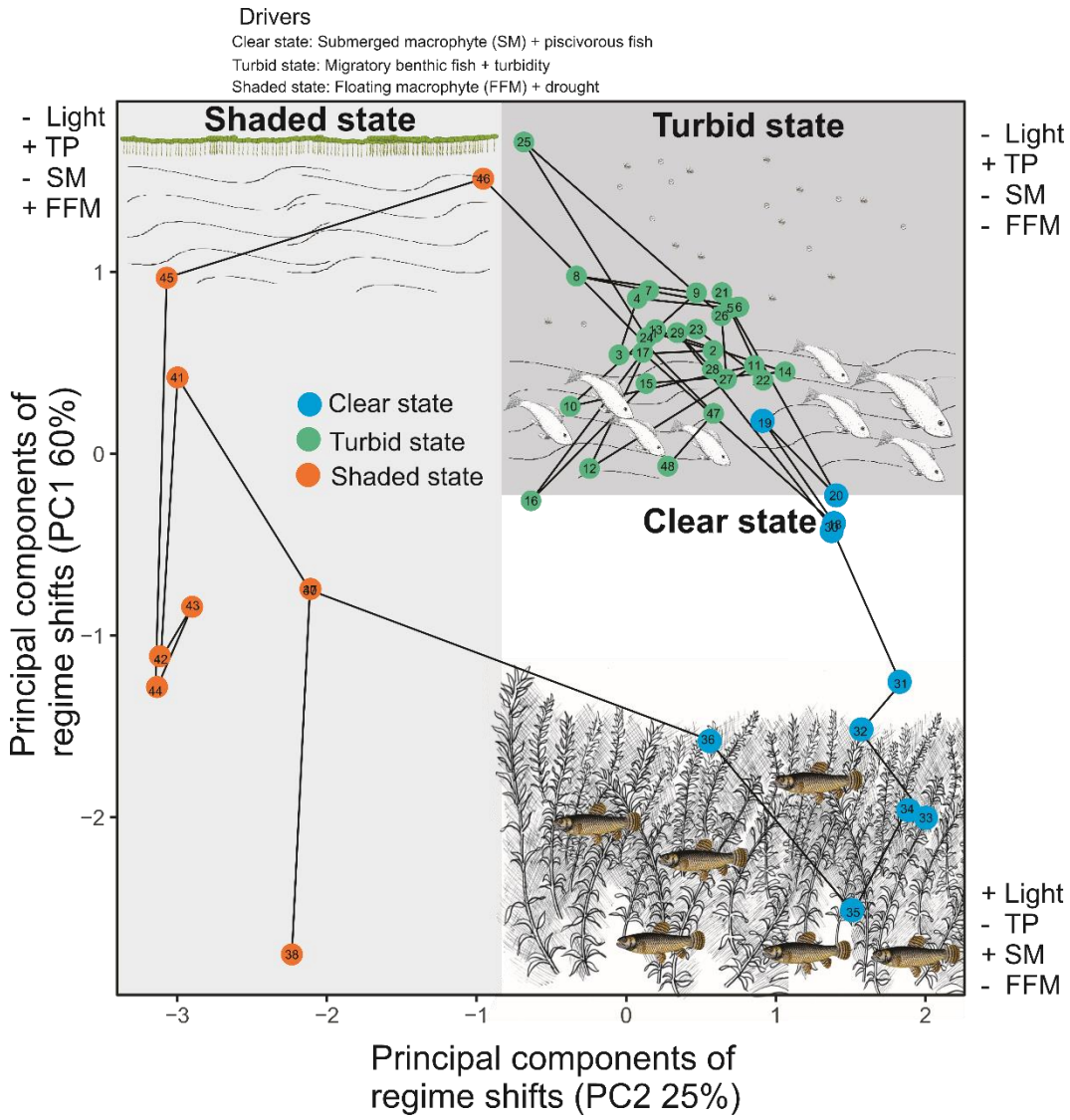
2.2 Materials and methods

2.2.1 Study site

Osmar is a shallow lake (60 m length, 1.1 m mean depth) located in the Upper Parana floodplain (22 º46´27. 53" S and 53º 19´ 57. 95"), Brazil (Figure S1). The lake is protected by a dense Atlantic Forest. The region has a tropical climate with a mean annual temperature of 22 °C (mean minimum and maximum temperatures of 10.3 and 33.6 °C, respectively) and a mean annual precipitation of 1500 mm (Moi et al., 2021a). The data used for the analysis originates from a long-term ecological research project (PELD-Sitio PIAP) and includes 12 years (2005 to 2016) of data,

During the 12-year period, Lake Osmar has undergone three alternative regimes, corresponding to a clear, turbid, and shaded state, as documented in two previous studies (Mormul et al., 2012; Moi et al., 2021a). The presence and transitions among the states are clearly observed in the bivariate plane of light availability, total phosphorus, and cover of submerged and floating macrophytes (Figure 1). Note that the sampling units were displaced from the top-right border of this plane (turbid state) to the bottom-right border (clear state), and after this to the top-left border (shaded state; Figure 1). This illustrates that as light availability, phosphorus concentrations and macrophyte cover change, the lake was pushed into distinct states. However, the regime shifts were driven by different mechanisms. The clear state was triggered by the presence of submerged macrophytes and high abundance of large piscivorous fish, which, directly and indirectly, reduced phosphorus concentrations and increased the water light availability (Mormul et al., 2012; Moi et al., 2021a). The turbid state was triggered by the high abundance of migratory benthic fish, which increased the phosphorus concentration and reduced the light availability (Mormul et al., 2012). The shaded state was triggered by low water levels and the presence of small floating macrophytes, which increased the phosphorus level and reduced the light availability (Moi et al., 2021a). The shifts among alternative states were

not associated with seasonality because (i) each regime remained dominant for more than two years, and (ii) the lake did not return to the previous state at the same time of the year (Figure 1).



F I G U R E 1. Principal component analysis (PCA) of the factors (phosphorus concentrations, light availability, submerged and floating macrophyte cover) that drive regime shifts between clear (blue circles), turbid (green circles) and shaded (orange circles) states. Each circle corresponds to a sampling unit. Note that the sampling units are displaced to different planes with the changes in phosphorus concentrations, light availability and macrophyte cover. Variables in the corners of the graph represent the drivers of the regimes [i.e., light availability, total phosphorus concentration (TP), submerged macrophytes (SMM) and free floating macrophytes (FFM)]. The images within each plane indicate the mechanisms that triggered the shift to each state; thus, the clear state was triggered by submerged macrophytes and large piscivorous fish, the turbid state was triggered by large migratory benthic fish and the shaded state was triggered by floating macrophytes and low water level. Also, only the sampling points that were not close to the borders of the bivariate plane were those that preceded the shift

between the clear and turbid states corresponding to bifurcation points (points 18, 19, 20 and 30).

2.2.2 Characteristics of each state

The clear state occurred from June 2009 to December 2009 and from June 2012 to March 2014 and was characterized by low turbidity, intermediate abundance of phytoplankton, high coverage of submerged macrophytes, and absence of small floating macrophytes (Figure S3). The turbid state occurred from June 2005 to November 2005, from March 2007 to February 2008, and from September 2008 to June 2009 and was characterized by high values of turbidity, large filamentous algae abundance, and absence of macrophytes (Figure S3). The shaded state occurred from June 2014 to December 2015 and was characterized by low turbidity, low abundance of phytoplankton, absence of submerged macrophytes, and high coverage of small floating macrophytes (Figure S3). We also recorded transitional states preceding the onset of the three states (clear, turbid, and shaded). These transitional states occurred several times during the monitoring period and always before the lake was pushed into a more stable regime. Therefore, the transitional states were not included in the subsequent analyses.

2.2.3 Fish and zooplankton sampling

The biological samplings were explicitly designed to assess the taxonomic and functional diversity of fish and zooplankton, as well as ecosystem functioning, during the three alternative states. During the 12 years, four annual standardized samples (summer, fall, winter, and spring) were collected, except in 2014 when only three sampling campaigns were conducted, resulting in 47 campaigns in total. The sampling of fish, zooplankton, and ecosystem variables were done using the same standardized methods throughout the whole study. The field study was properly realized with all required permissions from the Brazilian ministry of the environment (Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), National Council for the Control of Animal Experimentation (CONCEA)), and Ethics Committee on Animal Use under protocol number 1420221018 (ID 001974)). Fish were sampled with 20 m long seines with a mesh size of 0.5 cm in the littoral and middle zones of the lake for a 24h-period. As the lake is shallow, our sampling always included all lake compartments (i.e., sediment, pelagic, and littoral zones). Zooplankton were sampled in the subsurface of the pelagic zone using a motorized pump and a plankton net (68 µm), filtering 600 L water per sample. The samples were preserved in a 4% formaldehyde solution and buffered with calcium carbonate. To identify and enumerate (ind.m^{-3}) all organisms, the samples were processed under

an optical microscope with 100 x magnification. The abundance of individuals was estimated by analyzing minimum three subsamples, equivalent to 10% of the total sample, in a Sedgewick-Rafter chamber.

2.2.4 Functional traits

Fish and zooplankton were identified to species and categorized into functional groups according to the traits of the species (Oliveira et al., 2017; Braghin et al., 2018; Baumgartner et al., 2018; Tables S7 and S8). We selected the most important ecological traits that best reflect the importance of fish and zooplankton for the functioning of shallow lakes (Table S1). These traits are expected to differ among states, but this remains largely unexplored in natural ecosystems (see Appendix S1, for a full explanation of the traits). For fish, we used four functional trait combinations: body size (continuous, in cm), habitat use (benthic, benthopelagic, or pelagic), trophic guilds (piscivores, detritivores, omnivores, herbivores, insectivores, invertivores), and migration ability (migratory or non-migratory; Table S7). Body size was estimated by measuring the captured individuals, whereas the other traits were obtained from the literature (e.g., Oliveira et al., 2017; Baumgartner et al., 2018). For the functional categorization of zooplankton, we used five key functional traits: body size (continuous, in μm), habitat use (littoral or pelagic), feeding type (filter-feeder rotifers, sucker rotifers, predator rotifers, raptorial copepods, filter-feeder copepods, filter-feeder cladocerans, and scraper cladocerans), life span (short: a life span lower than five days, e.g., rotifers and Cladocera, long: a life span of up to one month, e.g., copepods), and predatory escape response (absent, low, medium, or maximum predatory escape; Table S8; Braghin et al., 2018). Body size was estimated by measuring the captured individuals, whereas the other functional traits were obtained from the literature (Braghin et al., 2018).

2.2.5 Ecosystem functions

To quantify how regime shifts affect ecosystem functioning, we scored the following five functions – nutrient concentrations, algae production, underwater light availability, and standing fish biomass – for each sampling period. To assess the potential for a trade-off between individual ecosystem functions, we calculated Spearman correlation coefficients between each pair of individual standardized functions. Of the possible 10 combinations of function pairs, we found only one strong correlation (underwater light availability with algae biomass = 0.83; Figure S2). This indicates a weak trade-off between individual functions, suggesting that the multifunctionality calculation was not biased by highly correlated functions. The five functions

are key properties of aquatic ecosystems (Moi et al., 2021b; Austin et al., 2021) and were measured 47 times during the study period.

2.2.5.1 Nutrient concentrations

Nutrient concentrations were quantified by *in situ* measurements of the total phosphorous (g L^{-1}) and total nitrogen (g L^{-1}) available in the water. Total phosphorus and nitrogen reflect all fractions of these nutrients under water, and their availability often limits primary producers and, consequently, primary production (Elser et al., 2009). We took water samples in each sampling period, and in the laboratory, nitrogen was analyzed via the persulphate method (Bergamin et al., 1978) and determined in a spectrophotometer in the presence of cadmium using a flow-injection system (Giné et al., 1980). Total phosphorus (TP) was measured according to Golterman et al. (1978).

2.2.5.2 Algae biomass

To obtain an indicator proxy for algae production, we measured the biomass of edible algae using the biovolume (individuals per mm L^{-1}) of nanoplankton: 2-60 µm and picoplankton: < 2 µm (Table S9). We focused on the biomass of small algae (such as Chlorophyceae) because they are the most abundant phytoplankton group in tropical shallow lakes (Moi et al., 2021a) and form the base of the food web of these ecosystems. Thus, they are the main food resource for small zooplankton that cannot feed efficiently on large algae (Lazzaro, 1997). Small edible algae were sampled in the pelagic zone using bottles and preserved in 10% acetic acid (Bicudo & Menezes, 2006). Biovolume was estimated by multiplying the abundance of each species by their mean volume. The algae volume was obtained from geometric models similar to three-dimensional shapes (Sun & Liu, 2003).

2.2.5.3 Underwater light availability

We quantified the underwater light availability as the depth of the euphotic zone, which represents the depth (m) of the lake where there is sufficient light incidence for autotrophs. The euphotic zone was calculated as Secchi depth multiplied by 1.7, where 1.7 is a correction factor for estimating the light available under water (Lansac-Tôha et al., 2021). The underwater light availability is a key resource that may limit primary producers, and thereby affect the primary production in aquatic ecosystems (Scheffer, 2004).

2.2.5.4 Standing fish biomass

To quantify fish biomass, all fish species were weighed using a microbalance (0.01 g precision). The standing biomass of the entire fish community (g m^{-2}) was then quantified by summing up the weight of all individuals and dividing it by the site area. Fish are important consumers in aquatic ecosystems, and their biomass is commonly used to reflect the ecosystem functioning (Benkwitt et al., 2020). Moreover, fish biomass is directly related to important ecosystem services such as fish production and food security (Duffy et al., 2016).

2.2.6 Multifunctionality

To test the effects of regime shifts on the simultaneous performance of multiple ecosystem functions, we calculated averaging multifunctionality (Byrnes et al., 2014). We first standardized all individual ecosystem variables between 0 and 1 (rawFunction−min(rawFunction)/(max(rawFunction)−min(rawFunction)) and then calculated their average to obtain a multifunctionality index (Byrnes et al., 2014). This index reflects changes in the average level of a suite of ecosystem functions. Very high levels of the averaging index (close to 1) mean that all functions reach their maximum level of performance simultaneously. In contrast, the lowest values (close to 0) mean all functions are at their minimum level of performance. High multifunctionality index in a given state (i close to 1) indicates that this state supports more functions operating at high performance levels. This implies that maintaining the lake in this state is more advantageous for maximizing their functioning. This is of key importance as ecosystems are managed and conserved to support multiple functions simultaneously (Hector & Bagchi, 2007).

2.2.7 Data analysis

The taxonomic richness of fish and zooplankton was calculated using the number of species captured in each sampling campaign. To account for possible differences in population densities between alternative states, we estimated species richness as the Chao index with abundance-based data using the ‘iNEXT’ package (Hsieh et al., 2016). The Chao index is based on rarefaction and extrapolation of Hill numbers, providing an unbiased estimate of asymptotic species richness and enabling comparison among alternative states with different numbers of individuals. The functional diversity of fish and zooplankton communities was calculated using Rao's quadratic entropy (RaoQ), which is a common measure for estimating functional diversity (Botta-Dukát, 2005). RaoQ has the advantage that it incorporates the weighted relative

abundance of each species and converts it to effective numbers. The trait matrix of fish and zooplankton had mixed variables (continuous and categorical); thus, we applied Gower's dissimilarity with Cailliez correction (Laliberté & Legendre, 2010). To further characterize the functional composition of the fish and zooplankton communities, we calculated community-weighted means (CWMs) for each functional trait (which was weighted by relative species abundances). We calculated the Rao's quadratic entropy (Rao's Q) and community-weighted means (CWMs) using the FD package in R (Laliberté et al., 2015).

We tested how the alternative states (fixed categorical: clear, shaded, and turbid) affect (i) taxonomic richness, (ii) functional diversity (FD), and (iii) community-weighted means (CWMs) of fish and zooplankton, (iv) five individual ecosystem functions (nitrogen and phosphorus concentrations, underwater light availability, algae biomass, and standing fish biomass), and (v) multifunctionality (averaging index) using linear-mixed effects models (LMEs) in the package *nlme* (Pinheiro et al., 2013). To control for seasonality effects in each state, we nested the seasons within year in each state as a random structure. This allowed the intercept to vary in each season within year independently for each state. In this floodplain ecosystem, exchange of biota in the lakes and water occurs during the flood period where the lakes may be connected with the rivers (Mormul et al., 2012). Thus, the temporal sampling performed in our study is considered independent. Moreover, we did not find temporal autocorrelation in our data using the function CAR1 in the 'CAR' package (Fox & Weisberg, 2019). We ensured that the model assumptions of variance homogeneity, normality, and outliers were met. We conducted post hoc comparisons between alternative stable states with Tukey's HSD using the *glht* function in the 'multcomp' package (Hothorn et al., 2013).

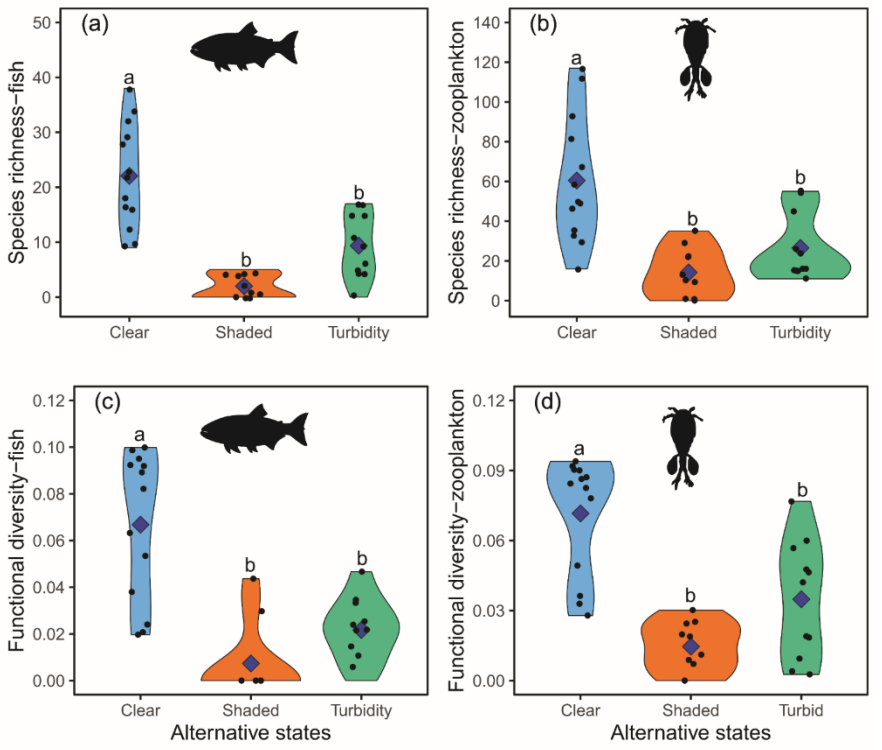
To evaluate the effects of the taxonomic and functional diversity of fish and zooplankton on ecosystem multifunctionality across the three states (clear, turbid, and shaded), we also employed mixed models. We explicitly included water level to account for seasonality in the data, such as flood and drought, which also may affect ecosystem multifunctionality (Moi et al., 2021b). To determine whether the effects of the predictors on multifunctionality change among states, we added interaction terms among the predictors (taxonomic and functional diversity) and state (clear, turbid, and shaded) into the models. We nested the seasons within year in each state as a random structure. We used a model selection approach to reduce the number of predictors, thereby obtaining a more parsimonious way of testing the relationships between taxonomic and functional diversity and water level versus multifunctionality. We ranked the set of candidate models consisting of every individual

predictor as well as their additive combinations and interactions with state as predictor variables influencing multifunctionality. A null model was also included into the model selection process (Table S2). We checked the multicollinearity between the predictors by calculating the variance inflation factor (VIF) for each predictor. VIF > 3 indicates possible collinearity, but all relationships had VIF < 2. The set of candidate models was constructed using LMEs and contrasted using sample-size corrected Akaike Information Criteria (AICc) (Burnham & Anderson 2002). Difference > 2 (ΔAICc < 2) was used to identify the best model using the function *ICtab* of the 'bbmle' package (Bolker, 2020). We only show the best models (i.e., those ΔAICc $\leq$ 2) graphically. Finally, to analyze the relationship between the community-weighted means of each trait of fish and zooplankton with the individual ecosystem functions, we performed Spearman correlations in each state. All analyses were performed in R (R Core Team, 2020).

2.3 Results

2.3.1 Taxonomic and functional diversity during regime shifts

There were marked changes in the taxonomic and functional diversity of fish and zooplankton communities over the 12-year study period. The values of both biodiversity indices increased from June 2009 to December 2009, and from June 2012 to March of 2014, coinciding with the clear state periods (Figure S4). The species richness of fish and zooplankton was higher in the clear state than in the turbid and shaded states (Table S4, Figures 2A,B). Likewise, the functional diversity of fish and zooplankton was significantly higher in the clear state than in the shaded and turbid states (Table S4, Figure 2C,D). Neither the taxonomic nor functional diversity of fish and zooplankton differed between the turbid and shaded states (Table S4, Figure 2).

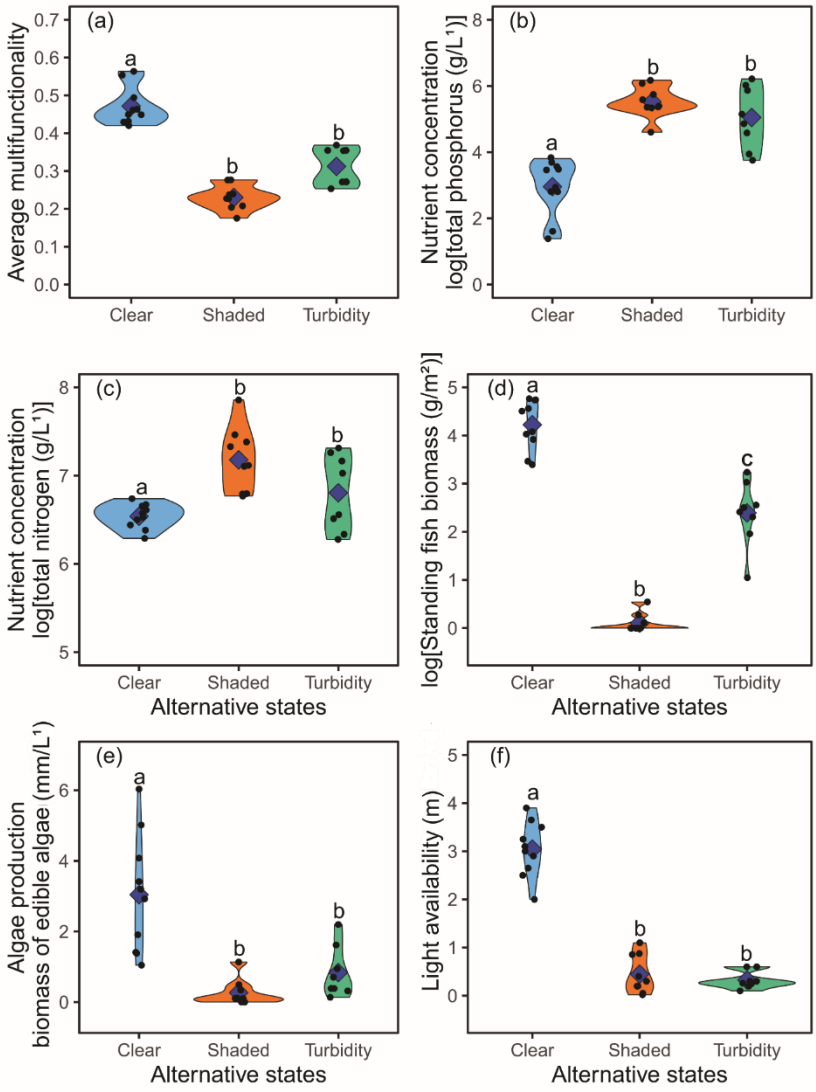


F I G U R E 2. Differences in taxonomic (A, B) and functional (FD; C, D) diversity of fish and zooplankton communities among the clear, shaded and turbid states. The blue triangle in the centre of each plot denotes mean values, and the different letters indicate statistically significant differences (LME/Tukey contrasts, $\alpha = 0.05$). Jitter function was used on the data to prevent overplotting.

There were significant differences in the CWM of most of the functional traits of fish and zooplankton among the three alternative states (Figure S5 and S6). For fish, the CWMs of body size, pelagic habitat preference, and feeding groups (apex piscivorous predators, omnivores, herbivores, insectivores, and invertivores) were higher in the clear state than in the turbid and shaded states (Table S5, Figure S5). By contrast, the CWMs of detritivores and migration ability were higher in the turbid state than in the clear and shaded states (Table S5, Figure S5). For zooplankton, the CWMs of body size, littoral habitat preference, feeding groups (filter-feeding copepods and filter-feeding cladocerans), no predator escape ability, and low predator escape ability were higher in the clear state than in the turbid and shaded states (Table S6, Figure S6). The CWMs of predatory rotifers and maximum predator escape ability were higher in the turbid state than in the clear and shaded states (Table S6, Figure S6). For both fish and zooplankton, all CWMs of functional traits were lowest in the shaded state (Figures S5 and S6).

2.3.2 Multifunctionality and individual ecosystem functions during regime shifts

There were significant differences in the multifunctionality and the five individual ecosystem functions when the lake was in the three different states. Notably, the multifunctionality was significantly higher during the clear state than during the shaded and turbid states (Table S4, Figure 3A). By contrast, the nutrient concentrations, including total phosphorus and total nitrogen, were significantly higher during the shaded and turbid states (Table S4, Figure 3B,C). Finally, the standing fish biomass as well as algae biomass and underwater light availability were significantly higher during the clear state than during the shaded and turbid states (Table S4, Figure 3D-F).

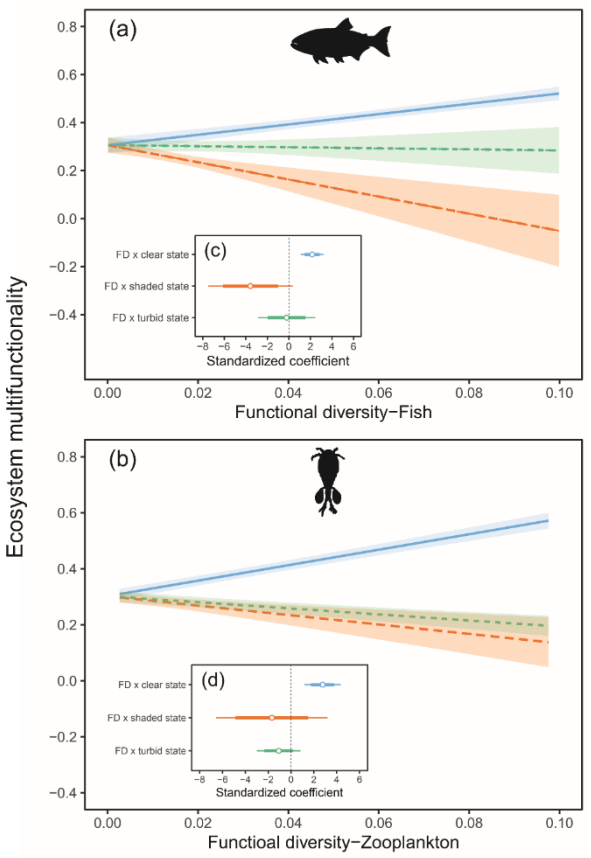


F I G U R E 3. Differences in (A) multifunctionality, (B) total phosphorus, (C) total nitrogen, (D) standing fish biomass, (E) algae biomass and (F) underwater light availability among the clear, shaded and turbid states. The blue triangle in the centre of each plot denotes mean values,

and the different letters indicate statistically significant differences (LME/Tukey contrasts, α = 0.05). Jitter function was used on the data to prevent overplotting.

2.3.3 Regime shifts driving the relationship between biodiversity and multifunctionality

The AIC model selection revealed that the functional diversity of fish and zooplankton and their interactions with states were the best predictors of the multifunctionality (Table S3). Together, functional diversity and their interactions with states explained up to 65% of the variation in the multifunctionality. The functional diversity of fish and zooplankton was strongly and positively associated with multifunctionality during the clear state (P = 0.003; Figure 4A,B). However, these relationships lost strength during the turbid and shaded states (Fig. 4). Consequently, the slope of the relationship between functional diversity and multifunctionality changed from being significantly positive during the clear state to being non-significant with negative trends during the turbid and shaded states (Figure 4C,D).

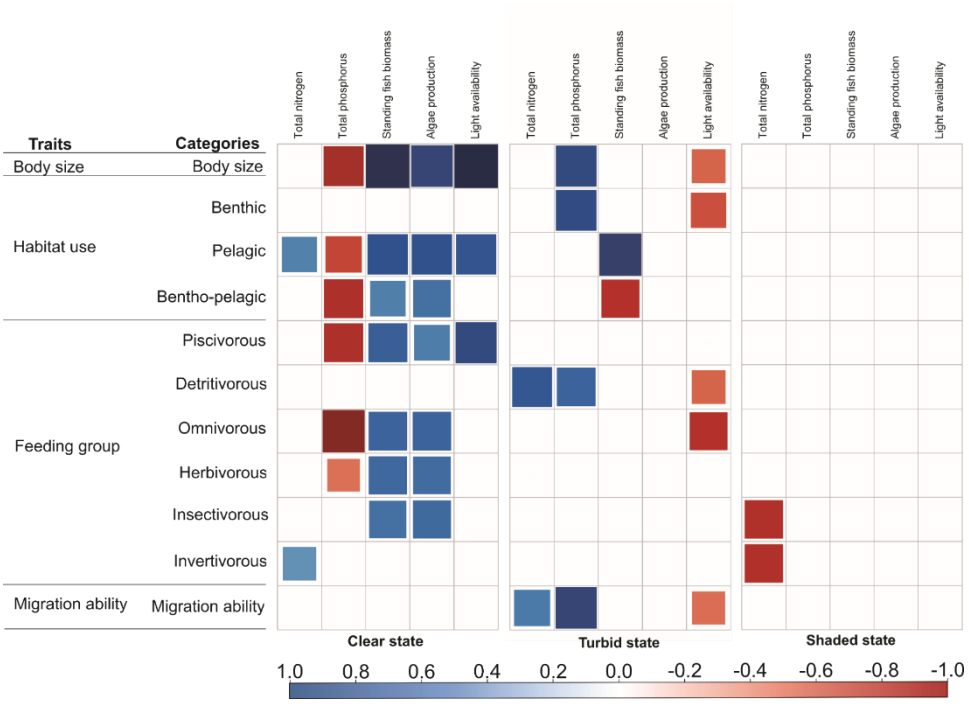


F I G U R E 4. The effects of (A) fish and (B) zooplankton functional diversity on ecosystem multifunctionality during the clear, turbid and shaded states. The solid-coloured line represents the effect of a linear mixed-effects model fit, while the shaded areas represent 95% confidence intervals. The graphic was constructed using the 'interact_plot' function from the interactions r package (Long, 2019). (C, D) Estimate coefficients for the effects of the interaction term between functional diversity and each alternative state on ecosystem multifunctionality. The

points represent estimates (non-scaled), thick lines represent 75% CIs and thin lines represent 95% CIs.

2.3.4 Relationship between traits and individual ecosystem functions

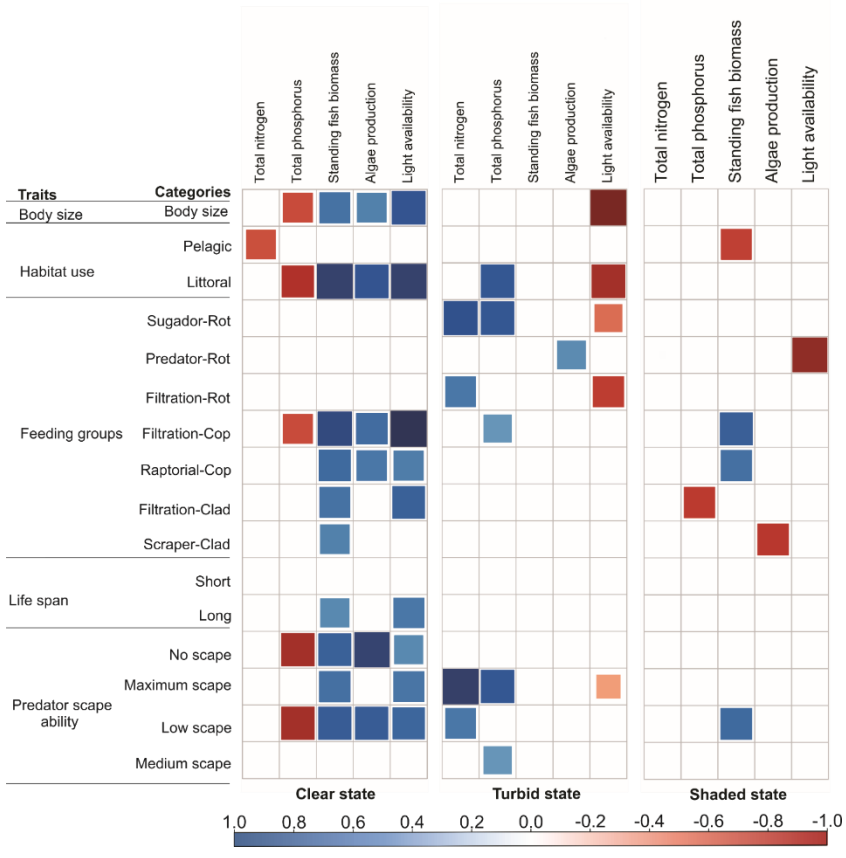
In addition to changes in the relationships between functional diversity and multifunctionality, there were also significant changes in the relationships between traits and individual ecosystem functions during the regime shifts. For example, there were many more significant correlations between traits and individual ecosystem functions during the clear state than during the turbid and shaded states (Figures 5 and 6). For fish, traits related to body size, habitat use (pelagic and bentho-pelagic), feeding groups (piscivorous, omnivorous, herbivorous, and insectivorous) were positively correlated with standing fish biomass, algae biomass, and underwater light availability, and negatively correlated with total phosphorus during the clear state (Figure 5). In contrast, during the turbid state, large-sized detritivores with migratory ability were positively correlated with total nitrogen and total phosphorus concentration, and negatively correlated with underwater light availability (Figure 5). During the shaded state, there were only two negative correlations between feeding groups (insectivorous and invertivorous fish) and total nitrogen (Figure 5).



F I G U R E 5. Significant correlations (Pearson; $p \leq 0.05$) between the community-weighted mean traits (CWMs) of fish with the five individual ecosystem functions (total phosphorus,

total nitrogen, standing fish biomass, algae biomass and underwater light availability) during each alternative stable state (clear, turbid and shaded). Colour squares illustrate significant correlations and white squares illustrate non-significant correlations.

For zooplankton, traits related to body size, habitat use (littoral), feeding groups (filtering copepods, raptorial copepods, and filtering cladocerans), life span (long), and predator escape ability (no escape, low, and maximum) had strong positive correlations with the standing fish biomass, algae biomass, and light availability during the clear state (Figure 6). The same traits were negatively correlated with total phosphorus during the clear state. During the turbid state, the traits related to body size, habitat use (littoral), feeding groups (sucker and filtering rotifers), and predator escape ability (low, medium, and maximum) were positively correlated with total nitrogen and total phosphorus and negatively correlated with underwater light availability (Figure 6).



F I G U R E 6. Significant correlations (Pearson; $p \leq 0.05$) between the community-weighted mean traits (CWMs) of zooplankton with the five individual ecosystem functions (total phosphorus, total nitrogen, standing fish biomass, algae biomass and underwater light availability) during each alternative stable state (clear, turbid and shaded). Coloured squares illustrate significant correlations and white squares illustrate non-significant correlations. Traits with Rot = rotifers, Clad = cladocerans and Cop = copepods.

2.4 Discussion

This study revealed that species richness and the functional diversity of fish and zooplankton were higher in the clear state than in the turbid and shaded states, indicating that the clear state sustains the highest number of species and unique sets of traits. In addition, the functional diversity of fish and zooplankton was strongly associated to ecosystem multifunctionality during the clear state. However, this relationship weakened during the turbid and shaded states. These findings demonstrate how regime shifts alter the functional diversity of shallow lakes, impairing the relationship between biodiversity and multifunctionality, ultimately decreasing multifunctionality. As regime shifts often occur abruptly, predicting this phenomenon for real-world ecosystems is a major challenge (Scheffer et al., 2009). Consequently, the impacts of regime shift on biodiversity and ecosystem functioning are little explored as this requires long-term and detailed ecosystem monitoring (Cooper et al., 2020). Our findings are a valuable contribution to understanding the effects of regime shifts on two facets of biodiversity (taxonomic and functional) and on ecosystem multifunctionality.

The higher taxonomic and functional diversity during the clear state was likely driven by the high coverage of submerged macrophytes. Macrophytes are known to increase habitat heterogeneity by providing space and refuge that facilitate species coexistence, thus increasing fish and zooplankton diversity (Meerhoff et al., 2007; Marsh et al., 2020). Furthermore, the trait diversity of fish and zooplankton also increases with habitat heterogeneity (Stuart-Smith et al., 2013; Porcel et al., 2020). We found that 63% (7 out of 11) and 50% (8 out of 16) of the functional traits of fish and zooplankton, respectively, were more abundant in the clear state than in the turbid and shaded states. Functional traits such as large body size, piscivorous and herbivorous feeding modes, as well as large-sized filter-feeding and low predator escape ability were abundant only during the clear water state. These traits may be indicators of the clear state because they were absent or occurred at negligible densities during the turbid and shaded states.

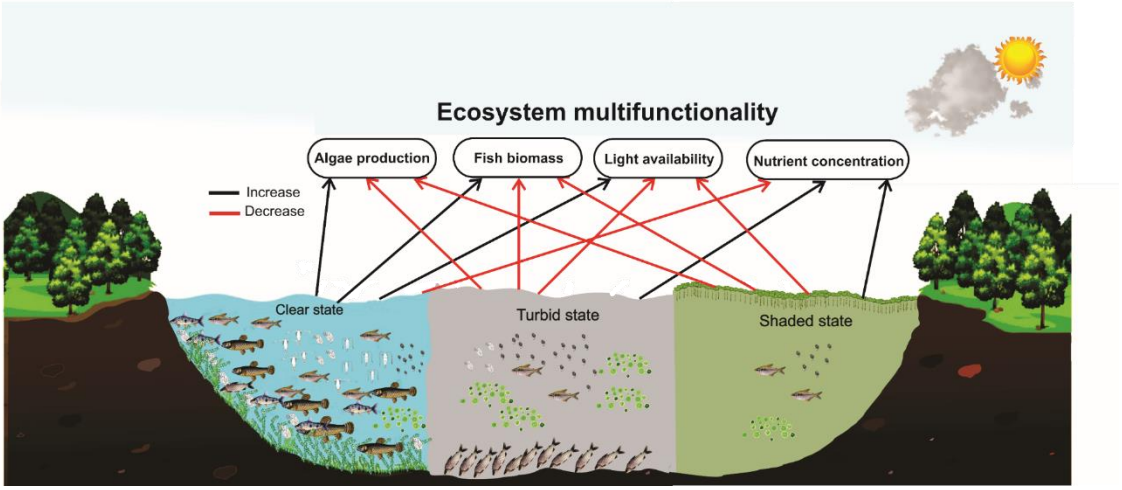
We found that the taxonomic and functional diversity of fish and zooplankton decreased during the turbid and shaded states. Because few traits were abundant during these two states, this indicates that only a few species with similar sets of traits were able to persist in the ecosystem during the turbid and shaded states. During the turbid state, fish were dominated by species with a benthic habitat preference, detritivorous feeding mode, and high migration ability, whereas the zooplankton were dominated by small filter-feeding rotifers with high

predator escape ability. These trait combinations are common in lake ecosystems in a turbid state (Mormul et al., 2012), as these are characterized by low refuge availability, which increases the predator-prey encounter rates (Figueiredo et al., 2020) and favors small-sized prey that can evade predation or remain undetected (Špoljar et al., 2018). The turbid state also sustains simplified food webs with low densities or even absence of apex piscivorous fish (Hobbs et al., 2012). This state is also characterized by high concentrations of detritus, which favors detritivorous fish (Mormul et al., 2012; Hobbs et al., 2012). Although isolated, the studied lake may connect to an adjacent river during intense flood events, alowing the entry of migratory benthic fish and providing suitable conditions for their development during the turbid state (Mormul et al., 2012). During the shaded state, low taxonomic and functional diversity of fish and zooplankton likely reflects the high environmental stress (Janse & Van Puijenbroek, 1998; Scheffer et al., 2003). The shaded state is characterized by dominance of small floating macrophytes, low water level, poor water quality (e.g., low O_2), and low habitat heterogeneity (Moi et al., 2021a). The combination of these stressors often causes high mortality of fish and zooplankton during the shaded state (Moi et al., 2021a), resulting in a decline in species richness, and homogenization of functional diversity.

There was a markedly higher multifunctionality, as well as a higher standing fish biomass, edible algae biomass, and underwater light availability during the clear state. Based on three lines of evidences, our findings indicate that this greater and healthier ecosystem functioning was due to higher functional diversity during the clear state. First, the relationship between functional diversity and multifunctionality was strongly positive during the clear state, but become flatted during the turbid and shaded states (Figure 4). Second, there were more significant links of fish and zooplankton traits with the individual ecosystem functions during the clear state (Figures 5 and 6). Third, species with key traits, such as large piscivorous fish and large filter-feeding zooplankton, were more abundant during the clear state. These findings suggest that fish and zooplankton assemblages were composed of functionally complementary species during the clear state, which allowed more ecosystem functions to be maintained by these two assemblages (Barry et al., 2019; Bagousse-Pinguet et al., 2019). Greater abundance of species with more influential traits is known to increase the efficiency of biodiversity in maintaining the ecosystem functioning (Loreau & Hector, 2001). Fish and zooplankton may increase multifunctionality in several ways, and their effects are stronger with the presence of more sets of functional traits (Moi et al., 2021b). These effects may include (i) bioturbation, (ii) presence of carcasses and faeces, (iii) translocation of nutrients among ecosystem

compartments, and (iv) indirect impact trophic cascades. All these pathways may maximize the edible algae biomass, standing fish biomass, and underwater light availability (Atkinson et al., 2017; Carpenter et al., 2001; Schmitz et al., 2010; Moi et al., 2021a). Moreover, there is evidence of a positive feedback between the clear state and biodiversity. We found that the clear state increased multiple biodiversity dimensions (Figure 6). In turn, higher diversity of functional traits, was beneficial for the clear state by promoting healthier functioning and maintaining high underwater light availability, which favours the maintenance of the clear state (Scheffer & van Nes, 2007).

Although nutrient concentrations increased during the turbid and shaded states, the standing fish biomass, edible algae biomass, and underwater light availability decreased (Figure 7). There were few links between the traits and the individual ecosystem functions during these two states. Combined with the fact that the positive relationship between functional diversity and multifunctionality become non-significant with negative trends during the turbid and shaded states, these results suggest that the loss of functional traits with regime shifts was closely accompanied by a decline in the multifunctionality. More broadly, these results highlight that regime shifts toward turbid or shaded states degrade the positive relationship between biodiversity and multifunctionality. This is because the functional diversity needed to maintain numerous ecosystem functions is reduced (Hilt et al., 2017). Our results add to recent empirical evidence from grasslands, temperate lakes, and rivers (Goto et al., 2020; Zhang et al., 2020; Freitag et al., 2021), indicating that regime shifts cause a biodiversity decline with negative consequences for important ecosystem functions. From an ecosystem management perspective, the clear state is noticeably more beneficial for human uses as it sustains a better and healthier ecosystem functioning, including higher production of fish biomass.



F I G U R E 7. Infographic representation of the characteristics of the three alternative states in Lake Osmar: clear (left, from June 2009 to December 2009 and from June 2012 to March 2014), turbid (centre: from June 2005 to November 2005, from March 2007 to February 2008 and from September 2008 to June 2009) and shaded (right: June 2014 to December 2015). During the clear state, the lake had a high taxonomic and functional diversities of fish and zooplankton as well as the highest primary productivity and biomass stock. By contrast, in the turbid state, the lake was dominated by migratory benthic fish and small zooplankton and had a high nutrient load. During the shaded state, the taxonomic and functional diversities of both fish and zooplankton markedly decreased, and no functional trait dominated. Likewise, the primary productivity and biomass stock decreased, but nutrient availability increased in the turbid and shaded states compared to the clear state.

Conclusions

Shallow lakes are the most abundant freshwater ecosystems worldwide (Verpoorter et al., 2014) and they provide multiple services to human well-being (Xu et al., 2017). Our shown that regime shifts alter the patterns of taxonomic and functional diversity in shallow lakes (Figure 7). Regime shifts toward turbid and shaded states weaken the relationships between biodiversity and multifunctionality by reducing the number of unique functional traits of fish and zooplankton. Our findings suggest that focusing on functional traits instead of relying only on traditional measures of species richness, provides a more mechanistic understanding of regime shifts and their consequences for ecosystem functioning. We draw this conclusion because sets of traits related to the large body size, feeding mode (including piscivorous, herbivorous, and insectivorous fish, and large filtering zooplankton), and habitat use (species that preferentially select shoreline habitats) were lost as the lake shifted toward turbid and shaded states. These groups of organisms and the associated traits tended to respond more strongly to these regime shifts. This is particularly concerning as these sets of traits were also those most strongly associated with ecosystem functions, indicating that loss of these traits will have negative consequences for the functioning of shallow lakes. In addition, the weakening of the relationship between functional diversity and multifunctionality resulting from regime shifts indicates that biodiversity conservation alone will likely not be sufficient to sustain multifunctionality if the underlying regime shifts are not controlled. Given that regime shifts are a global issue (Scheffer & van Nes, 2007; Hilt et al., 2017; Janssen et al., 2020), our results, based on detailed monitoring data from 12 years, could assist in the management of shallow lakes world-wide that face regime shifts.

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APPENDIX A – Details of the study area and results

Characteristic of the study area

The floodplain of the Upper Paraná River is located above the Itaipu hydroelectric plant. It is approximately 250 km long, occupies an area of 5,268 km^2 and exhibits numerous secondary canals, lakes, and rivers. In addition, the entire region is surrounded by vast forests that encompass a wide diversity of environments, including small shallow lakes. Our study was conducted in Lake Osmar, a small isolated shallow lake, located 120 meters from the channel of the Paraná River (22º46'27.53 "S and 53º19'57.95" O). During flood periods with an increase of the river's hydrometric level, the lake is connected to the Paraná River. Rainy periods usually occur between November to March (spring and summer) and dry periods between June and September (winter). In addition, there are several dams in the bed of the Paraná River, which can influence the water levels of the basin (Agostinho et al., 2004) and indirectly also the dynamics of lakes adjacent to the main river. The study area is monitored within the framework of the long-term ecological research project PELD - Sitio PIAP, according to which quarterly sampling is carried out. The data applied in the present study were obtained during the samplings conducted from March 2005 to December 2016.

Fish and zooplankton traits and their relationship with regime shifts

In this study, we selected four traits of fish (body size, habitat use, trophic guild, and migration ability) and five traits of zooplankton (body size, habitat use, feeding type, life span, and predatory escape response). We hypothesized that these trait combinations of fish and zooplankton communities would differ among the three environmental states of shallow lakes (clear, turbid, and shaded), a hypothesis that has not yet been tested in real-world ecosystems. For example, the body size (maximum total length) of fish and zooplankton is often small in the turbid and shaded state where small-sized fish and zooplankton are abundant, while large-sized fish and zooplankton are abundant in the clear state (Pace et al., 2013; Moi et al., 2020). Likewise, the feeding groups of fish and zooplankton differ among states; in the clear state, piscivorous fish are abundant and the occurrence of small planktivorous and omnivorous fish is therefore low, indirectly favoring high abundance of large-sized zooplankton (Carpenter et al., 2001). In the turbid and shaded states, piscivorous fish are often absent, and small planktivorous or omnivorous fishes might be abundant, which decreases the abundance of large-sized zooplankton (Carpenter et al., 2001; Moi et al., 2020). The type of habitat

occupation is also expected to change following regime shifts; the clear state provides an effective shelter for small fish and zooplankton, which might result in higher abundance of fish and of zooplankton preferring a littoral habitat (Meerhoff et al., 2003, 2007). By contrast, in the turbid and shaded states refuges are few, which might result in dominance of benthic fish and small pelagic zooplankton (Mormul et al., 2012; Moi et al., 2020). Also, the fish migration ability may change among states (Brönmark et al., 2010). For example, Mormul et al. (2012) found high abundance of migratory benthic fish during the turbid state and low abundance in the clear state. Finally, the traits considered in this study have repeatedly been found to explain a large proportion of the variation in lake ecosystem functioning (see table S1).

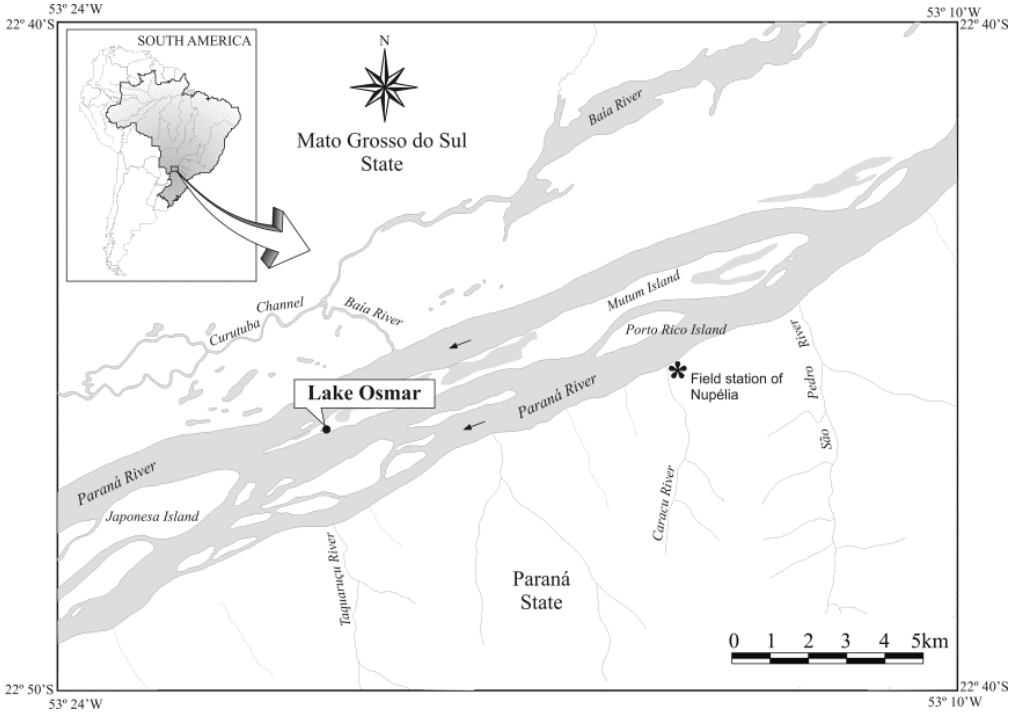

Figure S1. Map of the upper Paraná River floodplain highlighting Lake Osmar.

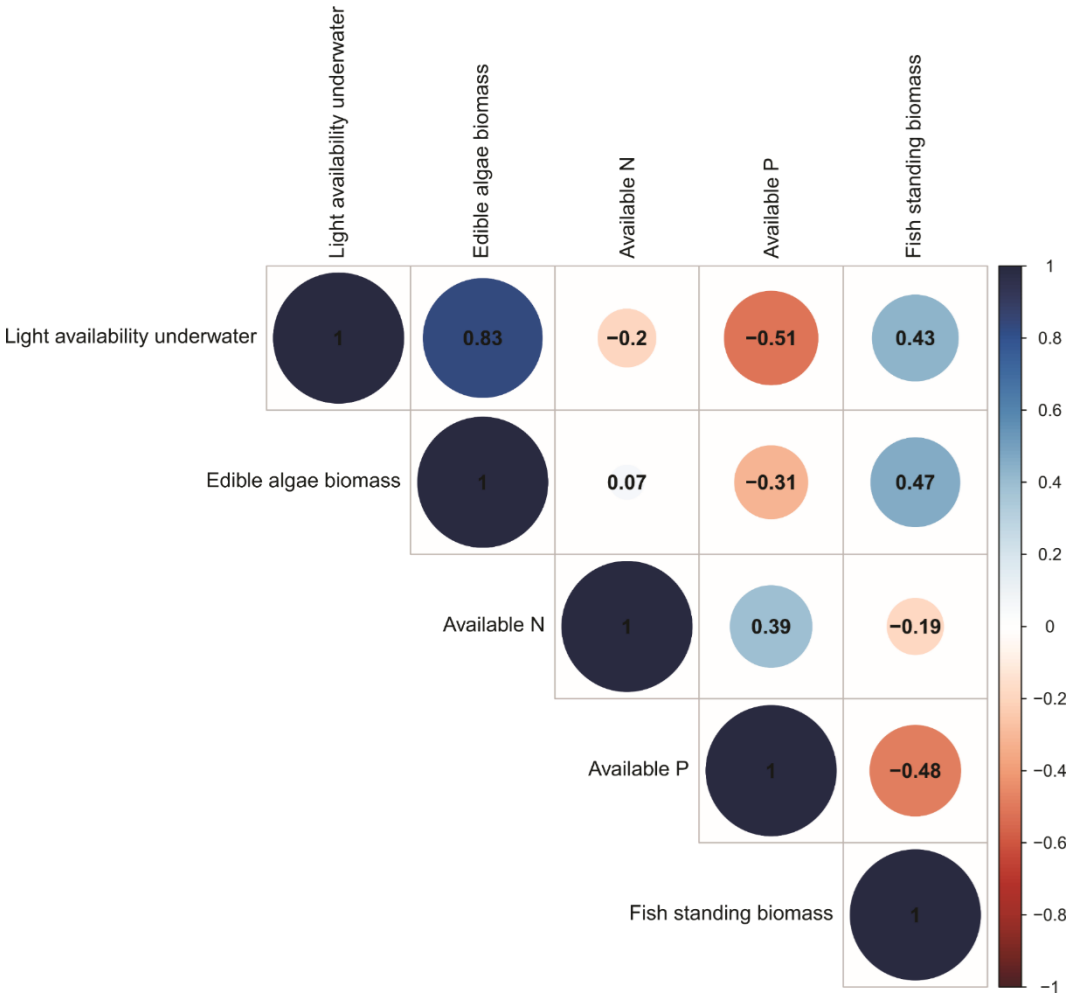

Figure S2. Correlations among five individual ecosystem functions used to create the multifunctionality index. Note that of the possible 10 combinations of pairs of functions, there are only one strong correlation (underwater light availability vs algae production = 0.83). This illustrates a weak trade-off between the individual functions, indicating that the multifunctionality calculation was not biased by highly correlated functions.

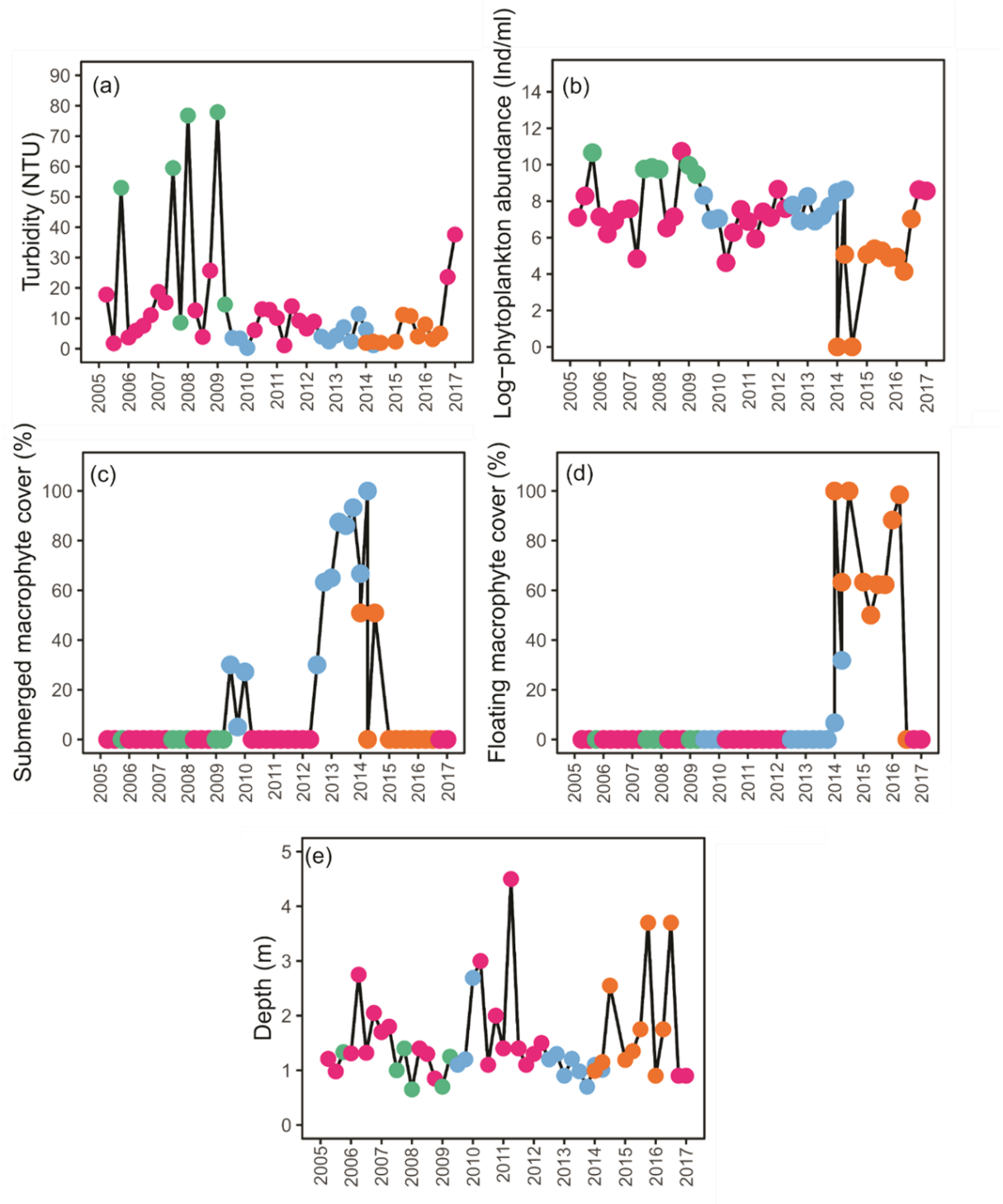

Figure S3. The variables triggering the shifts between the alternative stable states, (a) turbidity; (b) phytoplankton abundance, (c) submerged macrophyte cover, (d) floating macrophyte cover, and (e) depth. Each year had four sampling events (March, June, September, and December).

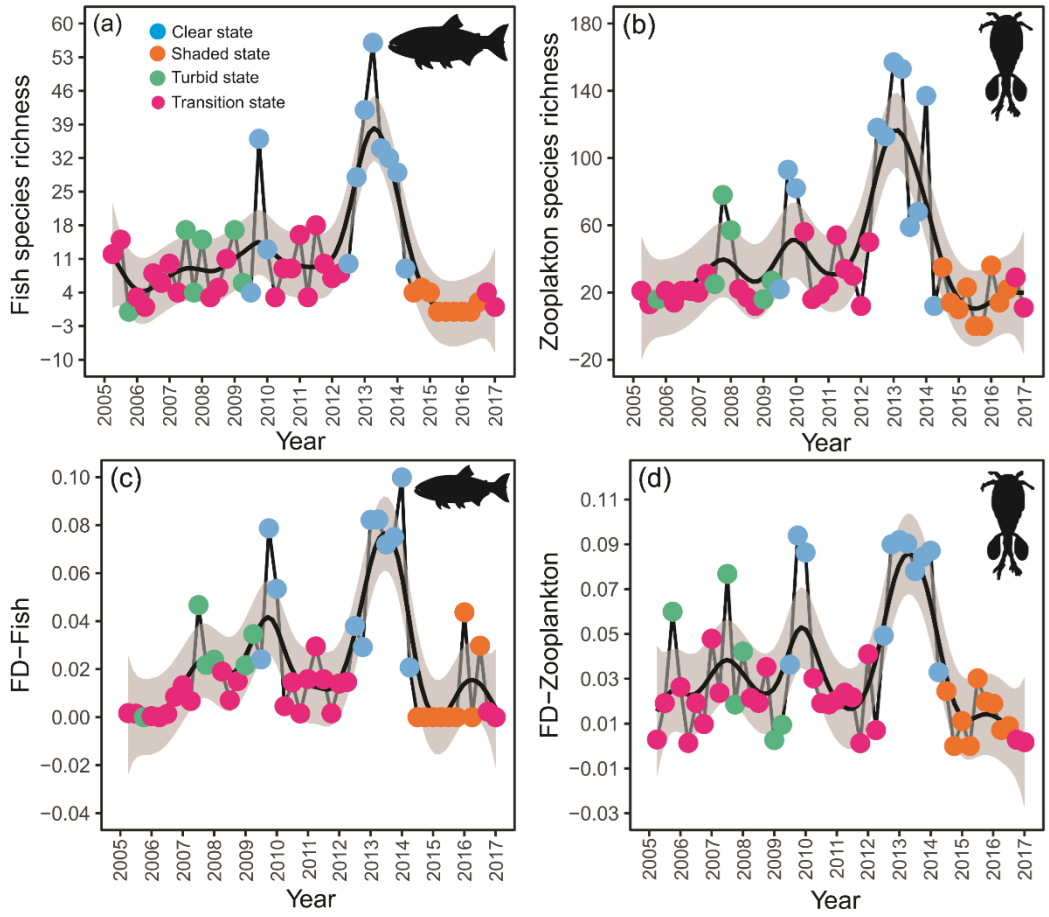

Figure S4. Temporal trends of the taxonomic and functional diversity of fish (a, c) and zooplankton (b, d) over the 12 study years (2005-2016). Each year had four sampling events (March, June, September, and December).

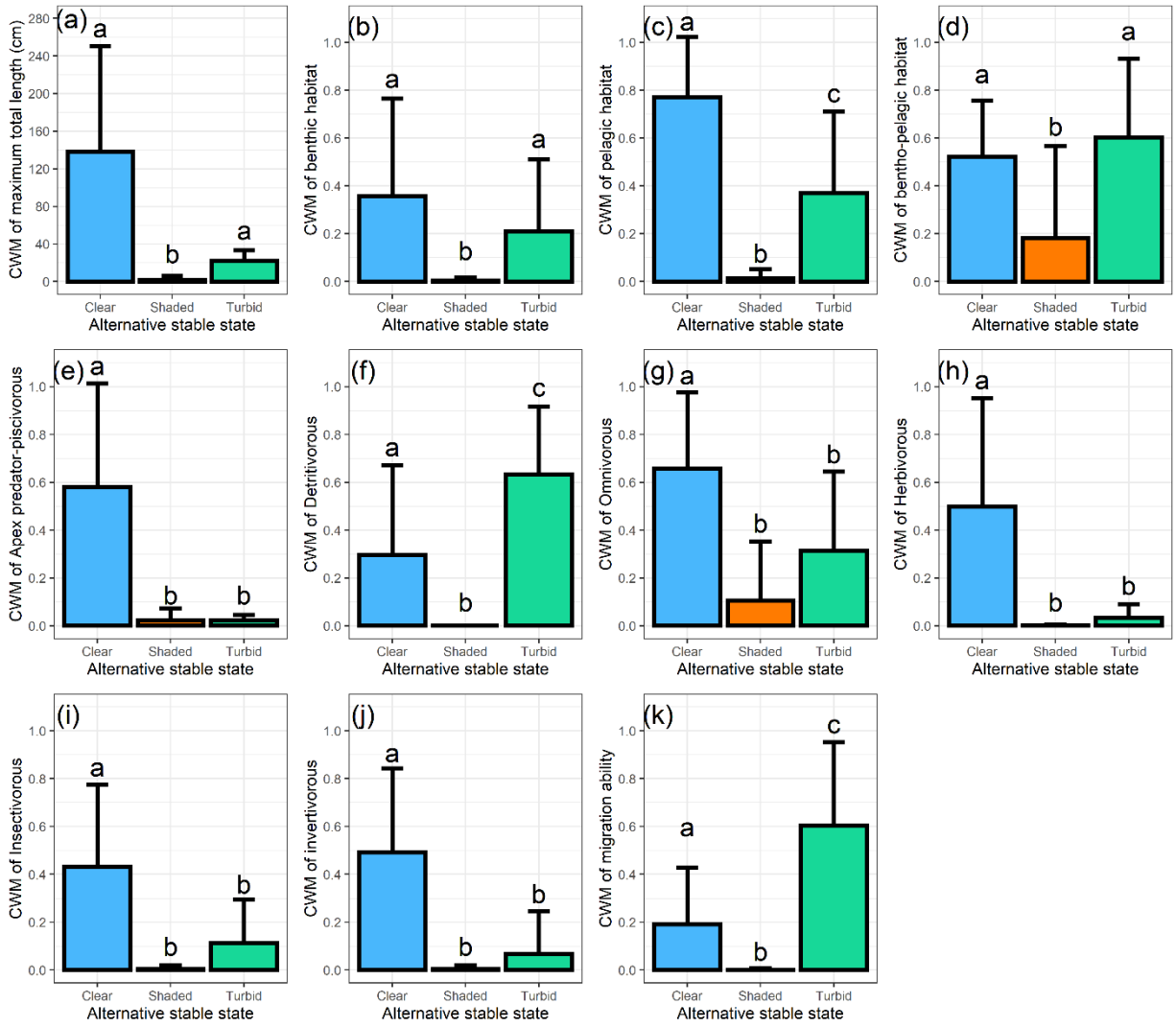

Figure S5. Mean ± SE of fish community-weighted means (CWMs) among three alternative states (clear water, shaded, and turbid). Each functional trait of fish was weighted by the relative abundance of species. Importantly, only maximum total length is a continuous variable, while other variables are dummies (binary). Different letters indicate statistically significant differences (one-way LME/Tukey contrasts, $\alpha = 0.05$).

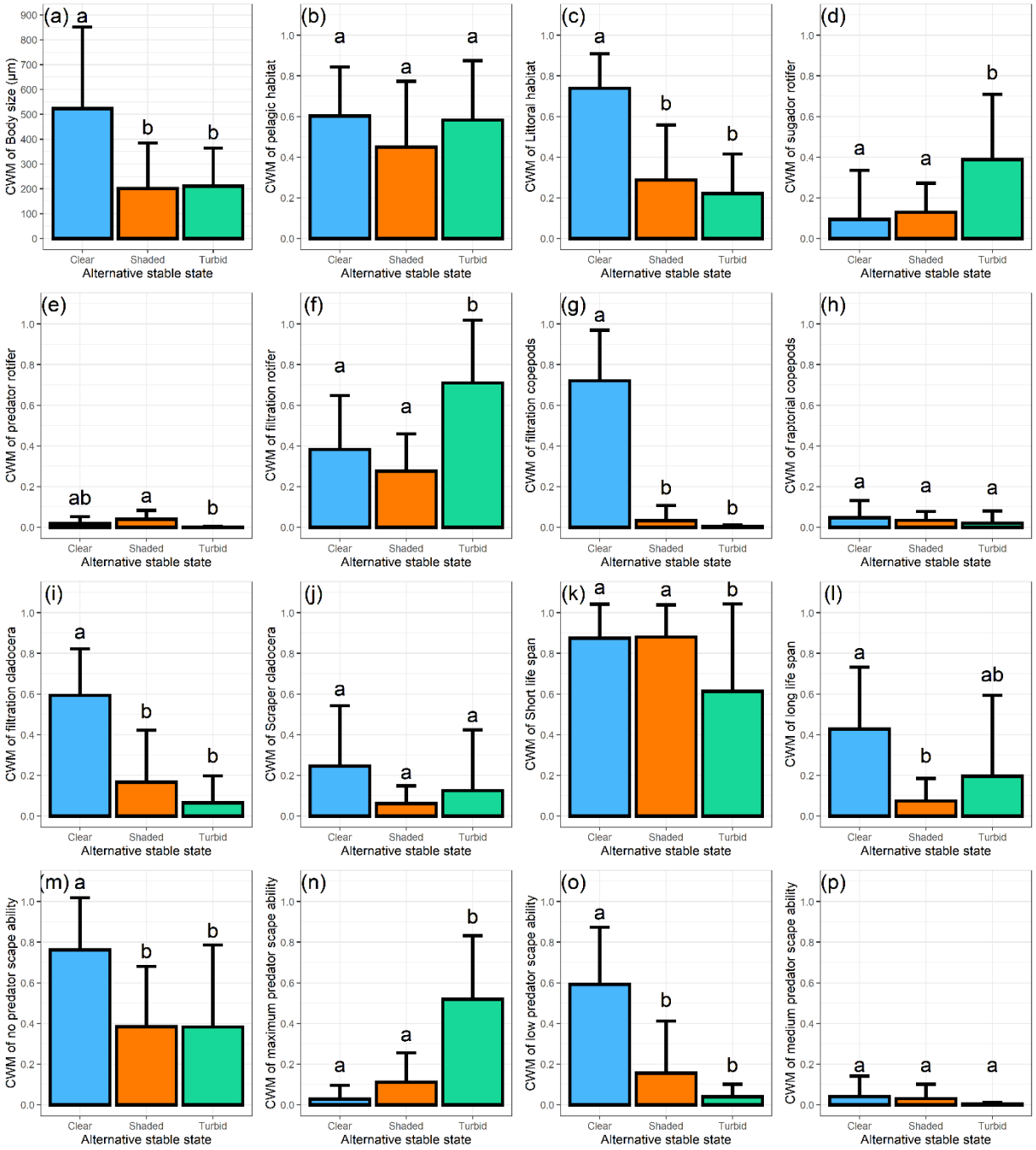

Figure S6. Mean ± SE of zooplankton community-weighted means (CWMs) among three alternative stable states (clear water, shaded, and turbid). Each functional trait of zooplankton was weighted by the relative abundance of species. Importantly, only maximum total length is a continuous variable, while other variables are dummies (0/1). Different letters indicate statistically significant differences (one-way LME/Tukey contrasts, $\alpha = 0.05$).

Table S1. Functional traits of fish and zooplankton communities used in this stud, and their likely influence on ecosystem functioning.

Functional traits	Type	Categories	Trait importance on ecosystem functioning
Fish			
Body size	Continuous	Centimeters (cm)	Fish body length is a functional trait that may impact ecosystem functioning as large-sized fish usually have stronger effects on ecosystem multifunctionality than small-sized fish (Moi et al., 2021). Thus, increased body size of fish causes shifts in ecosystem functioning through the following mechanisms: excretion, resuspension, and/or controlling the abundance of small-sized fishes and, consequently, their effects on ecosystem functioning (Vanni, 2002; Atkinson et al., 2017).
Habitat use	Categorical	Benthic Pelagic Benthopelagic	Fish occupying different types of habitats may create profound and distinct changes in ecosystem functioning. Pelagic fish are usually active and move throughout the lake compartments; consequently, they translocate large amounts of nutrients among compartments (Vanni et al., 2002; McIntyre et

			al., 2008). Likewise, benthic fish may act as important sources of nutrients through resuspension of sediment or bioturbation (Mormul et al., 2012).
Feeding group	Categorical	Apex predator-piscivorous Detritivorous Omnivorous Insectivorous Invertivorous	Different fish trophic guilds may exercise distinct effects on ecosystem functioning (da Silva et al., 2019). Apex predators (piscivores) may affect ecosystem functioning either directly via nutrient provisioning through faeces and carcass deposition or indirectly by altering the diversity of lower trophic groups (McIntyre et al., 2007; Atkinson et al., 2017). Indeed, a recent study showed that apex predators have stronger effects on ecosystem multifunctionality (nutrient availability, primary and secondary productivity; Moi et al., 2021). Detritivorous fish may have strong positive effects on nutrient availability and primary productivity through sediment suspension by bioturbation (Vanni et al., 2002; Mormul et al., 2012; Huang et al., 2020). Herbivorous fish also may affect ecosystem functions, such as decreasing primary productivity (Burkepile et al., 2013; Plass-Johnson et al., 2015).

Migration ability	Binary	Presence of migration	When abundant, the migratory fish may exercise strong positive effects on ecosystem functioning, thereby increasing the nutrient availability and primary productivity of shallow lakes (Mormul et al., 2012; Tamario et al., 2019).
Zooplankton			
Body size	Continuous	Micrometers (µm)	The body length of the zooplankton may affect primary and secondary productivity as well as nutrient availability though energetic transference over the food web (Litchman et al., 2013; Sodré et al., 2019). Larger zooplankton are expected to have stronger effects on nutrient availability and primary productivity because feeding and excretion rates tend to increase with body size (Kiørboe, 2011; Hebert et al., 2016).
Habitat use	Categorical	Littoral Pelagic	The type of habitat may influence the encounter rates of zooplankton with predators (e.g., fish), affecting their participation in the food web and their influence on energetic transference, which may impact ecosystem functions such as

			nutrient availability and secondary productivity (Hays et al., 1994; Sodré et al., 2019).
Feeding group	Categorical	Filtration rotifers Sucker rotifers Predator rotifers Raptorial copepods Filtration copepods Scraper cladocerans Filtration cladocerans	Feeding groups of zooplankton have different nutrient ratios and requirements. Thus, these groups may have distinct impacts on ecosystem processes such as nutrient availability, primary and secondary productivity (Hebert et al., 2017; Moi et al., 2021). In addition, predatory zooplankton (e.g., raptorial copepods) may indirectly impact ecosystem functioning by affecting their prey (Vanni, 2002).
Life span	Categorical	Short life span Long life span	Life span is related to the duration of the life cycle, and species with a long-life span may have more time to affect the functioning of the ecosystem than species with a short life span. However, species with short life span have a fast growth and develop large populations that may have strong impacts on ecosystem

			functioning in the short term (Litchman et al. 2013).
Predatory scape response	Categorical	No predatory scape Low Medium Maximum	The predatory escape response is a behavioral trait of zooplankton (Litchman et al., 2013). The efficiency with which zooplankton avoid predators may impact their effects on ecosystem functioning due to the fact that prey escaping predation can continue to excrete nutrients (affecting nutrient availability) and consume algae (affecting primary productivity).

Table S2. Models confronted in the model selection (see Table S7) including the influence of the predictors taxonomic fish richness (TR-F), taxonomic zooplankton richness (TR-Z), functional fish diversity (FD-F), functional zooplankton diversity (FD-Z), and water depth and their interactions with regime shifts (clear, turbid, and shaded) on the ecosystem multifunctionality indices, MFI (average, multifunctional ecosystem productivity (PC1), multifunctional fish biomass stock (PC2), and multifunctional nutrient availability (PC3)). Here, we tested all possible models, including single models (TR or FD or states), additive models (TR and FD + states), and multiplicative (TR:states and FD:states) models. Moreover, we also included combined models containing both fish and zooplankton.

Models	Variables
Model1	MFI ~ States
Model2	MFI ~ TR-F
Model3	MFI ~ FD-F
Model4	MFI ~ TR-Z
Model5	MFI ~ FD-Z
Model6	MFI ~ water depth
Model7	MFI ~ TR-F + states
Model8	MFI ~ FD-F + states
Model9	MFI ~ TR-Z + states
Model10	MFI ~ FD-Z + states
Model11	MFI ~ TR-F + FD-F
Model12	MFI ~ TR-Z + FD-Z
Model13	MFI ~ TR-F + TR-Z
Model14	MFI ~ FD-F + FD-Z
Model15	MFI ~ TR-F + FD-F + TR-Z + FD-Z
Model16	MFI ~ TR-F + FD-F + states
Model17	MFI ~ TR-Z + FD-Z + states
Model18	MFI ~ TR-F + TR-Z + states
Model19	MFI ~ FD-F + FD-Z + states
Model20	MFI ~ TR-F + FD-F + TR-Z + FD-Z + states
Model21	MFI ~ TR-F:states

Model22	MFI ~ FD-F:states
Model23	MFI ~ TR-Z:states
Model24	MFI ~ FD-Z:states
Model25	MFI ~ TR-F:states + TR-Z:states
Model26	MFI ~ FD-F:states + FD-Z:states
Model27	MFI ~ TR-F:states + TR-Z:states + FD-F:states + FD-Z:states
Model28	MFI ~ TR-F + states + TR-F:states
Model29	MFI ~ FD-F + states + FD-F:states
Model30	MFI ~ TR-Z + states + TR-Z:states
Model31	MFI ~ FD-Z + states + FD-Z:states
Model32	MFI ~ TR-F + FD-F + states + TR-F:states + FD-F:states
Model33	MFI ~ TR-Z + FD-Z + states + TR-Z:states + FD-Z:states
Model34	MFI ~ TR-F + TR-Z + states + TR-F:states + TR-Z:states
Model35	MFI ~ FD-F + FD-Z + states + FD-F:states + FD-Z:states
Model36	MFI ~ TR-F + FD-F + states + TR-F:states + FD-F:states + TR-Z + FD-Z + TR-Z:states + FD-Z:states
Model37	MFI ~ TR-F + FD-F + states + TR-F:states + FD-F:states + TR-Z + FD-Z + TR-Z:states + FD-Z:states + water depth
Null model	MFI ~ 1

Table S3. Contrasting the effects of taxonomic and functional diversity of fish and zooplankton, water level, and their interaction with regime shifts (clear, turbid, and shaded) on ecosystem multifunctionality. Detailed outcomes of the model selection performed using corrected Akaike Information Criteria (AICc) to assess the different contributions of taxonomic fish richness (TR-F), taxonomic zooplankton richness (TR-Z), functional fish diversity (FD-F), functional zooplankton diversity (FD-Z), and water depth and combinations of these predictors (Table S3) to ecosystem average multifunctionality, multifunctional ecosystem productivity (PC1), multifunctional fish biomass stock (PC2), and multifunctional nutrient availability (PC3). Model selection was performed using the function *'ICtab*' in the *'bbmle'* package. ΔAICc = difference between the model with the lowest score and subsequent models. Only the best subsets of models (ΔAICc < 2) are presented. Marginal R^2 (i.e., variance explained only by fixed effects) is also given.

Response	**Models**	**AICc**	**ΔAICc**	**df**	**Weight**
Multifunctionality					
Average	(i) FD-F:states + FD-Z:states	–49.982	0	9	0.96

Table S4. Pairwise comparisons of the effects of the alternative stable states (clear, shaded, and turbid) on the taxonomic and functional diversity of fish and zooplankton communities, and average multifunctionality, nutrient availability (PC1), primary production index (PC2), and biomass stock (PC3) (Tukey´s HSD).

Predictors	Estimate	Std.Error	z-value	P-value
Species richness-fish				
Shaded vs clear	–25.62	4.958	–5.168	< 0.001***
Turbid vs clear	–17.49	0.4.715	–3.709	< 0.001***
Turbid vs shaded	8.131	4.850	1.676	0.2412
Species richness-zooplankton				
Shaded vs clear	–85.42	14.24	–6.001	< 0.001***
Turbid vs clear	–72.82	11.80	–6.170	< 0.001***
Turbid vs shaded	12.61	12.25	1.029	0.556
Functional diversity-fish				
Shaded vs clear	–0.0497	0.008	–5.746	< 0.001***
Turbid vs clear	–0.0351	0.008	–4.089	< 0.001***
Turbid vs shaded	0.014	0.009	1.609	0.241
Functional diversity-zooplankton				
Shaded vs clear	–0.0566	0.009	–6.175	< 0.001***
Turbid vs clear	–0.0360	0.009	–3.928	< 0.001***
Turbid vs shaded	0.0206	0.009	2.126	0.084
Average multifunctionality				
Shaded vs clear	–0.241	0.042	–5.673	< 0.001***
Turbid vs clear	–0.159	0.046	–3.447	0.001**
Turbid vs shaded	0.082	0.047	1.735	0.191
Total phosphorus				
Shaded vs clear	2.560	0.356	7.177	< 0.001***
Turbid vs clear	2.134	0.385	5.531	< 0.001***
Turbid vs shaded	–0.425	0.394	–1.079	0.526
Total nitrogen				
Shaded vs clear	0.639	0.149	4.276	< 0.001***
Turbid vs clear	0.274	0.156	1.750	0.186
Turbid vs shaded	–0.365	0.160	–2.275	0.059
Standing biomass				
Shaded vs clear	–1.790	0.099	–18.042	< 0.001***
Turbid vs clear	–0.795	0.102	–7.764	< 0.001***
Turbid vs shaded	0.994	0.104	9.481	< 0.001***
Algae production				
Shaded vs clear	–2.775	0.509	–5.446	< 0.001***
Turbid vs clear	–2.205	0.526	–4.191	< 0.001***
Turbid vs shaded	0.570	0.539	1.059	0.541
Light availability				
Shaded vs clear	–2.598	0.202	–12.842	< 0.001***
Turbid vs clear	–2.709	0.223	–12.10	< 0.001***
Turbid vs shaded	–0.110	0.228	–0.483	0.879

Table S5. Pairwise comparisons of the effects of the alternative stable states (clear, shaded, and turbid) on the community-weighted means (CWMs) of the fish community (Tukey´s HSD). Results refer to Figure S5.

Predictors/Fish	Estimat	Std.Erro	z-value	P-value
Body size				
Shaded vs clear	–4.086	0.306	–13.350	< 0.001***
Turbid vs clear	–1.592	0.324	–4.914	< 0.001***
Turbid vs shaded	2.493	0.336	7.405	< 0.001****
Benthic habitat				
Shaded vs clear	–0.410	0.112	–3.642	< 0.001***
Turbid vs clear	–0.200	0.132	–1.512	0.283
Turbid vs shaded	0.209	0.136	1.538	0.271
Pelagic habitat				
Shaded vs clear	–0.757	0.105	–7.200	< 0.001***
Turbid vs clear	–0.401	0.102	–3.922	< 0.001***
Turbid vs shaded	0.355	0.109	3.254	0.003**
Bentho-pelagic habitat				
Shaded vs clear	–0.340	0.132	–2.574	0.027*
Turbid vs clear	0.076	0.131	0.585	0.827
Turbid vs shaded	0.417	0.139	3.002	0.007**
Apex predator-piscivorous				
Shaded vs clear	–0.525	0.082	–6.400	< 0.001***
Turbid vs clear	–0.549	0.110	–4.952	< 0.001***
Turbid vs shaded	–0.024	0.112	–0.213	0.975
Detritivorous				
Shaded vs clear	–0.326	0.117	–2.780	0.014*
Turbid vs clear	0.310	0.121	2.570	0.027*
Turbid vs shaded	0.637	0.126	5.037	< 0.001***
Omnivorous				
Shaded vs clear	–0.553	0.127	–4.329	< 0.001***
Turbid vs clear	–0.341	0.124	–2.746	0.016*
Turbid vs shaded	0.211	0.132	1.593	0.248
Herbivorous				
Shaded vs clear	–0.566	0.098	–5.739	< 0.001***
Turbid vs clear	–0.538	0.117	–4.574	< 0.001***
Turbid vs shaded	0.027	0.120	0.231	0.971
Insectivorous				
Shaded vs clear	–0.438	0.098	–4.440	< 0.001***
Turbid vs clear	–0.331	0.100	–3.309	0.002**
Turbid vs shaded	0.107	0.105	1.024	0.561
Invertivorous				
Shaded vs clear	–0.431	0.096	–4.474	< 0.001***
Turbid vs clear	–0.409	0.106	–3.847	< 0.001***
Turbid vs shaded	0.022	0.109	0.202	0.977
Migration ability				
Shaded vs clear	–0.379	0.113	–3.331	0.002**
Turbid vs clear	0.263	0.132	1.983	0.115
Turbid vs shaded	0.642	0.136	4.718	< 0.001***

Table S6. Pairwise comparisons of the effects of the alternative stable states (clear, shaded, and turbid) on the community-weighted means (CWMs) of the zooplankton community (Tukey´s HSD). Results refer to Figure S6.

Predictors/zooplankton	Estimate	Std.Erro	z-value	P-value
Body size				
Shaded vs clear	–0.872	0.276	−3.512	0.004**
Turbid vs clear	–0.947	0.301	−3.144	0.004**
Turbid vs shaded	–0.074	0.311	–0.238	0.969
Pelagic habitat use				
Shaded vs clear	–0.130	0.116	–1.118	0.503
Turbid vs clear	–0.005	0.121	–0.046	0.999
Turbid vs shaded	0.124	0.126	0.985	0.586
Littoral habitat use				
Shaded vs clear	–0.451	0.088	–5.080	< 0.001***
Turbid vs clear	–0.517	0.086	–5.979	< 0.001***
Turbid vs shaded	–0.065	0.092	–0.715	0.754
Sugador rotifers				
Shaded vs clear	0.035	0.104	0.340	0.938
Turbid vs clear	0.293	0.101	2.891	0.010*
Turbid vs shaded	0.258	0.108	2.283	0.045*
Predator rotifers				
Shaded vs clear	0.021	0.013	1.607	0.242
Turbid vs clear	–0.016	0.013	–1.206	0.449
Turbid vs shaded	–0.037	0.014	–2.678	0.020*
Filtration rotifers				
Shaded vs clear	–0.106	0.109	–0.968	0.720
Turbid vs clear	0.327	0.106	3.067	0.006**
Turbid vs shaded	0.433	0.113	3.807	< 0.001***
Filtration copepods				
Shaded vs clear	–0.687	0.067	–10.187	< 0.001***
Turbid vs clear	–0.716	0.065	–10.896	< 0.001***
Turbid vs shaded	–0.028	0.070	–0.409	0.912
Raptorial copepods				
Shaded vs clear	0.036	0.097	0.373	0.926
Turbid vs clear	0.175	0.095	1.843	0.156
Turbid vs shaded	0.139	0.101	1.369	0.357
Filtration cladocera				
Shaded vs clear	–0.426	0.089	–4.775	< 0.001***
Turbid vs clear	–0.528	0.086	–6.075	< 0.001***
Turbid vs shaded	–0.101	0.092	–1.099	0.515
Scraper cladocera				
Shaded vs clear	–0.184	0.107	–1.727	0.195
Turbid vs clear	–0.120	0.104	–1.155	0.480
Turbid vs shaded	0.064	0.111	0.580	0.831
Short life span				

Shaded vs clear	–0.026	0.114	–0.233	0.970
Turbid vs clear	–0.237	0.111	–2.118	0.086
Turbid vs shaded	–0.210	0.119	–1.761	0.183
Long life span				
Shaded vs clear	–0.354	0.126	–2.794	0.014*
Turbid vs clear	–0.232	0.123	–1.886	0.142
Turbid vs shaded	0.121	0.131	0.922	0.626
No predator scape ability				
Shaded vs clear	–0.378	0.135	–2.796	0.014*
Turbid vs clear	–0.379	0.131	–2.877	0.011*
Turbid vs shaded	–0.008	0.140	–0.006	0.987
Maximum predator scape ability				
Shaded vs clear	–0.016	0.104	–0.161	0.985
Turbid vs clear	0.358	0.102	3.509	0.001**
Turbid vs shaded	0.341	0.108	3.135	0.004**
Low predator scape ability				
Shaded vs clear	–0.388	0.088	–4.411	< 0.001***
Turbid vs clear	–0.540	0.098	–5.465	< 0.001***
Turbid vs shaded	–0.152	0.101	–1.494	0.293
Medium predator scape ability				
Shaded vs clear	–0.009	0.030	–0.298	0.952
Turbid vs clear	–0.034	0.030	–1.160	0.477
Turbid vs shaded	–0.025	0.032	–0.801	0.702

Table S7. Collected fish and their functional traits in Lake Osmar during three alterative states (clear, turbid, and shaded) over 12-year period.

Species	Length (cm)	Habitat use	Trophic guilds	Migration ability
Acestrorhynchus lacustri (Lütken 1875)	150	Benthopelagic	Piscivores	non-migrator
Aphyocharax anisitsi (Eigenmann and Kennedy 1903)	38.6	Benthopelagic	Invertivores	non-migrator
Aphyocharax dentatus (Eigenmann and Kennedy 1903)	48	Benthopelagic	Invertivores	non-migrator
Apistogramma commbrae (Regan 1906)	32.6	Benthopelagic	Omnivores	non-migrator
Astronotus crasssipinnis (Heckel 1840)	172.5	Benthopelagic	Omnivores	non-migrator
Astyanax lacustris (Lütken 1875)	79.8	Pelagic	Omnivores	non-migrator
Brachyhypopomus gauderio (Giora and Malabarba 2009)	150	Benthopelagic	Insectivores	non-migrator
Characidium zebra (Eigenmann 1909)	150.5	Pelagic	Invertivores	non-migrator
Cichlasoma paranaense (Kullander 1983)	110	Benthopelagic	Insectivores	non-migrator
Cichla kelberi (Kullander and Ferreira 2006)	190	Benthopelagic	Piscivores	non-migrator
Crenicichla britski (Kullander 1982)	130	Benthopelagic	Invertivores	non-migrator
Cyphocharax modestus (Fernández-Yépez 1948)	80	Benthopelagic	Detritivores	non-migrator
Cyphocharax inaequilabiatus (Fernández-Yépez 1948)	134	Benthopelagic	Piscivores	non-migrator
Hoplerythrinus unitaeniatus (Spix and Agassiz 1829)	150.2	Benthopelagic	Piscivores	non-migrator
Hoplias.sp2	250	Pelagic	Piscivores	non-migrator
Hoplias.sp3	170	Pelagic	Piscivores	non-migrator
Hoplias mbigua (Azpelicueta et al. 2015)	260	Pelagic	Piscivores	non-migrator
Hoplosternum littorale (Hacock 1828)	133.4	Benthic	Invertivores	non-migrator
Hephessobrycon eques (Steindachner 1882)	32	Benthopelagic	Herbivores	non-migrator
Hyphessobrycon guarani (Mahnert and Géry 1987)	33	Benthopelagic	Herbivores	non-migrator
Hyphessobrycon marginatus (Ellis 1911)	32	Benthopelagic	Insectivores	non-migrator
Hyphessobrycon ornatus	34	Benthopelagic	Herbivores	non-migrator
Laetacara araguaiae (Ottoni and Costa 2009)	32.6	Benthopelagic	Detritivores	non-migrator
Leporinus lacustris (Amaral Campos 1945)	210	Benthopelagic	Detritivores	non-migrator
Loricariichthys platymetopon (Isbrucker and Nijssen 1979)	210	Benthic	Detritivores	non-migrator
Megaleporinus piavussu (Britski et al. 2012)	190	Benthopelagic	Detritivores	non-migrator
Metynnis lippincottianus (Cope 1870)	149.3	Pelagic	Herbivores	non-migrator
Moenkhausia forestii (Benine et al. 2009)	30.6	Pelagic	insectivores	non-migrator

Moenkhausia aff. grandisquamis (Müller and Troschel 1845)	68.4	Pelagic	Invertivores	non-migrator
Moenkhausia cf intermedia (Eigenmann 1908)	68.4	Pelagic	Invertivores	non-migrator
Moenkhausia aff. Sanctaefilomenae (Steindachner 1907)	43	Pelagic	Invertivores	non-migrator
Moenkhausia bonita (Benine, Castro and Sabino 2004)	44	Pelagic	Detritivores	non-migrator
Odontostilbe avanhandava (Junior et al. 2018)	53	Pelagic	insectivores	non-migrator
Piabarchus stramineus	70	Pelagic	insectivores	non-migrator
Platanichthys platana (Regan 1917)	68.9	Pelagic	Detritivores	non-migrator
Prochilodus lineatus (Valenciennes 1837)	495	Benthic	Detritivores	Migrator
Psellogrammus kennedyi (Eignmann 1903)	30.5	Benthopelagic	Detritivores	non-migrator
Pseudoplatystoma corruscans (Spix and Agassiz 1829)	675	Benthic	Piscivores	Migrator
Pterygoplichthys ambrosettii (Holmberg 1893)	395	Benthic	Detritivores	non-migrator
Pyrrhulina australis (Eigenmann and Kennedy 1903)	29	Benthopelagic	Detritivores	non-migrator
Roeboides descalvadensis (Fowler 1932)	88.8	Benthopelagic	Piscivores	non-migrator
Satanoperna.sp	150	Benthopelagic	Invertivores	non-migrator
Schizodon altiparanae (Garavello and Britski 1990)	282	Benthopelagic	Herbivores	non-migrator
Schizodon borellii (Boulenger 1900)	207	Benthopelagic	Herbivores	non-migrator
Serrapinnus notomelas (Eigenmann 1915)	31.8	Pelagic	Detritivores	non-migrator
Serrapinnus sp1	31.8	Pelagic	Detritivores	non-migrator
Serrapinnus sp2	31.8	Pelagic	Detritivores	non-migrator
Serrapinnus calliurus (Boulenger 1900)	31.8	Benthopelagic	Detritivores	non-migrator
Serrasalmus marginatus (Valenciennes 1837)	160.2	Pelagic	Piscivores	non-migrator
Serrapinnus heterodon (Eigenmann 1915)	40	Pelagic	Detritivores	non-migrator
Steindachnerina brevipinna (Eigenmann and Eigenmann 1889)	105.8	Benthopelagic	Detritivores	non-migrator
Steindachnerina insculpta (Fernández-Yépez 1948)	90	Pelagic	Detritivores	non-migrator

The taxonomic classification of some of the species collected in our study is still discussed, and some names are being continuously revised. For a complete review of the species used in our study, see the recent taxonomic work of Ota et al. (2018). In this work, the authors reviewed all known fish species from the upper Paraná River.

Table S8. Collected zooplankton species and their functional traits in Lake Osmar during three alterative states (clear, turbid, and shaded) over a period of 12 years.

Species	Length (µm)	Habitat use	Feeding	Life span	Predator scape
Alona dentifera	480	Littoral	Scraper-clad	Short	Absent
Alona gutatta	250	Littoral	Scraper-clad	Short	Absent
Alona iheringula	200	Littoral	Filter-feeder-clad	Short	Absent
Alona intermedia	425	Littoral	Scraper-clad	Short	Absent
Alona ossiani	800	Littoral	Scraper-clad	Short	Absent
allonella dadayi	580	Littoral	Scraper-clad	Short	Absent
Argyrodiaptomus azevedoi	1704	Pelagic	Filter-feeder-cop	Long	Medium
Argyrodiaptomus furcatus	1354	Pelagic	Filter-feeder-cop	Long	Medium
Ascomorpha ecaudis	170	Pelagic	Sucker-rot	Short	Absent
Ascomorpha ovalis	176.5	Pelagic	Sucker-rot	Short	Absent
Ascomorpha saltans	165	Pelagic	Sucker-rot	Short	Absent
Asplanchna sieboldi	1500	Pelagic	Predator-rot	Short	Absent
Bosmina frey	301.18	Littoral	Filter-feeder-clad	Short	Low
Bosmina hagmanni	301.18	Pelagic	Filter-feeder-clad	Short	Low
Bosmina longirostris	500	Pelagic	Filter-feeder-clad	Short	Low
Bosmina sp1	301.18	Pelagic	Filter-feeder-clad	Short	Low
Bosminopsis deitersi	227.13	Pelagic	Filter-feeder-clad	Short	Low
Brachionus bidentata	368	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus budapestinensis	100	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus calyciflorus	201.19	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus caudatus	270	Littoral	Filter-feeder-rot	Short	Absent
Brachionus dolabratus	167	Littoral	Filter-feeder-rot	Short	Absent
Brachionus falcatus	430	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus forficula	145	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus havanensis	227.34	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus quadridentatus	143.75	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus satanicus	227.34	Pelagic	Filter-feeder-rot	Short	Absent
Brachionus tubicen	294.54	Pelagic	Filter-feeder-clad	Short	Low

Brachionus urceolaris	227.34	Pelagic	Filter-feeder-rot	Short	Absent
Cephalodella anebodica	170.4	Littoral	Sucker-rot	Short	Absent
Cephalodella gibba	130.7	Littoral	Sucker-rot	Short	Absent
Cephalodella hiulca	91	Littoral	Sucker-rot	Short	Absent
Ceriodaphnia cornuta	289.11	Pelagic	Filter-feeder-clad	Short	Low
Ceriodaphnia reticulata	369.555	Pelagic	Filter-feeder-clad	Short	Low
Ceriodaphnia richardi	369.555	Pelagic	Filter-feeder-clad	Short	Low
Ceriodaphnia silvestrii	450	Pelagic	Filter-feeder-clad	Short	Absent
Ceriodaphnia sp	344.55	Pelagic	Filter-feeder-clad	Short	Absent
Chydorus eurynotus	241.67	Littoral	Scraper-clad	Short	Absent
Chydorus nitidulus	260	Littoral	Scraper-clad	Short	Absent
Chydorus pubescens	287.5	Littoral	Scraper-clad	Short	Absent
Chydorus sp1	347.22	Littoral	Scraper-clad	Short	Absent
Colurella obtusa	101.5	Littoral	Filter-feeder-rot	Short	Absent
Conochilus coenobasis	112.5	Pelagic	Filter-feeder-rot	Short	Absent
Conochilus dossuaris	100	Pelagic	Filter-feeder-rot	Short	Absent
Conochilus natans	75	Pelagic	Filter-feeder-rot	Short	Absent
Conochilus unicornis	175	Pelagic	Filter-feeder-rot	Short	Absent
Coronatella monocantha	264	Littoral	Scraper-clad	Short	Absent
Coronatella poppei	306.67	Littoral	Scraper-clad	Short	Absent
Daphinia spinulosum	550.31	Pelagic	Filter-feeder-clad	Short	Low
Daphnia gessneri	598.22	Pelagic	Filter-feeder-clad	Short	Low
Daphnia laevis	1500	Pelagic	Filter-feeder-clad	Short	Low
Daphnia lumholtiz	500	Pelagic	Filter-feeder-clad	Short	Maximum
Diaphanosoma brevireme	612.5	Pelagic	Filter-feeder-clad	Short	Low
Diaphanosoma fluviatilis	538.28	Pelagic	Filter-feeder-clad	Short	Low
Diaphanosoma polyspina	630	Pelagic	Filter-feeder-clad	Short	Low
Diaphanosoma sp	571.8	Pelagic	Filter-feeder-clad	Short	Low
Dicranophoroides caudatus	310	Pelagic	Predator-rot	Short	Absent
Dicranophoroides claviger	187	Littoral	Predator-rot	Short	Absent
Dipleuchlanis propatula	508	Littoral	Filter-feeder-rot	Short	Absent

Disparalona hamata	510	Littoral	Scraper-clad	Short	Absent
Dunhevedia odontoplax	460	Littoral	Scraper-clad	Short	Absent
Enteroplea lacustris	431.5	Littoral	Sucker-rot	Short	Absent
Ephemeroporus barroisi	270	Littoral	Scraper-clad	Short	Absent
Ephemeroporus hybridus	260	Littoral	Scraper-clad	Short	Absent
Ephemeroporus tridentatus	310	Littoral	Scraper-clad	Short	Absent
Epiphanes macrourus	187.5	Littoral	Predator-rot	Short	Absent
Euchlanis clavatula	205.66	Littoral	Predator-rot	Short	Absent
Euchlanis dilatata	187.75	Littoral	Predator-rot	Short	Absent
Euchlanis incisa	229.25	Littoral	Predator-rot	Short	Absent
Euryalona brasiliensis	362.5	Littoral	Scraper-clad	Short	Absent
Euryalona orientalis	450	Littoral	Scraper-clad	Short	Absent
Filinia longiseta	140.61	Pelagic	Filter-feeder-rot	Short	Absent
Filinia opoliensis	220.16	Pelagic	Filter-feeder-rot	Short	Absent
Filinia saltator	149	Pelagic	Filter-feeder-rot	Short	Absent
Filinia terminalis	138.25	Pelagic	Filter-feeder-rot	Short	Absent
Floscularia sp	112.5	Pelagic	Filter-feeder-rot	Short	Absent
Gastropus hiptopus	96.87	Pelagic	Sucker-rot	Short	Absent
Gastropus stilifer	180	Pelagic	Sucker-rot	Short	Absent
Harringia rousseleti	200	Pelagic	Sucker-rot	Short	Absent
Hexarthra intermedia	234	Pelagic	Filter-feeder-rot	Short	Absent
Hexarthra mira	151.93	Pelagic	Filter-feeder-rot	Short	Absent
Ilyocryptus pinifer	265.97	Littoral	Filter-feeder-clad	Short	Absent
Karualona mulleri	462	Littoral	Scraper-clad	Short	Absent
Kellicottia bostoniensis	113.85	Pelagic	Filter-feeder-rot	Short	Absent
Keratella americana	159.74	Pelagic	Filter-feeder-rot	Short	Absent
Keratella cochlearis	107.16	Pelagic	Filter-feeder-rot	Short	Absent
Keratella lenzi	112.5	Pelagic	Filter-feeder-rot	Short	Absent
Keratella tropica	115.42	Pelagic	Filter-feeder-rot	Short	Absent
Kurzia longirostris	420	Littoral	Scraper-clad	Short	Absent
Lecane bulla	114.61	Littoral	Filter-feeder-rot	Short	Absent

Lecane cloterocerca	85	Littoral	Filter-feeder-rot	Short	Absent
Lecane cornuta	109.38	Littoral	Filter-feeder-rot	Short	Absent
Lecane crenata	112.66	Littoral	Filter-feeder-rot	Short	Absent
Lecane curvicornis	131.25	Littoral	Filter-feeder-rot	Short	Absent
Lecane elsa	150	Littoral	Filter-feeder-rot	Short	Absent
Lecane hamata	79.5	Littoral	Filter-feeder-rot	Short	Absent
Lecane hastata	86	Littoral	Filter-feeder-rot	Short	Absent
Lecane hornemanni	94	Littoral	Filter-feeder-rot	Short	Absent
Lecane inermis	114.86	Littoral	Filter-feeder-rot	Short	Absent
Lecane inopinata	68.5	Littoral	Filter-feeder-rot	Short	Absent
Lecane leontina	175	Littoral	Filter-feeder-rot	Short	Absent
Lecane ludwigii	134.38	Littoral	Filter-feeder-rot	Short	Absent
Lecane luna	126.95	Littoral	Filter-feeder-rot	Short	Absent
Lecane lunaris	101.94	Littoral	Filter-feeder-rot	Short	Absent
Lecane mira	145	Littoral	Filter-feeder-rot	Short	Absent
Lecane monostyla	69	Littoral	Filter-feeder-rot	Short	Absent
Lecane ohioensis	50	Littoral	Filter-feeder-rot	Short	Absent
Lecane papuana	107.7	Littoral	Filter-feeder-rot	Short	Absent
Lecane proiecta	113	Littoral	Filter-feeder-rot	Short	Absent
Lecane quadridentata	162.5	Littoral	Filter-feeder-rot	Short	Absent
Lecane rhytida	81	Littoral	Filter-feeder-rot	Short	Absent
Lecane signifera	113	Littoral	Filter-feeder-rot	Short	Absent
Lecane ungulata	157.5	Littoral	Filter-feeder-rot	Short	Absent
Lepadella ovalis	150	Littoral	Filter-feeder-rot	Short	Absent
Lepadella patela	130	Littoral	Filter-feeder-rot	Long	Absent
Macrochaetus albidus	1285	Littoral	Filter-feeder-rot	Short	Maximum
Macrochaetus collinsi	250	Littoral	Filter-feeder-rot	Short	Absent
Macrochaetus sericus	212	Littoral	Filter-feeder-rot	Short	Absent
Macrochaetus subquadratus	157	Littoral	Filter-feeder-rot	Short	Absent
Macrothris sp	481.25	Littoral	Scraper-clad	Short	Absent
Macrothrix elegans	300	Littoral	Scraper-clad	Short	Absent

Macrothrix squamosa	400	Littoral	Scraper-clad	Short	Absent
mesocyclops ogunnus	1185	Littoral	Raptorial-cop	Long	Maximum
metacyclops laticornis	772	Pelagic	Raptorial-cop	Long	Maximum
Moina micrura	440	Pelagic	Filter-feeder-clad	Short	Low
Moina minuta	385.67	Pelagic	Filter-feeder-clad	Short	Low
Moina reticulata	750	Pelagic	Filter-feeder-clad	Short	Low
Moina rostrata	525.22	Pelagic	Filter-feeder-clad	Short	Low
Moina sp	525.22	Pelagic	Filter-feeder-clad	Short	Low
Moinodaphnia macleayi	580	Pelagic	Filter-feeder-clad	Short	Low
Monommata arndti	210	Pelagic	Sucker-rot	Short	Absent
Monommata caeca	313.75	Littoral	Sucker-rot	Short	Absent
Monommata dentata	400	Littoral	Sucker-rot	Short	Absent
Mytilina mucronata	212.5	Littoral	Filter-feeder-rot	Short	Absent
Nicsmirnovius fitiztatricki	325	Littoral	Scraper-clad	Short	Absent
Notoalon globulosa	430	Littoral	Scraper-clad	Short	Absent
Notoalona sculpta	430	Littoral	Scraper-clad	Short	Absent
Notodiaptomus amazonicus	1245.2633	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus cearensis	1100	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus conifer	1050	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus deitersi	1240	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus henseni	1208.07	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus incompositus	1029	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus isabelae	1202.263333	Pelagic	Filter-feeder-cop	Long	Medium
Notodiaptomus sp	1149.51	Littoral	Filter-feeder-cop	Long	Medium
Notodiaptomus spinuliferus	1466	Littoral	Filter-feeder-cop	Long	Medium
Notomatta copeus	1120	Littoral	Sucker-rot	Short	Absent
Notommata pachyura	482	Littoral	Sucker-rot	Short	Absent
paracyclops chiltoni	739	Littoral	Raptorial-cop	Long	Maximum
Paracyclops fimbriatus	900	Littoral	Raptorial-cop	Long	Maximum
paracyclops sp	900	Littoral	Raptorial-cop	Long	Maximum
Phompholyx complanata	90	Pelagic	Filter-feeder-rot	Short	Absent

Phompholyx triloba	83.5	Pelagic	Filter-feeder-rot	Short	Absent
Plationus macrachantus	122.5	Littoral	Filter-feeder-rot	Short	Absent
Plationus patulus	122.5	Littoral	Filter-feeder-rot	Short	Absent
Platyias leloupi	218.75	Pelagic	Filter-feeder-rot	Short	Absent
Platyias quadricornis	141.67	Pelagic	Filter-feeder-rot	Short	Absent
Ploesoma truncatum	131.5	Pelagic	Filter-feeder-rot	Short	Absent
Polyarthra dolichoptera	96.5	Pelagic	Filter-feeder-rot	Short	Absent
Polyarthra remata	92.5	Pelagic	Filter-feeder-rot	Short	Absent
Polyarthra vulgaris	115.34	Pelagic	Filter-feeder-rot	Short	Absent
Proales minima	95	Littoral	Filter-feeder-rot	Short	Absent
Ptygura sp	350	Littoral	Filter-feeder-rot	Short	Absent
Sarsilatona punctatus	1812	Pelagic	Filter-feeder-clad	Short	Low
Sarsilatona serricauda	700	Littoral	Filter-feeder-clad	Short	Absent
Scapholeberis armata	700	Littoral	Filter-feeder-clad	Short	Low
Simocephalus latirostris	1600	Pelagic	Filter-feeder-clad	Short	Low
Simocephalus serrulatus	2005	Pelagic	Filter-feeder-clad	Short	Low
Simocephalus sp	1850	Pelagic	Filter-feeder-clad	Short	Low
Sinantherina spinosa	1050	Pelagic	Filter-feeder-rot	Short	Absent
Synchaeta longipes	144.96	Pelagic	Filter-feeder-rot	Short	Absent
Synchaeta oblonga	110.35	Pelagic	Filter-feeder-rot	Short	Absent
Synchaeta pectinata	86.03	Pelagic	Filter-feeder-rot	Short	Absent
Synchaeta stylata	238.5	Pelagic	Filter-feeder-rot	Short	Absent
Testudinalla greeni	214.125	Littoral	Sucker-rot	Short	Absent
Testudinalla patina	350	Littoral	Filter-feeder-rot	Short	Absent
Testudinalla tridentata	185	Littoral	Sucker-rot	Short	Absent
Testudinella mucronata	181.5	Littoral	Filter-feeder-rot	Short	Absent
Testudinella ohlei	140	Littoral	Filter-feeder-rot	Short	Absent
Trichocerca bicristata	300	Littoral	Sucker-rot	Short	Absent
Trichocerca bidens	133.33	Littoral	Sucker-rot	Short	Absent
Trichocerca collaris	130	Littoral	Filter-feeder-rot	Short	Absent
Trichocerca cylindrica	325	Littoral	Sucker-rot	Short	Absent

Trichocerca elongata	237.5	Littoral	Sucker-rot	Short	Absent
Trichocerca heterodactyla	225	Littoral	Sucker-rot	Short	Absent
Trichocerca iernis	135	Littoral	Sucker-rot	Short	Absent
Trichocerca inermis	88.5	Littoral	Sucker-rot	Short	Absent
Trichocerca longiseta	222	Littoral	Sucker-rot	Short	Absent
Trichocerca macera	224	Littoral	Sucker-rot	Short	Absent
Trichocerca pusilla	175	Littoral	Sucker-rot	Short	Absent
Trichocerca ruttneri	85.5	Littoral	Sucker-rot	Short	Absent
Trichocerca scipio	408	Littoral	Sucker-rot	Short	Absent
Trichocerca similis	195.65	Littoral	Sucker-rot	Short	Absent
Trichotria tetractis	295	Littoral	Predator-rot	Short	Absent
tropocyclops prasinus	500	Littoral	Raptorial-cop	Long	Maximum

Table S9. Species of algae used to calculate the biomass of small edible algae. Note that all algae were measured, and only algae smaller than 60 µm were considered as they are more easily consumed by zooplankton in tropical lakes.

Species	Group
Acanthoceras magdeburgensis	Nanoplankton (2-63 µm)
Achnanthidium minutissimum	Nanoplankton (2-63 µm)
Ancyonema sp.	Nanoplankton (2-63 µm)
Aulacoseira alpigena	Nanoplankton (2-63 µm)
Aulacoseira distans	Nanoplankton (2-63 µm)
Cocconeis sp.	Nanoplankton (2-63 µm)
Cyclotella sp.	Nanoplankton (2-63 µm)
Cyclotella sp1	Nanoplankton (2-63 µm)
Cymbella sp.	Nanoplankton (2-63 µm)
Discostella sp.	Nanoplankton (2-63 µm)
Discostella stelligera	Nanoplankton (2-63 µm)
Encyonema mesianum	Nanoplankton (2-63 µm)
Eunotia sp1	Nanoplankton (2-63 µm)
Fragilaria capucina	Nanoplankton (2-63 µm)
Fragilaria sp.	Nanoplankton (2-63 µm)
Fragilaria/Synedra sp.	Nanoplankton (2-63 µm)
Frustulia sp.	Nanoplankton (2-63 µm)
Gomphonema augur	Nanoplankton (2-63 µm)
Gomphonema gracile	Nanoplankton (2-63 µm)
Gomphonema parvulum	Nanoplankton (2-63 µm)
Gomphonema sp.	Nanoplankton (2-63 µm)
Navicula cryptocephala	Nanoplankton (2-63 µm)
Navicula schroeterii	Nanoplankton (2-63 µm)
Navicula sp.	Nanoplankton (2-63 µm)
Nitzschia acicularis	Nanoplankton (2-63 µm)
Nitzschia cf. *ignorata*	Nanoplankton (2-63 µm)
Nitzschia clausii	Nanoplankton (2-63 µm)
Nitzschia gracilis	Nanoplankton (2-63 µm)
Nitzschia palea	Nanoplankton (2-63 µm)
Nitzschia sp.	Nanoplankton (2-63 µm)
Nitzschia sp1	Nanoplankton (2-63 µm)
Orthoseira sp.	Nanoplankton (2-63 µm)
Thalassiosira sp.	Nanoplankton (2-63 µm)
Urosolenia eriensis	Nanoplankton (2-63 µm)
Urosolenia sp.	Nanoplankton (2-63 µm)
Actinastrum aciculare	Nanoplankton (2-63 µm)
Actinastrum gracillimum	Nanoplankton (2-63 µm)
Actinastrum hantzschii	Nanoplankton (2-63 µm)
Acutodesmus acuminatus	Nanoplankton (2-63 µm)
Ankistrodesmus bernardii	Nanoplankton (2-63 µm)
Ankistrodesmus falcatus	Nanoplankton (2-63 µm)
Ankistrodesmus fusiformes	Nanoplankton (2-63 µm)
Ankistrodesmus gracilis	Nanoplankton (2-63 µm)
Ankistrodesmus spiralis	Nanoplankton (2-63 µm)

Ankyra ancora	Nanoplankton (2-63 µm)
Ankyra judayi	Nanoplankton (2-63 µm)
Chlamydomonas sp.	Nanoplankton (2-63 µm)
Chlamydomonas sp1	Nanoplankton (2-63 µm)
Chlorogonium cf. *fusiforme*	Nanoplankton (2-63 µm)
Closteriopsis sp	Nanoplankton (2-63 µm)
Coelastrum indicum	Nanoplankton (2-63 µm)
Coelastrum microporum	Nanoplankton (2-63 µm)
Coelastrum pseudomicroporum	Nanoplankton (2-63 µm)
Coelastrum reticulatum	Nanoplankton (2-63 µm)
Coenochloris planconvexa	Nanoplankton (2-63 µm)
Coenochloris planctonicus	Nanoplankton (2-63 µm)
Coenocystis planctonica	Nanoplankton (2-63 µm)
Coenocystis sp.	Nanoplankton (2-63 µm)
Crucigenia fenestrata	Nanoplankton (2-63 µm)
Crucigenia sp.	Nanoplankton (2-63 µm)
Crucigenia tetrapedia	Nanoplankton (2-63 µm)
Crucigeniella crucifera	Nanoplankton (2-63 µm)
Crucigeniella pulchra	Nanoplankton (2-63 µm)
Crucigeniella rectangularis	Nanoplankton (2-63 µm)
Crucigeniella sp.	Nanoplankton (2-63 µm)
Desmatractum indutum	Nanoplankton (2-63 µm)
Desmodemus spinosus	Nanoplankton (2-63 µm)
Desmodesmus armatus	Nanoplankton (2-63 µm)
Desmodesmus armatus	Nanoplankton (2-63 µm)
Desmodesmus brasiliensis	Nanoplankton (2-63 µm)
Desmodesmus cf. *histrix*	Nanoplankton (2-63 µm)
Desmodesmus communis	Nanoplankton (2-63 µm)
Desmodesmus denticulatus	Nanoplankton (2-63 µm)
Desmodesmus denticulatus	Nanoplankton (2-63 µm)
Desmodesmus intermedius	Nanoplankton (2-63 µm)
Desmodesmus intermedius	Nanoplankton (2-63 µm)
Desmodesmus maximus	Nanoplankton (2-63 µm)
Desmodesmus opoliensis	Nanoplankton (2-63 µm)
Desmodesmus serratus	Nanoplankton (2-63 µm)
Desmodesmus sp.	Nanoplankton (2-63 µm)
Dictyosphaerium ehrenbergianum	Nanoplankton (2-63 µm)
Dictyosphaerium elegans	Nanoplankton (2-63 µm)
Dictyosphaerium pulchellum	Nanoplankton (2-63 µm)
Dictyosphaerium sp.	Nanoplankton (2-63 µm)
Dictyosphaerium tetrachotomum	Nanoplankton (2-63 µm)
Dimorphococcus cordatus	Nanoplankton (2-63 µm)
Dimorphococcus sp.	Nanoplankton (2-63 µm)
Euastropsis sp.	Nanoplankton (2-63 µm)
Eudorina elegans	Nanoplankton (2-63 µm)
Eutetramorus fottii	Nanoplankton (2-63 µm)
Eutetramorus sp.	Nanoplankton (2-63 µm)
Flagelado	Nanoplankton (2-63 µm)

Fusola viridis	Nanoplankton (2-63 µm)
Golenkinia radiata	Nanoplankton (2-63 µm)
Gonium cf. *pectorale*	Nanoplankton (2-63 µm)
Gonium sp.	Nanoplankton (2-63 µm)
Lagerheimia ciliata	Nanoplankton (2-63 µm)
Micractinium bornhemiense	Nanoplankton (2-63 µm)
Micractinium pusillum	Nanoplankton (2-63 µm)
Monoraphidium arcuatum	Nanoplankton (2-63 µm)
Monoraphidium caribeum	Nanoplankton (2-63 µm)
Monoraphidium contortum	Nanoplankton (2-63 µm)
Monoraphidium convolutum	Nanoplankton (2-63 µm)
Monoraphidium griffithii	Nanoplankton (2-63 µm)
Monoraphidium irregulare	Nanoplankton (2-63 µm)
Monoraphidium komarkovae	Nanoplankton (2-63 µm)
Monoraphidium minutum	Nanoplankton (2-63 µm)
Monoraphidium pusillum	Nanoplankton (2-63 µm)
Monoraphidium tortile	Nanoplankton (2-63 µm)
Neochloris sp.	Nanoplankton (2-63 µm)
Nephroclamys sp.	Nanoplankton (2-63 µm)
Oocystis borgei	Nanoplankton (2-63 µm)
Oocystis lacustris	Nanoplankton (2-63 µm)
Oocystis solitaria	Nanoplankton (2-63 µm)
Oocystis sp.	Nanoplankton (2-63 µm)
Pachycladella sp.	Nanoplankton (2-63 µm)
Paradoxia multiseta	Nanoplankton (2-63 µm)
Polyedriopsis spinulosa	Nanoplankton (2-63 µm)
Pseudodidimocystis bicellularis	Nanoplankton (2-63 µm)
Pyrobotrys elongata	Nanoplankton (2-63 µm)
Quadrigula closterioides	Nanoplankton (2-63 µm)
Rhombocystis complanata	Nanoplankton (2-63 µm)
Rhombocystis sp.	Nanoplankton (2-63 µm)
Scenedesmus acunae	Nanoplankton (2-63 µm)
Scenedesmus arcuatus	Nanoplankton (2-63 µm)
Scenedesmus ecornis	Nanoplankton (2-63 µm)
Scenedesmus indicus	Nanoplankton (2-63 µm)
Scenedesmus javanensis	Nanoplankton (2-63 µm)
Scenedesmus linearis	Nanoplankton (2-63 µm)
Scenedesmus obtusus	Nanoplankton (2-63 µm)
Scenedesmus ovalternus	Nanoplankton (2-63 µm)
Scenedesmus sp.	Nanoplankton (2-63 µm)
Scenedesmus sp1	Nanoplankton (2-63 µm)
Schroederia antillarum	Nanoplankton (2-63 µm)
Schroederia setigera	Nanoplankton (2-63 µm)
Schroederia sp.	Nanoplankton (2-63 µm)
Spermatozopsis exsultans	Nanoplankton (2-63 µm)
Sphaerellopsis agloe	Nanoplankton (2-63 µm)
Sphaerellopsis cf. *mucosa*	Nanoplankton (2-63 µm)
Sphaerellopsis cilyndrica	Nanoplankton (2-63 µm)

Sphaerellopsis sp.	Nanoplankton (2-63 µm)
Stauridium tetras	Nanoplankton (2-63 µm)
Tetrachlorella sp.	Nanoplankton (2-63 µm)
Tetradesmus cf. *wisconsinensis*	Nanoplankton (2-63 µm)
Tetraedron caudatum	Nanoplankton (2-63 µm)
Tetranephis sp.	Nanoplankton (2-63 µm)
Tetranephris brasiliensis	Nanoplankton (2-63 µm)
Tetrastrum elegans	Nanoplankton (2-63 µm)
Tetrastrum heteracanthum	Nanoplankton (2-63 µm)
Tetrastrum komarekii	Nanoplankton (2-63 µm)
Tetrastrum triangulare	Nanoplankton (2-63 µm)
Treubaria quadrispina	Nanoplankton (2-63 µm)
Treubaria sp.	Nanoplankton (2-63 µm)
Treubaria sp1	Nanoplankton (2-63 µm)
Treubaria triappendiculata	Nanoplankton (2-63 µm)
Westella botryoide	Nanoplankton (2-63 µm)
Dinobryon sp.	Nanoplankton (2-63 µm)
Kephyrion sp.	Nanoplankton (2-63 µm)
Mallomonas cf.	Nanoplankton (2-63 µm)
Mallomonas sp	Nanoplankton (2-63 µm)
Mallomonas sp1	Nanoplankton (2-63 µm)
Mallomonas sp3	Nanoplankton (2-63 µm)
Mallomonas sp4	Nanoplankton (2-63 µm)
Synura sp.	Nanoplankton (2-63 µm)
Chroomonas sp.	Nanoplankton (2-63 µm)
Cryptomonas brasiliensis	Nanoplankton (2-63 µm)
Cryptomonas curvata	Nanoplankton (2-63 µm)
Cryptomonas erosa	Nanoplankton (2-63 µm)
Cryptomonas marssonii	Nanoplankton (2-63 µm)
Cryptomonas sp.	Nanoplankton (2-63 µm)
Cryptomonas sp2	Nanoplankton (2-63 µm)
Plagioselmis sp.	Nanoplankton (2-63 µm)
Aphanocapsa delicatissima	Nanoplankton (2-63 µm)
Aphanocapsa elachista	Nanoplankton (2-63 µm)
Aphanocapsa holsatica	Nanoplankton (2-63 µm)
Aphanocapsa incerta	Nanoplankton (2-63 µm)
Aphanocapsa koordersii	Nanoplankton (2-63 µm)
Aphanocapsa parasitica	Nanoplankton (2-63 µm)
Aphanocapsa sp.	Nanoplankton (2-63 µm)
Aphanothece sp.	Nanoplankton (2-63 µm)
Chroococcus limneticus Lemm.	Nanoplankton (2-63 µm)
Chroococcus microscopicus	Nanoplankton (2-63 µm)
Chroococcus minimus	Nanoplankton (2-63 µm)
Chroococcus sp.	Nanoplankton (2-63 µm)
Coelomoron pusillum	Nanoplankton (2-63 µm)
Coelomoron tropicale	Nanoplankton (2-63 µm)
Cyanodictyon sp.	Nanoplankton (2-63 µm)
Cyanogranis ferruginea	Nanoplankton (2-63 µm)

Cyanothece sp.	Nanoplankton (2-63 µm)
Cylindrospermopsis raciborskii	Nanoplankton (2-63 µm)
Dolichospermum circinalis	Nanoplankton (2-63 µm)
Eucapsis starmachii	Nanoplankton (2-63 µm)
Gloeocapsa sp.	Nanoplankton (2-63 µm)
Gloeothece membranacea	Nanoplankton (2-63 µm)
Konvophoron sp.	Nanoplankton (2-63 µm)
Limnothrix sp.	Nanoplankton (2-63 µm)
Merismopedia tenuissima	Nanoplankton (2-63 µm)
Merismopedia warminigiana	Nanoplankton (2-63 µm)
Planktolyngbya limnetica	Nanoplankton (2-63 µm)
Pseudanabaena moliniformes	Nanoplankton (2-63 µm)
Pseudanabaena mucicola	Nanoplankton (2-63 µm)
Pseudanabaena sp.	Nanoplankton (2-63 µm)
Pseudanabaena sp1	Nanoplankton (2-63 µm)
Rhabdogloea sp.	Nanoplankton (2-63 µm)
Romeria gracilis	Nanoplankton (2-63 µm)
Snowella atomus	Nanoplankton (2-63 µm)
Snowella lacustris	Nanoplankton (2-63 µm)
Gymnodinium sp.	Nanoplankton (2-63 µm)
Peridinium	Nanoplankton (2-63 µm)
Peridinium cf	Nanoplankton (2-63 µm)
Peridinium sp.	Nanoplankton (2-63 µm)
Peridinium sp4	Nanoplankton (2-63 µm)
Peridinium sp8	Nanoplankton (2-63 µm)
Peridinium umbonatum	Nanoplankton (2-63 µm)
Colacium sp.	Nanoplankton (2-63 µm)
Euglena	Nanoplankton (2-63 µm)
Euglena cf. *proxima*	Nanoplankton (2-63 µm)
Euglena sp	Nanoplankton (2-63 µm)
Euglena sp1	Nanoplankton (2-63 µm)
Euglena sp2	Nanoplankton (2-63 µm)
Euglena sp3	Nanoplankton (2-63 µm)
Euglena sp4	Nanoplankton (2-63 µm)
Euglena viridis	Nanoplankton (2-63 µm)
Lepocinclis caudata	Nanoplankton (2-63 µm)
Lepocinclis ovum	Nanoplankton (2-63 µm)
Lepocinclis salina	Nanoplankton (2-63 µm)
Lepocinclis sp.	Nanoplankton (2-63 µm)
Monomorphina pyrum	Nanoplankton (2-63 µm)
Monomorphina sp.	Nanoplankton (2-63 µm)
Phacus acuminatus	Nanoplankton (2-63 µm)
Phacus cf. hamatus	Nanoplankton (2-63 µm)
Phacus cf	Nanoplankton (2-63 µm)
Phacus contortus	Nanoplankton (2-63 µm)
Phacus curvicauda	Nanoplankton (2-63 µm)
Phacus horridus	Nanoplankton (2-63 µm)
Phacus margaritatus	Nanoplankton (2-63 µm)

Phacus orbicularis	Nanoplankton (2-63 µm)
Phacus pleuronectes	Nanoplankton (2-63 µm)
Phacus pyrum	Nanoplankton (2-63 µm)
Phacus sp1	Nanoplankton (2-63 µm)
Phacus sp2	Nanoplankton (2-63 µm)
Phacus sp3	Nanoplankton (2-63 µm)
Phacus suecicus	Nanoplankton (2-63 µm)
Strombomonas deflandrei	Nanoplankton (2-63 µm)
Strombomonas ensifera	Nanoplankton (2-63 µm)
Strombomonas gibberosa	Nanoplankton (2-63 µm)
Strombomonas girardiana	Nanoplankton (2-63 µm)
Strombomonas ovalis	Nanoplankton (2-63 µm)
Strombomonas scabra	Nanoplankton (2-63 µm)
Strombomonas schauinslandii	Nanoplankton (2-63 µm)
Strombomonas sp	Nanoplankton (2-63 µm)
Strombomonas verrucosa	Nanoplankton (2-63 µm)
Trachelomonas abrupta	Nanoplankton (2-63 µm)
Trachelomonas allia	Nanoplankton (2-63 µm)
Trachelomonas armata	Nanoplankton (2-63 µm)
Trachelomonas armata	Nanoplankton (2-63 µm)
Trachelomonas armata	Nanoplankton (2-63 µm)
Trachelomonas armata	Nanoplankton (2-63 µm)
Trachelomonas atomaria	Nanoplankton (2-63 µm)
Trachelomonas cervicula	Nanoplankton (2-63 µm)
Trachelomonas curt	Nanoplankton (2-63 µm)
Trachelomonas curvata	Nanoplankton (2-63 µm)
Trachelomonas cylindrica	Nanoplankton (2-63 µm)
Trachelomonas dastuguei	Nanoplankton (2-63 µm)
Trachelomonas hemisphaerica	Nanoplankton (2-63 µm)
Trachelomonas hirta	Nanoplankton (2-63 µm)
Trachelomonas hispida	Nanoplankton (2-63 µm)
Trachelomonas hispida	Nanoplankton (2-63 µm)
Trachelomonas hispida	Nanoplankton (2-63 µm)
Trachelomonas horrida	Nanoplankton (2-63 µm)
Trachelomonas intermedia	Nanoplankton (2-63 µm)
Trachelomonas kellogii	Nanoplankton (2-63 µm)
Trachelomonas lacustris	Nanoplankton (2-63 µm)
Trachelomonas lacustris	Nanoplankton (2-63 µm)
Trachelomonas lemmermannii	Nanoplankton (2-63 µm)
Trachelomonas levefrei	Nanoplankton (2-63 µm)
Trachelomonas magdaleniana	Nanoplankton (2-63 µm)
Trachelomonas minuscula	Nanoplankton (2-63 µm)
Trachelomonas naviculiformis	Nanoplankton (2-63 µm)
Trachelomonas oblonga	Nanoplankton (2-63 µm)
Trachelomonas parvicollis	Nanoplankton (2-63 µm)
Trachelomonas planctonica	Nanoplankton (2-63 µm)
Trachelomonas pseudobulla	Nanoplankton (2-63 µm)
Trachelomonas pulcherrima	Nanoplankton (2-63 µm)

Trachelomonas pulchra	Nanoplankton (2-63 µm)
Trachelomonas pusilla	Nanoplankton (2-63 µm)
Trachelomonas rugulosa	Nanoplankton (2-63 µm)
Trachelomonas sculpta	Nanoplankton (2-63 µm)
Trachelomonas similis	Nanoplankton (2-63 µm)
Trachelomonas similis	Nanoplankton (2-63 µm)
Trachelomonas sp.	Nanoplankton (2-63 µm)
Trachelomonas sp1	Nanoplankton (2-63 µm)
Trachelomonas sp2	Nanoplankton (2-63 µm)
Trachelomonas sp3	Nanoplankton (2-63 µm)
Trachelomonas sp4	Nanoplankton (2-63 µm)
Trachelomonas superba	Nanoplankton (2-63 µm)
Trachelomonas syaneyensies	Nanoplankton (2-63 µm)
Trachelomonas sydneyensis	Nanoplankton (2-63 µm)
Trachelomonas volvocina	Nanoplankton (2-63 µm)
Trachelomonas volvocinopsis	Nanoplankton (2-63 µm)
Trachelomonas woycickii	Nanoplankton (2-63 µm)
Trachelomonas woycickii	Nanoplankton (2-63 µm)
Gonyostomum sp1	Nanoplankton (2-63 µm)
Gonyostomum sp3	Nanoplankton (2-63 µm)
Raphidoficea não identificada	Nanoplankton (2-63 µm)
Characiopsis cf. *longipes*	Nanoplankton (2-63 µm)
Goniochloris cf. *sculpta*	Nanoplankton (2-63 µm)
Goniochloris cochleata	Nanoplankton (2-63 µm)
Goniochloris contorta	Nanoplankton (2-63 µm)
Goniochloris fallax	Nanoplankton (2-63 µm)
Goniochloris mutica	Nanoplankton (2-63 µm)
Goniochloris spinosa	Nanoplankton (2-63 µm)
Isthmochloron gracile	Nanoplankton (2-63 µm)
Isthmochloron lobulatum	Nanoplankton (2-63 µm)
Isthmochloron neustonica	Nanoplankton (2-63 µm)
Isthmochloron sp.	Nanoplankton (2-63 µm)
Ophiocytium capitatum	Nanoplankton (2-63 µm)
Pseudostaurastrum enorme	Nanoplankton (2-63 µm)
Tetraedriella jovetti	Nanoplankton (2-63 µm)
Tetraedriella regularis	Nanoplankton (2-63 µm)
Tetraedriella spinigera	Nanoplankton (2-63 µm)
Tetraplektron acutum	Nanoplankton (2-63 µm)
Tetraplektron cf	Nanoplankton (2-63 µm)
Tetraplektron sp	Nanoplankton (2-63 µm)
Tetraplektron sp.	Nanoplankton (2-63 µm)
Tetraplektron torsum	Nanoplankton (2-63 µm)
Tetraplektron tribulus	Nanoplankton (2-63 µm)
Actinotaenium cf. *wollei*	Nanoplankton (2-63 µm)
Actinotaenium sp.	Nanoplankton (2-63 µm)
Closterium sp1	Nanoplankton (2-63 µm)
Closterium sp3	Nanoplankton (2-63 µm)
Cosmarium cf. *candianum*	Nanoplankton (2-63 µm)

Cosmarium cf	Nanoplankton (2-63 µm)
Cosmarium cf. *semliae*	Nanoplankton (2-63 µm)
Cosmarium contractum	Nanoplankton (2-63 µm)
Cosmarium protractum	Nanoplankton (2-63 µm)
Cosmarium pseudoconnatum	Nanoplankton (2-63 µm)
Cosmarium pseudopyramidatum	Nanoplankton (2-63 µm)
Cosmarium punctulatum	Nanoplankton (2-63 µm)
Cosmarium rectangulare	Nanoplankton (2-63 µm)
Cosmarium regnesi	Nanoplankton (2-63 µm)
Cosmarium sp.	Nanoplankton (2-63 µm)
Cosmarium sp1	Nanoplankton (2-63 µm)
Cosmarium sp3	Nanoplankton (2-63 µm)
Cosmarium sp4	Nanoplankton (2-63 µm)
Cosmarium sp6	Nanoplankton (2-63 µm)
Cosmarium sp7	Nanoplankton (2-63 µm)
Cosmocladium cf. *constrictum*	Nanoplankton (2-63 µm)
Euastrum rectangulare	Nanoplankton (2-63 µm)
Euastrum sp.	Nanoplankton (2-63 µm)
Staurastrum gracile	Nanoplankton (2-63 µm)
Staurastrum margaritaceum	Nanoplankton (2-63 µm)
Staurastrum muticum	Nanoplankton (2-63 µm)
Staurastrum quagrangulare	Nanoplankton (2-63 µm)
Staurastrum rotula	Nanoplankton (2-63 µm)
Staurastrum setigerum	Nanoplankton (2-63 µm)
Staurastrum sp	Nanoplankton (2-63 µm)
Staurastrum sp.1	Nanoplankton (2-63 µm)
Staurastrum sp.2	Nanoplankton (2-63 µm)
Staurastrum tetracerum	Nanoplankton (2-63 µm)
Staurastrum trifidum	Nanoplankton (2-63 µm)
Staurodesmus cf. *megacanthus*	Nanoplankton (2-63 µm)
Staurodesmus cuspidatus	Nanoplankton (2-63 µm)
Staurodesmus dejectus	Nanoplankton (2-63 µm)
Staurodesmus dickiei	Nanoplankton (2-63 µm)
Staurodesmus lobatus	Nanoplankton (2-63 µm)
Staurodesmus sp.	Nanoplankton (2-63 µm)
Staurodesmus sp1	Nanoplankton (2-63 µm)

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3 HUMAN PRESSURE DRIVES BIODIVERSITY-MULTIFUNCTIONALITY RELATIONSHIPS IN LARGE NEOTROPICAL WETLANDS

ABSTRACT

Many studies have shown that biodiversity regulates multiple ecological functions that are needed to maintain the productivity of a variety of ecosystem types. What is unknown is how human activities may alter the 'multifunctionality' of ecosystems through both direct impacts on ecosystems and indirect effects mediated by the loss of multifaceted biodiversity. Using an extensive database of 72 lakes spanning four large Neotropical wetlands in Brazil, we demonstrate that species richness and functional diversity across multiple larger (fish and macrophytes) and smaller (microcrustaceans, rotifers, protists, and phytoplankton) groups of aquatic organisms are positively associated with ecosystem multifunctionality. Whereas the positive association between smaller organisms and multifunctionality broke down with increasing human pressure, this positive relationship was maintained for larger organisms despite the increase in human pressure. Human pressure impacted multifunctionality both directly and indirectly through reducing species richness and functional diversity of multiple organismal groups. These findings provide further empirical evidence about the importance of aquatic biodiversity for maintaining wetland multifunctionality. Despite the key role of biodiversity, human pressure reduces the diversity of multiple groups of aquatic organisms, eroding their positive impacts on a suite of ecological functions that sustain wetlands.

3.1 Introduction

Human activities are causing biodiversity to decline worldwide[1,2], which has led to an interest in how biodiversity loss might alter the functioning of ecosystems[3]. Most studies have revealed positive and saturating effects of biodiversity on single ecosystem functions[4]. Empirical evidence suggests that species are ecologically unique and can play complementary roles in natural systems, thus varying in their contributions to different functions[3-5]. As a consequence, the effect of biodiversity on ecosystem functioning is stronger – and the relationship is non-saturating – when multiple functions are considered (hereafter 'multifunctionality')[5-8]. Therefore, it has been increasingly recognized that biodiversity and multifunctionality are strongly associated. This recognition has led to the prediction that as

biodiversity declines in human-dominated ecosystems, their ability to sustain multiple ecosystem functions is impaired, ultimately altering the biodiversity-multifunctionality relationship[3,9-13]. Current evidence supporting the anthropogenic impacts on biodiversity-multifunctionality relationships are scarce and comes mostly from experimental manipulations of single trophic levels[10-13]. It is possible that these studies under-estimate human impacts on biodiversity and ecosystem multifunctionality since natural systems are comprised of multiple organismal groups of varying trophic levels, and different trophic levels may combine to have stronger impacts on multifunctionality[5-7]. Further research applying a multitrophic perspective is needed to develop a more mechanistic understanding of the consequences of human pressures for biodiversity-ecosystem functioning relationships in natural systems worldwide.

Here, we used a unique dataset from 72 lakes distributed across four large Neotropical wetlands of Brazil (Amazon, Araguaia, Pantanal and Paraná) to test how the cumulative effect of multiple human pressures impacts the relationship between biodiversity and multifunctionality. These four wetlands provide a unique opportunity to test the influence of human pressures across broad spatial scales as the lakes span a 3,700,000 km² gradient of distinct human activities (Fig. 1). We quantified human pressure on the wetland using the Human Footprint (HFP) index[14], which was extracted for each lake individually (see Methods). The HFP is a recently developed index that incorporates eight different human pressures: (i) built environments, (ii) crop land, (iii) pasture land, (iv) human density, (v) night-time lights, (vi) railways, (vii) roads, and (viii) navigable waterways into a standardized cumulative index of human pressure[14]. This index provides an interesting opportunity to understand how human pressures are affecting biodiversity-multifunctionality relationships in natural to human-dominated systems.

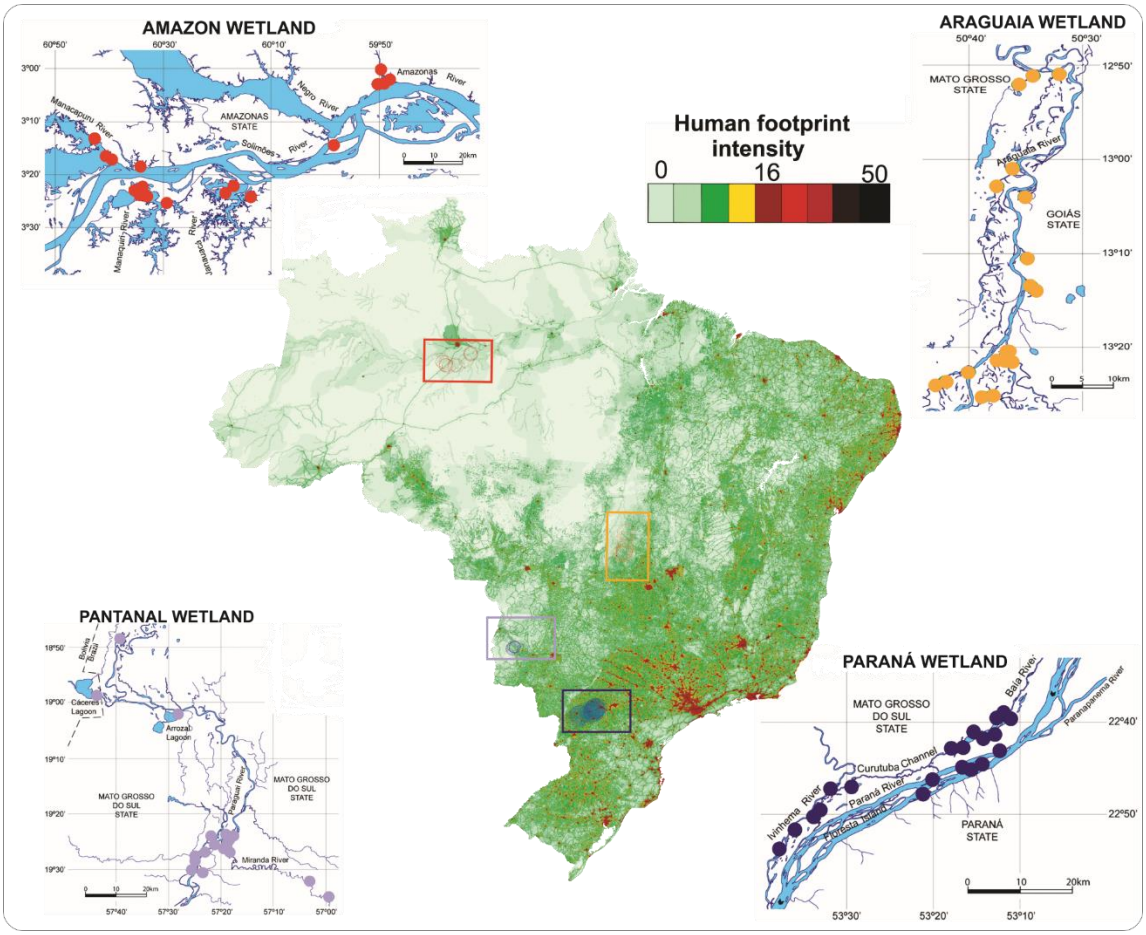

Fig. 1 | intensity of the HFP across Brazil and four Neotropical wetlands. Activity data maps of the Amazon, Araguaia, Pantanal and Paraná wetlands (built environments, crop land, pasture land, human population density, nighttime lights, railways, roads and navigable waterways) used in the HFP analysis in this study were extracted from ref. 14. The HFP data ranged from 0 to 50 according to the pressure of a suite of human activities. The HFP data on the four focal wetlands included low intensity (HFP < 1) and moderate/high intensity of human pressures (HFP < 18). Overall, Amazon and Araguaia had a relatively low/mean HFP intensity, while Pantanal and Paraná had mean/high HFP intensity. Coloured rectangles represent each of the focused wetlands. The points within the rectangles highlight the sampling lakes in each wetland (n = 72 lakes).

We compiled data on the species richness and functional diversity of seven taxonomic groups, including fish, aquatic macrophytes, microcrustaceans, rotifers, phytoplankton, ciliates, and testate amoebae. These data comprised 1,465 plant, animal, and microbial species. Because biodiversity-multifunctionality relationships can be multi-dimensional[6-7], we also used measures of multidiversity (joint diversity of all organismal groups, both for species richness and functional diversity)[15]. Studies considering multidiversity have found strong biodiversity-multifunctionality relationships[6-8]. To estimate functional diversity, we focused on a core set of

independent organismal traits that mediate the species response to human pressures (Supplementary Table 1): body size, resource-use (e.g., feeding groups, growth forms, and mixotrophy), and mobility (e.g., migration ability, propagation method, and cell motility) traits. These traits are often linked to multiple ecosystem functions in wetlands. For instance, body size, feeding groups, and migration ability are related to metabolism, multitrophic biomass production, and nutrient cycling[16-17]. We further quantified ecosystem multifunctionality by using a set of 11 variables that included nutrient concentrations (*in situ* measurements of N and P water concentrations), metabolism (daily changes in water O_2 concentration), biomass at multiple trophic levels (algae, herbivores, carnivores, detritivores, and omnivores), microorganism abundance (bacterial cell densities), availability of photosynthetically active radiation (light availability underwater), and variation in habitat complexity under water (variation in plant above-bottom cover). Together, these variables measure environmental characteristics that are directly linked to ecosystem functions. A detailed rationale for each variable is provided in Supplementary Table 2. We quantified multifunctionality using three common approaches: (i) the averaging multifunctionality index, (ii) the multi-threshold multifunctionality index, and (iii) multiple single functions. The averaging approach takes the average of the standardized values of each single function. In contrast, the multi-threshold considers the number of functions that simultaneously surpass a range of thresholds, which are expressed as a percentage of the highest observed level of functioning (here, 1-99%). These three approaches are complementary, and when taken together, they provide a robust estimation of how multiple functions (averaging and multi-pillar approach), as well as single functions, respond to biodiversity enhancement[5-8,18].

Because no studies have examined the broad-scale relationships between biodiversity and ecosystem multifunctionality across wetlands, we first established whether species richness and the functional diversity of the seven organismal groups were, in fact, related to multifunctionality as previous narrow-scale evidence suggests[17,19]. For this, we employed multiple linear mixed models considering species richness and functional diversity as predictors and multifunctionality as the response. After confirming a consistent relationship, we also used linear mixed model to determined how human pressures alter these biodiversity-multifunctionality relationships. Lastly, we used structural equation models (SEMs) to investigate the direct and indirect biodiversity-mediated pathways by which human pressure can influence multifunctionality in wetlands.

3.2 Results and discussion

Across four hyperdiverse Neotropical wetlands, we found significant positive relationships between the diversity of single groups of aquatic organisms and the multidiversity of all groups with ecosystem multifunctionality (Figs. 2 and 3, and Supplementary Table 3). This finding was consistent for both species richness and functional diversity (Figs. 2 and 3). Our model averaging procedure revealed that the biodiversity of organismal groups was best predictors of multifunctionality, even after accounting for influence of other well-known drivers of multifunctionality such as space, climate (precipitation and temperature), and aquatic properties (conductivity, pH and water level; Supplementary Table 4). The positive association between aquatic biodiversity and multifunctionality persisted regardless of how the measures of multifunctionality were weighted (Supplementary Figs. 1 and 2). The multi-threshold approach provided additional evidence showing that the mean minimum threshold at which the species richness of organismal groups had its strongest effects on multifunctionality averaged 57% (range 5-92%, Supplementary Fig. 3). Similarly, the mean minimum threshold at which functional diversity had its strongest effects on multifunctionality was 91% (range 70-99%, Supplementary Fig. 4). The diversity of aquatic organism groups was also positively associated with most of the individual ecosystem functions, although each organismal group was more closely associated with specific functions (Supplementary Tables 5 and 6). Here, fish diversity was strongly related to multitrophic biomass, macrophyte diversity was most strongly related to light availability and habitat complexity, whereas microorganism diversity was most related to nutrient concentrations and ecosystem metabolism (Extended Data Figs. 1 and 2). Finally, aquatic biodiversity had stronger effects on multifunctionality than other multifunctionality drivers (Extended Data Figs. 3 and 4; SEM: total effect of composite species richness on multifunctionality 0.79, total effect of composite functional diversity on multifunctionality 0.72). Collectively, our broad-scale dataset revealed strong and consistent associations between the diversity of multiple groups of aquatic organisms and ecosystem multifunctionality. These results underline the important role of multiple elements of biodiversity in driving the ecosystem functioning in Neotropical wetlands[15-16,18], as in other ecosystem types such as drylands[8] and forest[7].

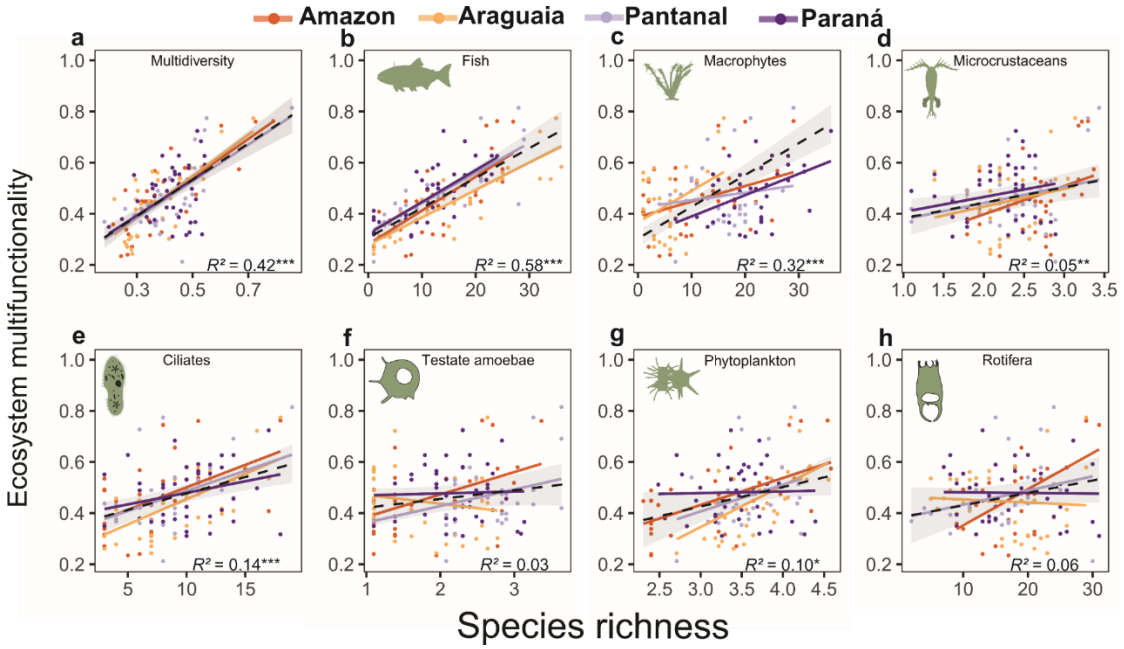

Fig. 2 | Relationship between the species richness of aquatic organisms and multifunctionality in Neotropical wetlands. The linear association between multifunctionality and the species richness of the seven selected taxonomic groups and the composite metric of their joint richness (multi-diversity, standardized between 0 and 1)15 in four Neotropical wetlands; n = 72 lakes. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show the 95% confidence interval for the overall trend. R2 = marginal (that is, variance of the fixed effects). *P < 0.05, **P < 0.01, ***P < 0.01. The richness of microcrustaceans, testate amoebae and phytoplankton was log-transformed before the analysis. Full model results are provided in Supplementary Table 3. Multifunctionality is represented by the averaging index, which reflects changes in the average level of the 11 ecosystem functions. Very high averaging index levels (close to 1) mean that all functions reach their maximum level of performance simultaneously. By contrast, the lowest values (close to 0) mean all functions are at their minimum level of performance. Illustration credit: João Vitor Fonseca da Silva (https://gqromero.wixsite.com/lab/team-3).

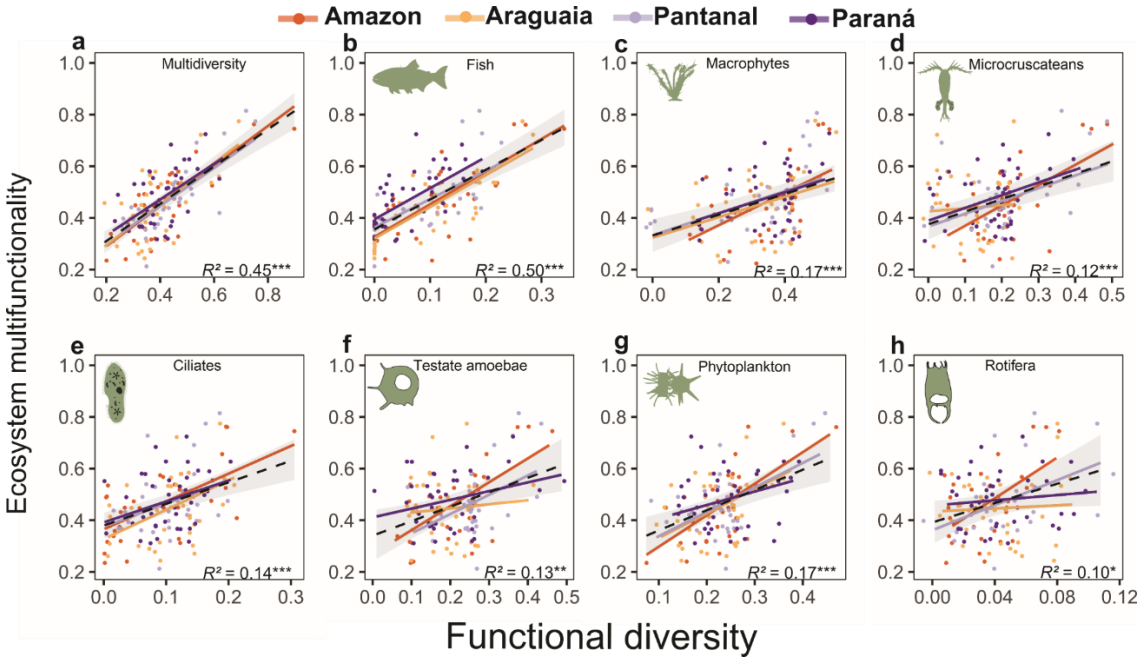

Fig. 3 | Relationship between the functional diversity of aquatic organisms and multifunctionality in Neotropical wetlands. The linear association between multifunctionality and the functional diversity of the seven selected taxonomic groups and the composite metric of their joint functional diversity (multi-diversity; standardized between 0 and 1)15 in four Neotropical wetlands; n = 72 lakes. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show the 95% confidence interval for the overall trend. R2 = marginal (that is, variance of the fixed effects). *P < 0.05, **P < 0.01, ***P < 0.01. The richness of microcrustaceans, testate amoebae and phytoplankton was log-transformed before the analysis. Full model results are provided in Supplementary Table 3. Multifunctionality is represented by the averaging index, which reflects changes in the average level of the 11 ecosystem functions. Very high averaging index levels (close to 1) mean that all functions reach their maximum level of performance simultaneously. By contrast, the lowest values (close to 0) mean all functions are at their minimum level of performance. Illustration credit: João Vitor Fonseca da Silva (https://gqromero.wixsite.com/lab/team-3).

The close association between biodiversity and multifunctionality, suggests that biodiversity loss might impact the ability of wetlands to maintain their functioning[4-8]. Analysis of the relationship between HFP and biodiversity revealed a decline in species richness and functional diversity with increasing HFP (Supplementary Figs. 5 and 6). To test how this affected the relationship between biodiversity and multifunctionality, we examined how interaction HFP x biodiversity influenced the slope of biodiversity-multifunctionality relationships. While the isolated effect of species richness on multifunctionality was positive for most organismal groups, the interactive HFP x species effect was negative (Fig. 4a). Similarly, the isolated effect of functional diversity on multifunctionality was positive, but the

interactive HFP x functional diversity effect was strongly negative (Fig. 5a). This suggests that human pressure can alter the relationship of both species' richness and functional diversity with multifunctionality. By decomposing the effect of biodiversity on multifunctionality through low, medium, and high HFP intensity, we found that the positive effect of species richness and functional diversity on multifunctionality declined from low to high HFP intensity (Fig. 4b and Fig. 5b). In particular, the effect of the diversity of smaller organisms (such as microcrustaceans, testate amoebae, ciliates, and rotifera) on multifunctionality shifted from positive at low HFP intensity to neutral or negative at high HFP intensity (Figs 4 and 5). By contrast, the positive effect of the diversity of larger organisms (such as fish and macrophytes) on multifunctionality was maintained despite increased HFP. These results illustrate how the ability of smaller organisms to promote multifunctionality is sensitive to human pressure and simultaneously highlight the importance of larger organisms for maintaining ecosystem functioning in a human-dominated world[20].

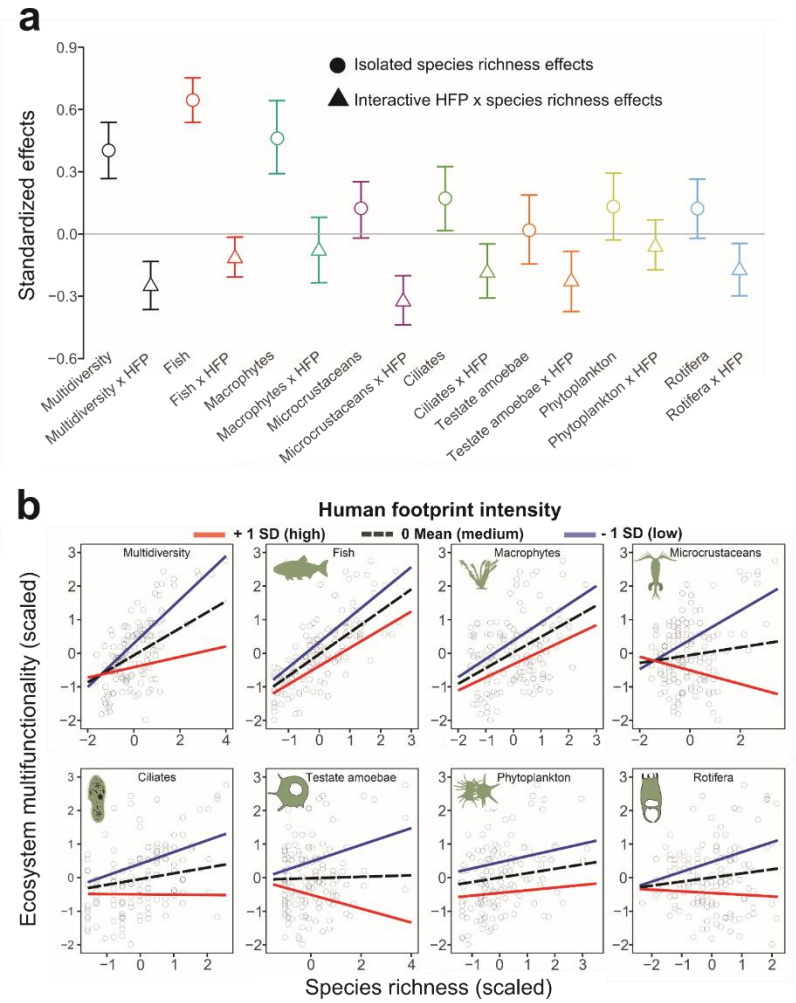

Fig. 4 | Effect of HFP on the relationship between species richness and multifunctionality in Neotropical wetlands. **a**, Standardized coefficients (mean ± s.e.m.(standard error)) from LMMs for the isolated effect of species richness and the interactive HFP × species richness effect on multifunctionality. Model summary statistics are provided in Supplementary Table 7. The colors of the standardized coefficients represent the different groups of aquatic organisms. **b**, Ecosystem multifunctionality as a function of the species richness of single organismal groups and multi-diversity on wetlands subject to low (solid blue line), medium (dashed black line) and high (solid red line) HFP intensity. The lines are predicted (fitted) values from LMMs in which the effect of species richness on multifunctionality is mediated at three levels of HFP: (1) medium: mean = 0; (2) high: the standard deviation above the mean = +1; and (3) low: the standard deviation below the mean = −1. Species richness and HFP were mean centred to remove the high collinearity48. All variables were scaled to interpret parameter estimates at a comparable scale. Multifunctionality is represented by the averaging index. Illustration credit: João Vitor Fonseca da Silva (https://gqromero.wixsite.com/lab/team-3).

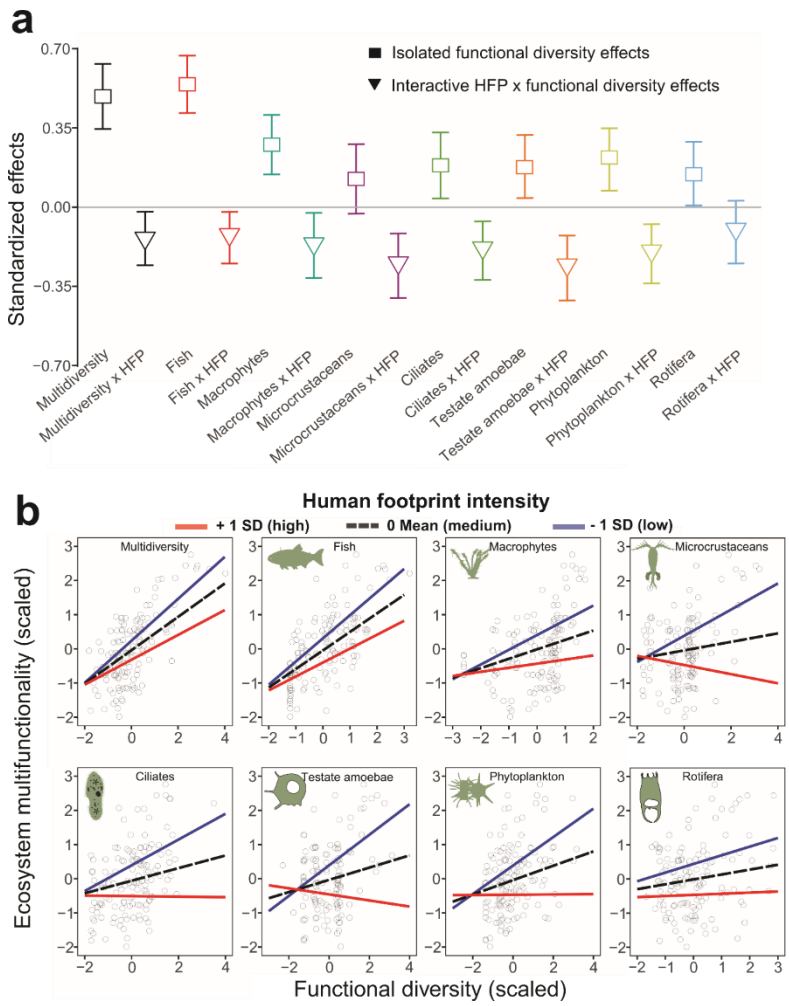

Fig. 5 | Effect of HFP on the relationship between functional diversity and multifunctionality in Neotropical wetlands. **a**, Standardized coefficients (mean ± s.e.m.(standard error)) from LMMs for the isolated effect of functional diversity and the interactive HFP × functional diversity effect on multifunctionality. Model summary statistics are provided in Supplementary Table 7. The colors of the standardized coefficients represent the different groups of aquatic

organism. **b**, Ecosystem multifunctionality as a function of the functional diversity of single organismal groups and multi-diversity on wetlands subject to low (solid blue line), medium (dashed black line) and high (solid red line) HFP intensity. The lines are predicted (fitted) values from LMMs in which the effect of species richness on multifunctionality is mediated at three levels of HFP: (1) medium: mean = 0; (2) high: the standard deviation above the mean = +1; and (3) low: the standard deviation below the mean = −1. Functional diversity and HFP were mean centred to remove the high collinearity48. All variables were scaled to interpret parameter estimates at a comparable scale. Multifunctionality is represented by the averaging index. Illustration credit: João Vitor Fonseca da Silva (https://gqromero.wixsite.com/lab/team-3).

The changes in the magnitude and direction of the relations between biodiversity and multifunctionality suggest that such relationships can be context-dependent in wetlands[21]. This is more evident for smaller groups of aquatic organisms as their effects on multifunctionality changed from positive at low HFP intensity to negative at high HFP intensity. Using a structural equation model, we disentangled the direct and biodiversity-mediated, indirect pathways by which human pressures affect multifunctionality. We demonstrate that the direct effect of HFP on multifunctionality was consistently negative across all wetlands (Fig. 6a, Supplementary Tables 8-10). This is consistent with the fact that the studied wetlands cover regions with intensive human activities (Fig. 1). Most of the studied wetlands cover areas of simultaneous crops of soy and sugarcane, and pasturelands grazed by cattle[22-25] and Paraná wetland is located downstream of one of the most populated areas on the planet[22]. Consequently, multiple human pressures can jointly affect the integrity of these wetlands by decreasing biodiversity and ecosystem multifunctionality (Supplementary Fig. 7).

Beyond their direct negative effect on multifunctionality, HFP had large indirect negative effects on the multifunctionality mediated by declining species richness and functional diversity (Fig. 6). Although indirect negative effects of human pressure were driven by the decline in the diversity of most organismal groups, these effects were strongly mediated by fish diversity (Fig. 6b,d). This is consistent with the fact that fish diversity has greatest influence on functioning of wetlands[16,17], and loss in fish diversity is known to impact multiple ecosystem functions[26]. The negative indirect biodiversity-mediated effects of human pressure on multifunctionality were also consistent across wetlands (Supplementary Table 11). Combined with the fact that the positive effects of biodiversity on multifunctionality decreased with increasing HFP (Fig. 4), our results highlight that, if the human pressures continue to increase[27], preservation of biodiversity for maintaining multifunctionality will not be sufficient unless they are accompanied by a reduction of human pressures. Seen in the light of the increasing human

influence on natural landscapes, our results illustrate the importance of considering multiple pathways through which human pressures can influence ecosystem multifunctionality.

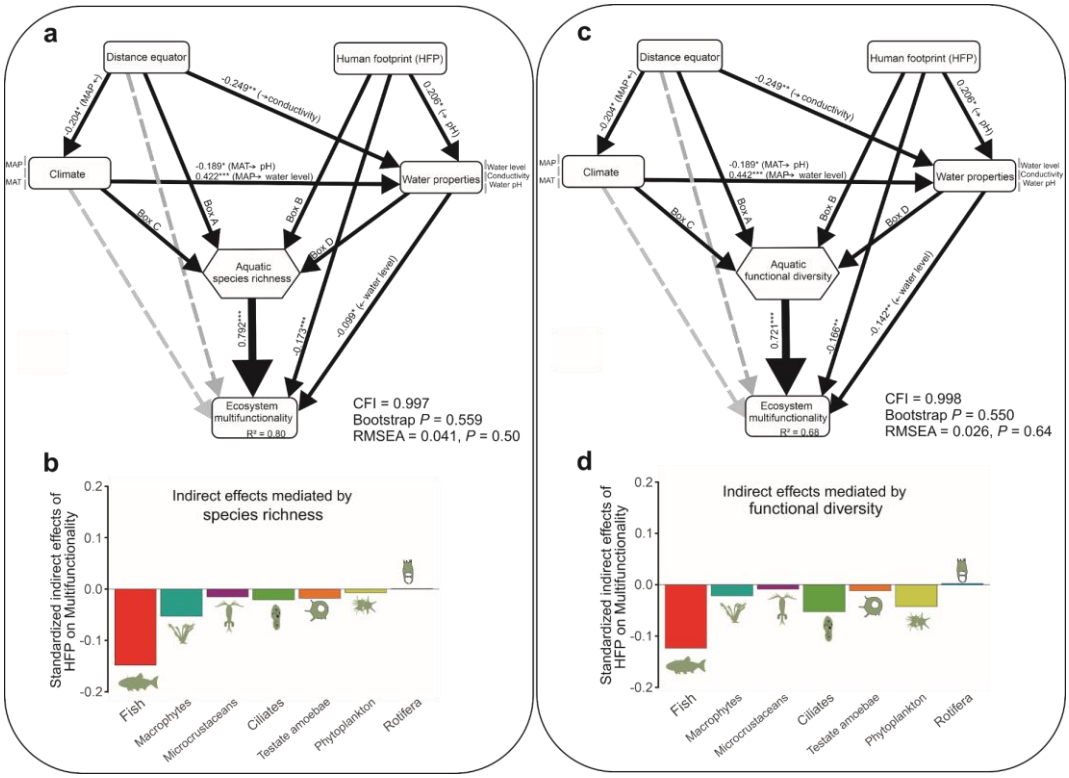

Fig. 6 | The relationship between HFP, climate and water properties and biodiversity and ecosystem multifunctionality. **a,c,** SEM allowed to disentangle the direct and indirect biodiversity-mediated effects of HFP on multifunctionality. Aquatic species richness **(a)** and functional diversity **(c)**, represented by a hexagon, were obtained through composite variables[48], including information about the diversity of seven taxonomic groups of aquatic organisms (Methods). We accounted for multiple ecosystem drivers, including distance from the equator, climate (temperature and precipitation) and aquatic properties (pH, conductivity and water level). We grouped the different categories of drivers (climate, space and water properties) into the same box for graphic simplicity; nevertheless, it does not represent latent variables. Solid black and dashed grey arrows represent significant pathways ($P \leq 0.05$) and non-significant pathways ($P \geq 0.05$), respectively. The thickness of the significant pathways (arrows) represents the magnitude of the standardized regression coefficient. Numbers adjacent to arrows are the standardized effect size. R2 for component models are given in Supplementary Table 12. Significance levels are $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. For simplicity, we grouped the effects of ecosystem drivers (distance, HFP, climate and water properties) on the diversity of each of the seven taxonomic group in boxes. Specifically, Box A represents the effect of distance from the equator, Box B the effect of HFP, Box C the effect of climate and Box D the effect of water properties. Full model outputs and information about boxes A–D are provided in Supplementary Tables 8 and 9. CFI, comparative fit index; RMSEA, the root mean square error of approximation; MAP, mean annual precipitation; MAT, mean annual temperature **b,d,** The standardized indirect effects of the human footprint on multifunctionality mediated by species richness **(b)** and the functional diversity **(d)** of each organismal group used to compute the composite diversity (Supplementary Table 11). Illustration credit: João Vitor Fonseca da Silva (https://gqromero.wixsite.com/lab/team-3).

3.3 Conclusion

We have provided the first empirical evidence of a positive broad-scale relationship between the diversity of multiple groups of aquatic organisms and the multifunctionality of wetland ecosystems. We demonstrate that a positive association between aquatic biodiversity and multifunctionality occurs for both single metrics of diversity as for those combined into a multidiversity. These positive relationships are also apparent for the seven groups of aquatic organisms, although larger organisms are more strongly linked to multifunctionality than smaller organisms. Collectively, our findings highlight the importance of aquatic biodiversity for maintaining ecosystem multifunctionality and their associated services[28]. It is imperative that biodiversity conservation be a key management priority in wetlands[29] and that ecosystem management targets the joint conservation of multiple components of aquatic biodiversity, from vertebrates to plants and microorganisms. We have also shown that human pressures degrade the positive relationship between biodiversity and multifunctionality, which occur both directly and indirectly as human pressures reduce the biodiversity needed to maintain numerous ecosystem functions. These findings demonstrate that human pressures are degrading multifunctionality through multiple pathways. Consequently, conserving the functioning of wetlands will be a major challenge as human pressures continue to increase in these ecosystems worldwide[29-30]. More broadly, reducing human pressures must be addressed urgently in wetlands as these systems rank among the most diverse and productive ecosystems globally, providing a suite of functions and services essential for human well-being.

3.4 Material and methods

3.4.1 Study sites and data collection.

The study comprised the four largest South American wetlands – Amazon, Araguaia, Pantanal, and Paraná – encompassing a subcontinental spatial area of approximately 3,700,000 km² and 72 lake ecosystems (Fig. 1). These wetlands are subject to distinct intensities of human pressure. Amazon is a global biodiversity hotspot and is more preserved than Araguaia and Pantanal that are both subject to moderate human pressure (Fig. 1). Paraná includes 150 constructed dams[31] and faces the strongest human pressure among the four wetlands. The climate ranges from subtropical to tropical, with a mean annual temperature of 16 - 29ºC and a mean precipitation of 1,300 - 2,000 mm year-1[32]. The field data were collected between August and May 2011 and 2012. The wetland lakes were surveyed under the Brazilian program "National System for Research in Biodiversity" (Sisbiota Brazil). The field surveys were designed to include lakes representing a wide range of climate, human pressure, and environmental conditions. They followed a standardized sampling protocol and the sampling effort was the same in all lakes[32]. In order to provide a comprehensive assessment of aquatic communities, we performed one sampling

during the dry season and another during the wet season in each lake. The sampling included fish, aquatic macrophytes, microcrustaceans (cladocerans and copepods), rotifers, phytoplankton, testate amoebae, and ciliates (see Supplementary Methods).

3.4.2 Diversity measure.

We quantified the species richness of the seven taxonomic groups in all 72 lakes. After identifying each individual to species level, we determined 325 fish species, 87 macrophyte species, 99 microcrustacean species, 124 rotifer species, 598 phytoplankton species, 124 testate amoebae species, and 108 ciliate species. Sample coverage was equal for all wetlands, and we calculated estimated species richness as the Chao index with abundance-based data using the R package iNEXT[33], which is based on rarefaction and extrapolation of Hill numbers and provides an unbiased estimate of asymptotic species richness and enables comparisons among wetlands with different numbers of individuals. We used the Chao species richness because richness is the most commonly used and simplest metric of biodiversity[5-8]. We also measured the key functional traits for all organismal groups. We focused on the traits that are known to govern the patterns of spatial distribution and individual fitness, and which also influence ecosystem processes (Supplementary Table 1)[16,34]. These traits fall into the three broad categories: (i) body size (maximum body length for animal taxa or cell volume for phytoplankton), (ii) resource and habitat use traits (feeding groups for animal taxa, growth form for macrophytes, nitrogen fixation or mixotrophy for microorganisms), and (iii) mobility traits (dispersal ability for animal taxa, propagation means for macrophytes, and cell motility for microorganisms). In order to determine the functional diversity (FD) of each organismal group, we calculated functional dispersion – i.e., the mean distance in multidimensional trait space of the individual species to the centroid of all species[35]. This measure provides a robust estimate of functional diversity. Because the relationship between biodiversity and ecosystem functioning can be multi-dimensional on both the predictor (biodiversity) and response side (multifunctionality)[6-7], we estimated a multidiversity index including the diversity of the seven organismal groups[15]. We first standardized the diversity values of each organismal group between 0 and 1 (species richness or functional diversity) by scaling them to the maximum observed value, and then we average these standardized diversity values[15]. This procedure ensures that the diversity of each organism group contributes equally to the multidiversity. We calculated separately the multidiversity index for species richness and functional diversity. The multidiversity index has been widely used because it reflects very well the biodiversity-multifunctionality relationships in multitrophic ecosystems[8,11,15,17].

3.4.3 Assessing ecosystem functions and properties.

In each lake, 11 ecosystem variables regulated by aquatic organisms and belonging to a wide range of ecosystem functions and properties were measured (Supplementary Table 2). These functions and properties included: (i) nutrient concentrations represented by *in situ* measurements of total phosphorous (mg L^{-1}) and total nitrogen (mg L^{-1}) available in the water. Total phosphorus and nitrogen cover all fractions of these nutrients, including nitrate, nitrite, ammonia, particulate phosphate, dissolved organic phosphate, and orthophosphate. We took water samples in each lake and in the laboratory, nitrogen was quantified according to Mackereth et al.[36], while phosphorus was quantified following[37]. (ii) Ecosystem metabolism represented by the daily variation of dissolved oxygen in the water (mg L^{-1} day^{-1}), which was measured from dawn to dusk in each lake using a digital oximeter portable YSI

aid (Digimed). We use the mean of daily oxygen variation as it represents the change in the metabolic underwater regime[38]. (iii) Multitrophic standing biomass was represented by the biomass of algae, carnivorous fish, omnivorous fish, herbivorous fish, and detritivorous fish. Algae standing biomass was quantified using biovolume (individuals per mm L^{-1}) of identified algae species. Biovolume was estimated by multiplying the abundance of each species by their mean volume[39]. Fish were classified into trophic groups using information from feeding trials and gut content analysis[16,32]. Afterwards, the fish counts within each trophic group were converted to biomass (g m^{-2}) using published species-specific length–weight relationships[40]. (iv) Availability of photosynthetically active radiation represented by light availability under water (m). We quantified light availability under water by the depth of the euphotic zone, which represents the depth (m) of the lake where there is sufficient light incidence for autotrophs. The euphotic zone was calculated as Secchi depth multiplied by 1.7, where 1.7 is a correction factor for estimating the light available under water[32]. (v) Microorganism abundance (cells mL^{-1}) was quantified using bacterial abundance. To record the accumulative abundance of bacteria, we took water samples at the subsurface (approximately 30 cm below the air-water interface) at the central, deepest region of each lake using polyethylene flasks. Bacteria were analyzed from water samples treated with a fixative solution composed of alkaline Lugol's solution, borate buffered formalin, and sodium thiosulfate that was filtered through black Nuclepore filters (0.2 and 0.8 μm, respectively) and stained with fluorochrome DAPI (4,6- diamidino-2-phenylindole[41]. Bacterial quantification was done with an epifluorescence microscope at a magnification of ×1000 (Olympus BX51). (vi) Variation of underwater habitat complexity was quantified based on variations in the above-ground cover of aquatic plants (m^{-2}). We estimated the area of all leaves and culm of each plant species. We then summed the area of all leaves and culm to obtain the above-ground area cover by each individual. We calculated the standard deviation of the above-ground area cover between all plant species and used this standard deviation as a proxy of variation in the above-ground vegetal cover.

3.4.4 Pairwise correlation between ecosystem functions.

To assess the potential for a trade-off between individual ecosystem characteristics, we calculated Pearson correlation coefficients between each pair of individual standardized functions. Of the possible 45 combinations of pairwise functions, we found only seven strong correlations ($r = 0.5$; Supplementary Fig. 8). To remove any bias in our multifunctionality index, the highly correlated functions were down-weighted in its calculation (Supplementary Fig. 9), as described in Manning et al.[42]. Ecosystem functions were grouped into clusters according to their correlations. This weighted approach indicated three different clear clusters: (1) aboveground plant cover, (2) available N and P, light availability underwater, daily oxygen variation, and algal biomass, and (3) carnivore biomass, omnivore biomass, detritivore biomass, omnivore biomass, and bacterial abundance. Weighted multifunctionality was then calculated as the average of all variables within each cluster. For instance, each function within cluster 2 was weighted with a weight of 0.2. These functions were then averaged into a standardised variable. We repeated the analyses of the relationship between biodiversity and multifunctionality for the weighted multifunctionality to determine whether the results differed between weighted and non-weighted multifunctionality (see ref.[42]).

3.4.5 Assessing ecosystem multifunctionality.

To obtain robust and quantitative multifunctionality indexes, we used three multifunctionality approaches: (1) the averaging multifunctionality index, (2) the multi-threshold multifunctionality index, and (3) the multiple single functions index[18]. To obtain the averaging ecosystem multifunctionality index, we standardized all 11 ecosystem functions between 0 and 1 (rawFunction − min(rawFunction) / (max(rawFunction) − min(rawFunction)) and then calculated their means. The averaging ecosystem multifunctionality index is the most commonly used index in the multifunctionality literature[5,18], but has the limitations that the number of functions with high performance are impossible to obtain and it does not allow for potential trade-offs between functions. To take these limitations into account, we used the multi-threshold index. This index calculates how many functions simultaneously exceed a predefined percentage of the maximum observed value of each individual function. Because the selection of any threshold is arbitrary, analysing multiple thresholds of maximum functioning is recommended[18]. We analysed the effect of the diversity of each organismal group on multifunctionality across the full range of thresholds from 1% to 99%. We used the mean of the three largest values of each ecosystem variable across all lakes as the observed maximum to reduce the impact of potential outliers.

3.4.6 Assessing the Human Footprint on wetlands.

We used the global Human Footprint (HFP) map as a surrogate of the cumulative human-induced pressure on the wetlands[14]. This map is constructed from an ensemble of eight human pressure: (i) the extent of built environments, (ii) crop land, (iii) pasture land, (iv) human population density, (v) night-time lights, (vi) railways, (vii) roads, and (vii) navigable waterways. To facilitate comparison among pressures, each pressure was weighted (details on the weightings are provided below). The pressures were weighted according to their relative intensity[14]. For example, (i) constructed environments are areas related to urban settlements such as buildings and urban areas. The pressure of built environments was assigned a score of 10 (i.e., a score of 10 is assigned if there are built environments, otherwise a score of 0 is assigned). (ii) Crop land is characterized by monocultures with high inputs of pesticides and fertilizers. In terms of HFP, the crop land pressures received a score between 0 and 7, where 7 indicates intensive agriculture and 0 indicates the absence of crop lands. (iii) Pasture land includes some of the major land uses worldwide and is characterized by cattle and sheep farming. The pressure of pastures on wetlands was assigned a score of 4, which was scaled from 0 to 4 using the %pasture for each 1 km^2 pixel. (iv) Human population is an important underlying driver of the global change of natural ecosystems. Human density was mapped using gridded population downscaled to match the 1 km^2 resolution. All areas with a population above 1,000 people/km^2 were assigned a pressure score of 10. For less populated areas, the pressure score is logarithmically scaled using the following estimation: Pressure score = 3.333 x log (population density + 1). (v) Night-time lights include electric infrastructure related to more rural areas that are not part of built environments. To calculate the pressure of night-time lights, the areas were divided into 10 quantiles of increased night-time light intensity associated with scores between 1 and 10, while areas with no lights were assigned a zero score. (vi) Railways are essential human infrastructures that influence natural ecosystems. The direct pressure of railways was assigned a score of 8 for a distance of 0.5 km on either side of the railway. (vii) Likewise, roads modify the landscape where they are built. The direct and indirect pressure of roads on wetlands was assigned a score of 8 for 0.5 km (direct impact), while nearby areas up to 15 km received a score value that decayed exponentially on either side of the road (indirect impact). (viii) Navigable waterways act as conduits for people to access nature, resulting in impacts on wetlands.

The pressure of navigable waterways was assigned a score of 4, which decayed exponentially out to 15 km away from the water banks. For full details of HFP estimation see refs[2,14].

The average HFP of the 1 km² pixels (cell-size resolution) overlapping each lake was extracted to derive the cumulative pressure, and this average HFP ranged between 0 and 50 (cumulative sum of all individual human pressures). The average HFP was extracted using the 'raster' R package[43] through a global HFP map that was available for the year 2009. The eight human pressures are not mutually exclusive, and may co-occur in the same wetland or vary among and within wetlands. The HFP was initially developed to represent human pressures in terrestrial systems[14], but most of these human pressures extensively affect wetland ecosystems. For instance, Brazil has experienced rapid expansion of urban areas[44]. Along with the increase in human populations in the vicinity of wetlands, there has been an increased pressure on these ecosystems from sewage, cattle and sheep pastures, railways, roads, and navigable waterways[45]. We found negative correlations between the individual pressures with biodiversity and multifunctionality, which suggest that the use of the HFP in our study is robust (Supplementary Fig. 7).

3.4.7 Statistical analyses.

3.4.7.1 Linking aquatic biodiversity to multifunctionality.

To determine the direct link between aquatic biodiversity and average multifunctionality across four wetland ecosystems, we fitted a series of linear mixed effects models (LMMs) to the surveyed data. We tested the relationship of (i) species richness and (ii) functional diversity of single organismal groups, and (iii) multidiversity with the ecosystem multifunctionality. The models were run using the function lme in the 'nlme' package[46]. We included wetlands and two sampling periods as our random structure, and allowed the intercept and slopes to vary by wetland. The assumptions of normality, linearity, and homoscedasticity were verified using graphical diagnostics (QQ plots and residual plots). To determine the importance of other biotic and abiotic variables besides biodiversity for multifunctionality, we included other well-known drivers of multifunctionality such as space (distance from equator), climate (temperature and mean annual precipitation), and aquatic properties (pH, conductivity, and water level; see Supplementary Methods). We performed a model averaging procedure that calculated all possible subset models and chose from this set those subset models with the lowest values (ΔAICc $\leq$ 2) of the Akaike Information Criterion corrected for small sample size (AICc). This analysis was conducted using the R-package MuMIn[47].

Using LMMs we also assessed the relationship of species richness and functional diversity of single organismal groups and multidiversity with each of the 11 individual ecosystem functions. This allowed us to compare the multifunctionality results to the performance of individual functions. *Priori* to these analyses, we standardized all individual ecosystem functions (z-scored: mean-centred and divided by the SD) to better meet model assumptions. Even so, for some functions, the residuals were highly heteroscedastic. We then modelled the variance using the function varIdent, with diversity nested by wetlands as the stratum. We considered quadratic terms for some ecosystem functions to evaluate potential nonlinear relationships.

We also modelled aquatic diversity against the number of functions above a threshold using generalized linear mixed effects model (GLMMs), assuming a Gaussian error distribution in the MASS package[48]. Because we wanted to know whether the relationships between species richness and functional diversity with ecosystem multifunctionality varied as a function of organismal group and among the four wetlands, we fitted the GLMM individually to each organismal group. We then extracted and plotted the linear coefficient (fitted values) of the relationship between biodiversity and each threshold level (1 to 99%; 99 thresholds) to each wetland system. This led us to examine changes in the shape of the fitted curve for each wetland at multiple thresholds.

3.4.7.2 Effect of human pressure on biodiversity-multifunctionality relationships.

We conducted linear mixed-effect models between human footprint (HFP) and biodiversity (species richness and functional diversity of single organismal groups and multidiversity). We found strong negative effects of HFP on biodiversity (Supplementary Figs. 5 and 6), allowing us to determine whether HFP altered the relationship between biodiversity and ecosystem multifunctionality. We then added interaction terms for HFP × species richness and HFP × functional diversity of each single organismal group and multidiversity to the mixed-effects models and measured the estimated coefficients of these interactions on ecosystem multifunctionality. Since biodiversity and HFP are both continuous variables, analyse their interactions could result in an interaction predictor that is collinear with the main effect[49]. Thus, we centered these variables by subtracting the sample mean from all input variable values. The mean of the centered variables is zero and the collinearity is reduced. We also scaled all the variables, dividing them by their standard deviations to interpret parameter estimates from models at a comparable scale. Since HFP is a continuous covariate, there are an infinite number of values we can use to analysis the effect of biodiversity on multifunctionality. For a better interpretation of the interactive effect, we selected three values (thresholds) of the scaled HFP: (i) a mean value (0), a value of standard deviation above the mean (1), and a value of standard deviation below the mean (–1). This is a common approach to analyse interaction between continuous predictors[50]. These three HFP values can be interpreted as three levels of HFP intensity, low intensity (below average), moderate intensity (on average) a high intensity (above average). The slopes of each relationship between HFP and species richness, functional diversity, and ecosystem multifunctionality are similar among wetlands, suggesting absence of any bias in our results (Supplementary Fig. 10).

3.4.7.3 Pathways by which human pressure affects multifunctionality.

To disentangle the direct and biodiversity-mediated pathways by which HFP affects multifunctionality, we ran structural equation modelling (SEM) using the R package lavaan[51]. Considering that all seven organismal groups worked in combination to determine multifunctionality (Fig. 2 and 3), we used their diversity to construct composite variables in our SEM. We combined the species richness and functional diversity of the seven organismal groups to construct a composite index for species richness and functional diversity, respectively. A composite index collapses the effects of multiple related variables into a single composite effect, thus representing a good way to analyse complex multivariate relationships in SEM[52]. We accounted for six ecosystem drivers: distance from equator, climate (mean annual temperature and precipitation), and aquatic characteristics (pH, conductivity, and water level) in the SEM. The SEM was fitted based on a meta-model (Supplementary Fig. 11). We calculated the standardized direct coefficients for each pathway within the model. We also estimated the

indirect effect of HFP on multifunctionality mediated by diversity (species richness and functional diversity) of single organismal groups. To do so, we multiplied the coefficient of HFP on diversity (species richness and functional diversity) of a given organism group by the standardized loading of this organism group on composite. Finally, we multiplied the above result by the coefficient of composite on multifunctionality (Supplementary Table 11). We applied multigroup analysis in the SEM to evaluate whether (i) the effects of selected predictors (HFP, biodiversity, climate, space, and aquatic properties) on multifunctionality, as well as (ii) the effect of HFP on biodiversity varied across wetlands. We considered the four wetlands as the grouping variable (Amazon, Araguaia, Pantanal, and Paraná). We constructed a SEM model in which all parameters were free to differ between wetlands and a model in which all parameters were fixed (i.e., constrained to a single value determined by all wetlands). We compared the free model with the constrained model, where non-significant differences indicated no variation in pathway coefficients by wetlands, whereas significant difference indicated that pathway coefficients varied by wetlands. Because we found significant differences between the free and restricted/constrained model for both species richness and functional diversity, our next step was to understand which pathways differed. We only analysed the differences (multigroup) of the pathways including multifunctionality and biodiversity (species richness and functional diversity; Supplementary Table 10). Differences between other pathways within the model were not analysed. We evaluated the SEM fit using the comparative fit index (CFI; the model has a good fit when CFI $\geq$ 0.95) and the root MSE of approximation test (RMSEA; the model has a good fit when RMSEA $\leq$ 0.05). For our species richness model, the CFI was 0.997 and the RMSEA was 0.041, and for our functional diversity model the CFI was 0.998 and the RMSEA was 0.026, indicating a good model fit. All analyses were conducted in R version 3.4.4[53].

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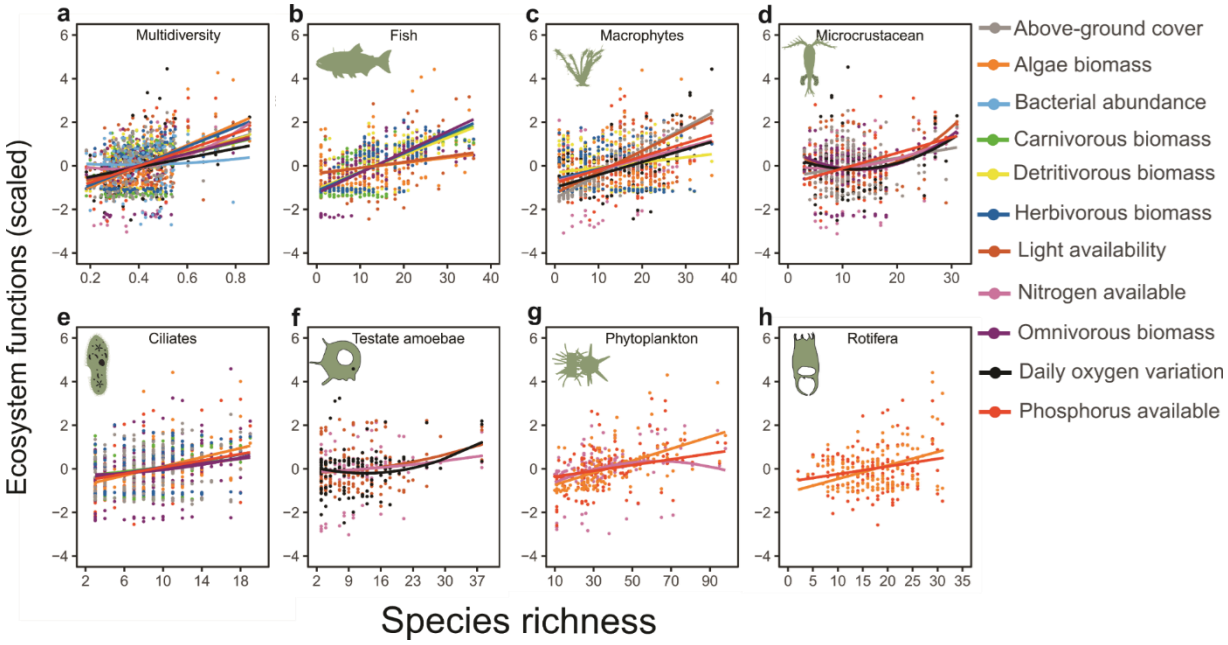
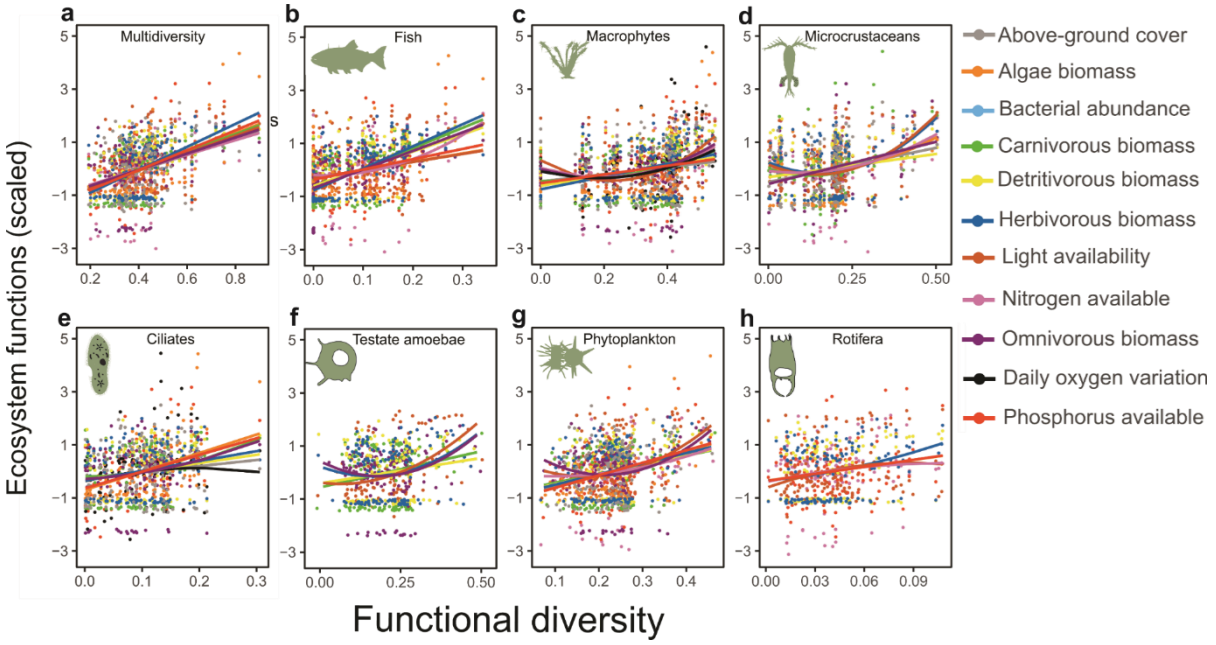
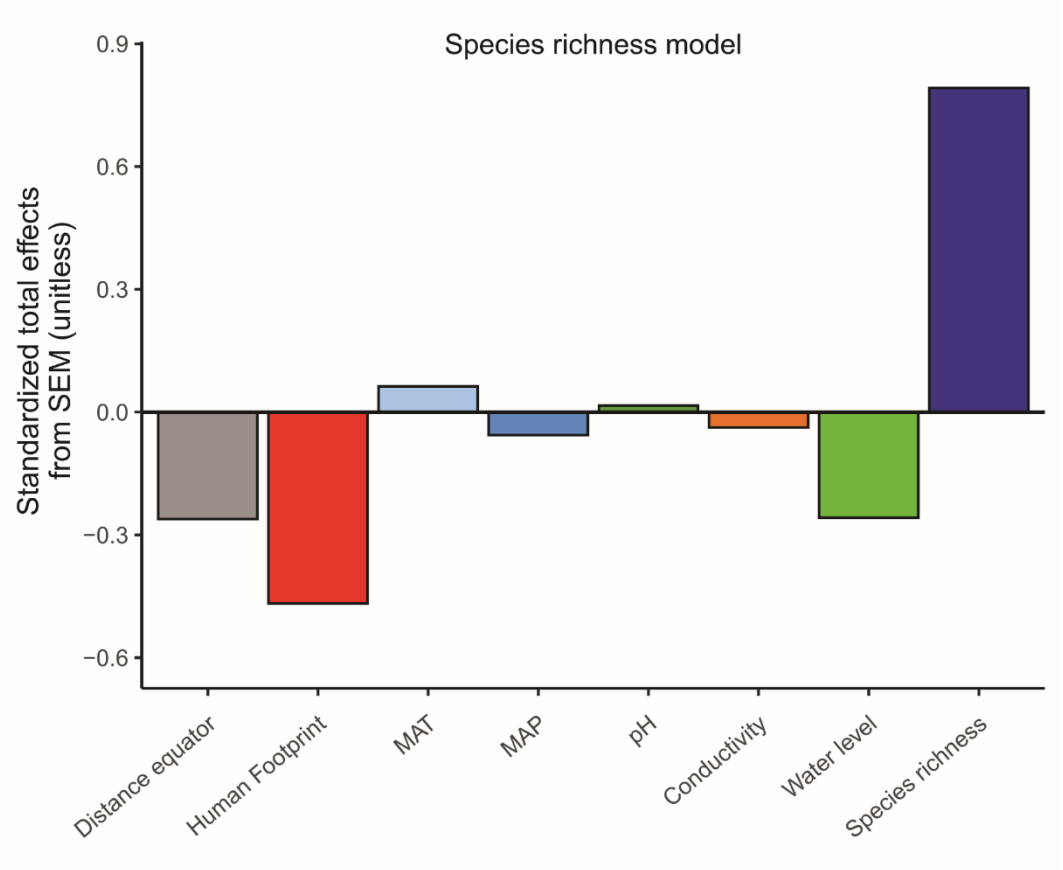


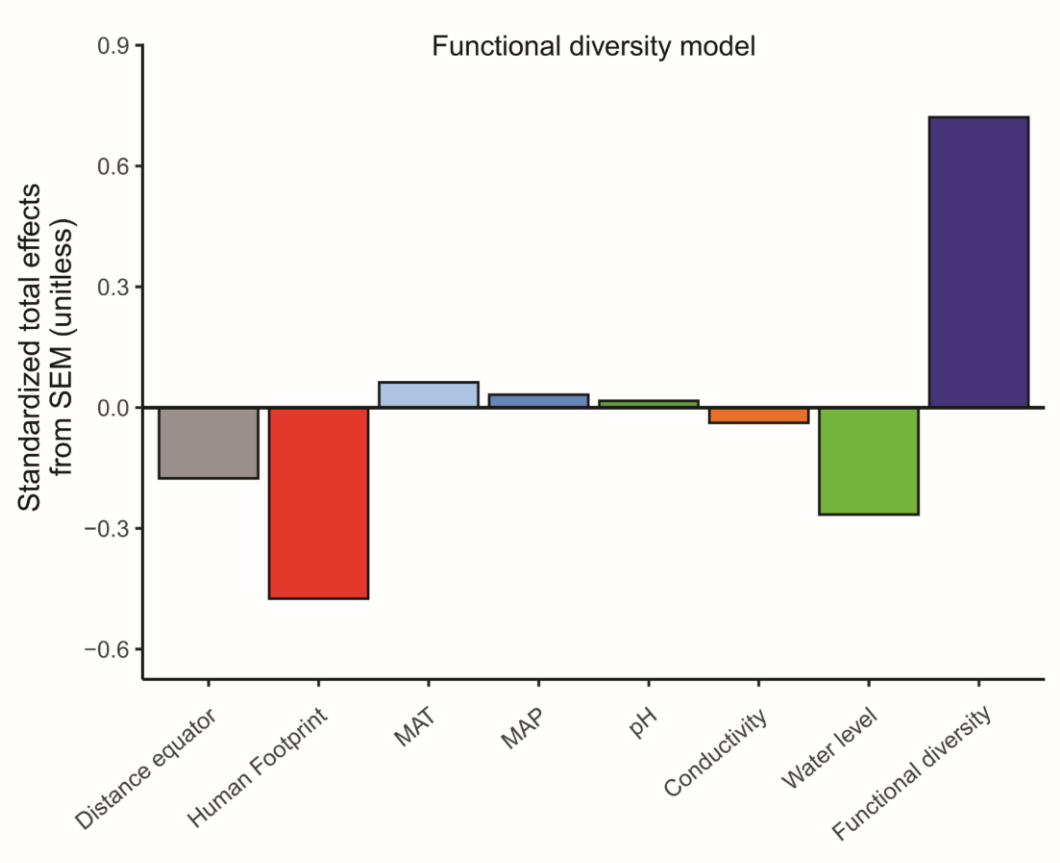
Extended Data Fig. 1 | The relationship between the species richness of aquatic organisms and single ecosystem functions in Neotropical wetlands. Significant links between the species richness of single organismal group and multi-diversity (joint richness of seven organismal groups) with 11 individual ecosystem functions. Solid coloured lines are extracted from linear mixed-effect models and show the significant relationships with each organismal group and ecosystem function. Non-significant relationships are not shown. Full model results are provided in Supplementary Table 5. All single ecosystem functions are scaled (z-score standard) for better graphical interpretation.



Extended Data Fig. 2 | The relationship between the functional diversity of aquatic organisms and single ecosystem functions in neotropical wetlands. Significant links between the functional diversity of single organismal group and multidiversity (joint functional diversity of seven organismal groups) with 11 individual ecosystem functions. Solid colored lines are extracted from linear mixed-effect models and show the significant relationships with each organismal group and ecosystem function. Non-significant relationships are not shown. Full model results are provided in Supplementary Table 6. All single ecosystem functions are scaled (z-score standard) for better graphical interpretation.



Extended Data Fig. 3 | Importance of species richness and ecosystem drivers for multifunctionality in neotropical wetlands. Standardized total effects (direct plus indirect effects) of seven ecosystem drivers and species richness to multifunctionality. The results were derived from the structural equation models (Fig. 5a). Species richness represents a composite variable that includes information about the species richness of seven groups of aquatic organisms. For the complete estimated model, see Supplementary Table 8.



Extended Data Fig. 4 | Importance of functional diversity and ecosystem drivers for multifunctionality in neotropical wetlands. Standardized total effects (direct plus indirect effects) of seven ecosystem drivers and functional diversity to multifunctionality. The results were derived from the structural equation models (Fig. 5c). Functional diversity is a composite variable that includes information about the functional diversity of seven groups of aquatic organisms. For the complete estimated model, see Supplementary Table 9.

APPENDIX B – Details of the study area and results

(1) Taxonomic groups

Phytoplankton

Quantitative phytoplankton samples were taken with bottles at the subsurface (~20 cm) of the limnetic region at each sampling site. The samples were preserved *in situ* with acidified Lugol's solution[1]. We counted individuals from random screens using an inverse microscope according to[2,3]. To estimate phytoplankton abundances. Species identification was done using specialized literature[1, 4-8]**.**

Macrophytes

At all sampling sites, we recorded the aquatic macrophytes present from a boat sailing at slow speed along the entire lake shoreline. Submerged species were recorded with a grapple, treble hooks, and a rake attached to an aluminum stick that was dragged along the lake bottom. Species that could not be identified in the field were collected for later identification in the laboratory and kept in the Herbarium of the State University of Maringá (HUEM). Species identification followed[9-14].

Ciliates

To sample ciliates, five liters of water were collected at the subsurface of the limnetic region (10-20 cm) from each lake using polyethylene flasks. The samples were stored in a cooler and taken to the laboratory where they were concentrated into 100 mL using a micro-plankton net (5 μm). Ciliates were counted and identified *in vivo* within a maximum period of 4 h after sampling using an optical microscope (Olympus CX-41) and following the live counting technique[15]. Ten replicates of 100 μL drops were counted per site. Species identification was done using[15-21].

Testate amoebae, rotifers, and microcrustaceans

These taxa were sampled in the limnetic region of the lakes using a motor pump from a boat moving at constant speed (to take a composite sample from each site) and a plankton net (68 μm) to filter and concentrate 600 L of water per sample[22]. The samples were preserved in formaldehyde (4%) buffered with calcium carbonate. Abundance and species richness of rotifers, amoebae testate, and microcrustacean were determined using a Sedgewick-Rafter

counting chamber under an optical microscope. At least 80 individuals were counted in each of three sequential samples obtained with a Hensen-Stempell pipette (2.5 mL). Species identification followed[22-30].

Fish

Fish were caught using 20 m trawl nets (20 m x 1.5 m) and seine nets (0.54 cm mesh size) deployed in the littoral zone of the lakes with a standardized effort of three drags per sampling site. Subsequently, the fish were anesthetized with benzocaine solution. The fish were identified to species level and counted. Voucher specimens of all species have been deposited in the ichthyological collection of the Research Centre in Ichthyology, Limnology, and Aquaculture (Nupélia), State University of Maringá (UEM). Species identification was done follow[32,33].

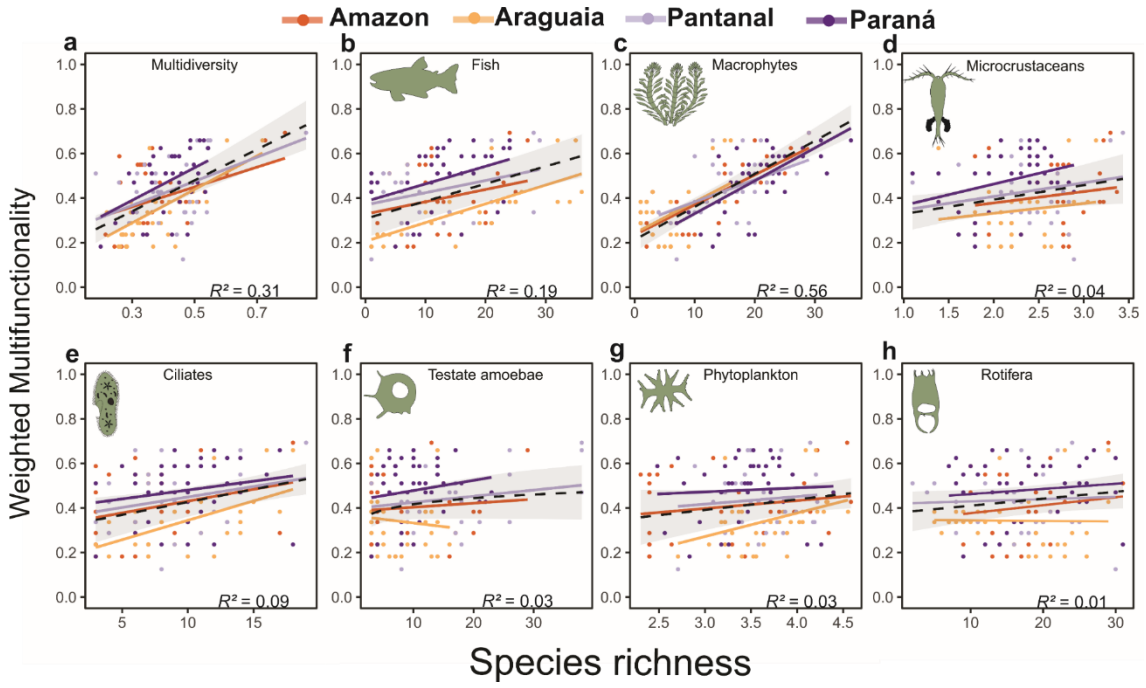
(ii) Functional traits measurements

A core set of organismal traits were measured across the seven taxonomic groups considered (fish, aquatic macrophytes, microcrustaceans, rotifers, phytoplankton, ciliates, and testate amoebae). We selected those traits related to three sets of functional categories: (i) body size, (ii) resource acquisition, and (iii) mobility. These three trait categories are expected to exert strong effects on ecosystem functioning[34,35]. Body size is a key trait as it relates to important functions such as metabolism[36], reproduction rates[37], and biotic interactions[38-40]. Resource acquisition traits, e.g., growth forms of plants, feeding groups, and mouth position of animals may well reflect the diversity of poll resource and feeding diet across co-occurring taxa[41-43]. Mobility is a key trait as it is related to the dispersal of organisms, which is critical for ecosystem colonization and influences the response of organisms to human pressures[44-46]. We selected functional traits for all taxonomic groups.

Ecosystem drivers

To determine the importance of biodiversity for multifunctionality in neotropical wetlands, we measured key drivers of multifunctionality, such as space, climate (mean annual de temperature and mean annual de precipitation), and aquatic properties (including conductivity, pH and water level). In particular, the mean annual temperature (MAT) and mean annual of precipitation (MAP) were extracted from the WorldClim database (http://www.worldclim.org) at a resolution of 1 km² (30 arcsec). Moreover, conductivity, pH, and water level were all

measured in situ using a portable digital potentiometer (Digimed), and a water level ruler, respectively.

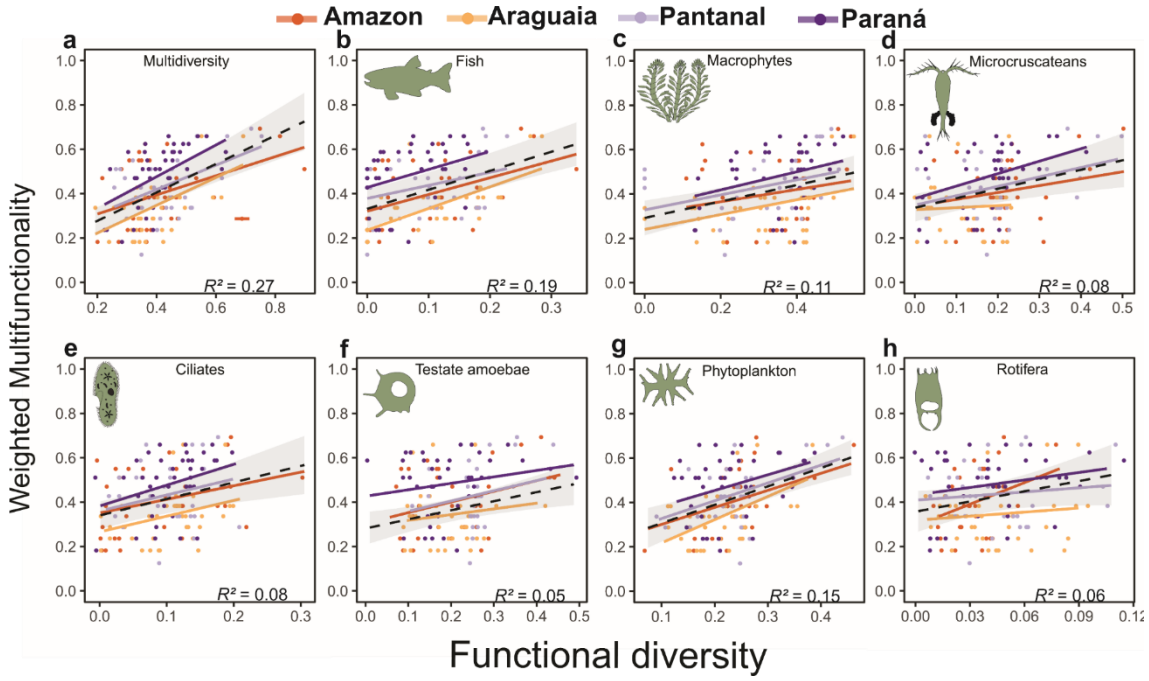


Predictors	Weighted ecosystem multifunctionality						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Species richness							
Multidiversity	0.693	0.502	0.885	0.097	128	7.15	<0.001***
Fish	0.007	0.005	0.010	0.001	128	6.06	<0.001***
Macrophytes	0.014	0.012	0.017	0.001	128	11.1	<0.001***
Microcrustaceans	0.064	0.008	0.120	0.028	128	2.29	0.024*
Ciliates	0.011	0.004	0.018	0.003	128	3.13	0.002**
Testate amoebae	0.004	0.001	0.008	0.001	128	2.64	0.009**
Phytoplankton	0.047	−0.010	0.105	0.029	128	1.62	0.108
Rotifera	0.003	−0.000	0.006	0.001	128	1.66	0.099

P < 0.05*; *P* < 0.01**; *P* < 0.001***

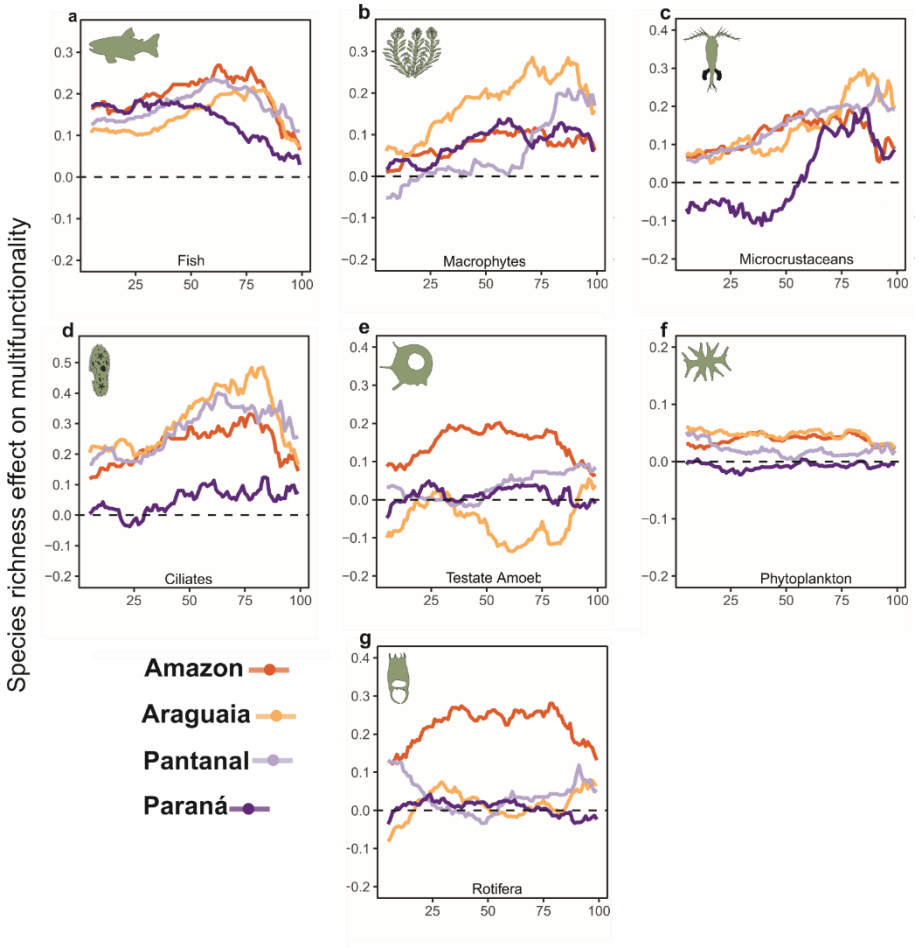
Supplementary Figure 1. The relationship between the species richness of aquatic organisms and weighted multifunctionality in neotropical wetlands**.** The linear link between weighted multifunctionality and the species richness of the seven selected organismal groups; n = 72 lakes. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show 95% confidence interval for the overall trend. R^2 = marginal (i.e., variance of the fixed effects). *P < 0.05, **P < 0.01, ***P

< 0.01. Full model results are in above table. The richness of microcrustacean, testate amoebae, and phytoplankton was log-transformed to improve normality. Multifunctionality is represented by averaging index. This index reflects changes in the average level of the 11 ecosystem functions.



Predictors	Weighted ecosystem multifunctionality						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Functional diversity							
Multidiversity	0.642	0.449	0.835	0.097	128	6.59	<0.001***
Fish	0.857	0.556	1.15	0.146	128	5.88	<0.001***
Macrophytes	0.371	0.195	0.548	0.089	128	4.16	<0.001***
Microcrustaceans	0.427	0.160	0.695	0.135	128	3.16	0.002**
Ciliates	0.744	0.306	1.18	0.222	128	3.36	0.001**
Testate amoebae	0.409	0.142	0.677	0.135	128	3.03	0.003**
Phytoplankton	0.830	0.506	1.15	0.163	128	5.08	<0.001***
Rotifera	1.53	-0.198	3.26	0.874	128.	1.75	0.082

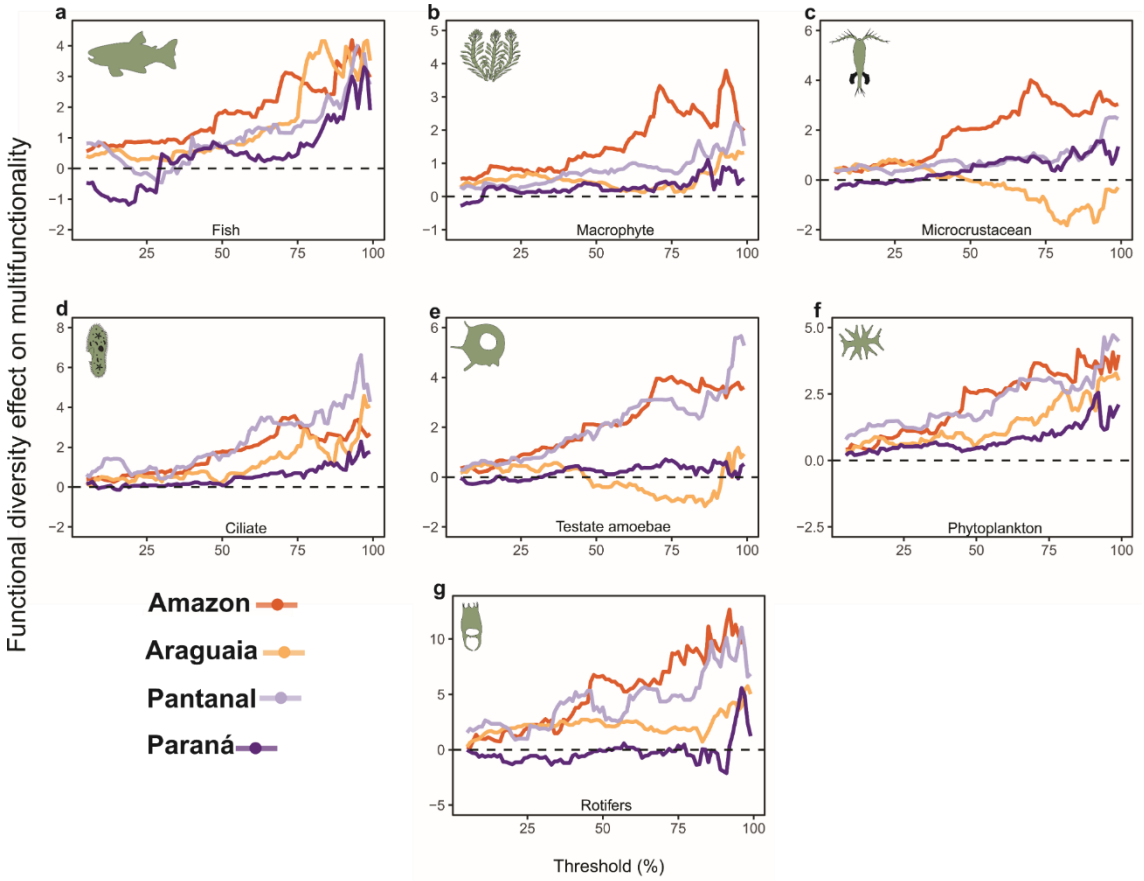
Supplementary Figure 2. The relationship between the functional diversity of aquatic organisms and weighted multifunctionality in neotropical wetlands**.** The linear link between weighted multifunctionality and the functional diversity of the seven selected organismal groups; n = 72 lakes. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show 95% confidence interval for the overall trend. R^2 = marginal (i.e., variance of the fixed effects). *P < 0.05, **P < 0.01, ***P < 0.01. Trends from LMM show that the links between species richness and functional diversity with average multifunctionality are mostly positive for all groups of aquatic organisms and wetlands. Full model results are in above table. Multifunctionality is represented by averaging index. This index reflects changes in the average level of the 11 ecosystem functions.



Organismal groups	Wetland	N_{func}	T_{min}	T_{max}	T_{mde}	R_{mde}
Species richness						
Fish	Amazon	11	–	–	63%	0.26
	Araguaia	11	–	–	78%	0.21
	Pantanal	11	–	–	60%	0.23
	Paraná	11	–	99%	27%	0.18
Macrophytes	Amazon	11	51%	–	72%	0.13
	Araguaia	11	25%	–	71%	0.26
	Pantanal	11	80%	–	87%	0.17
	Paraná	11	39%	–	60%	0.07
Microcrustaceans	Amazon	11	19%	–	79%	0.19
	Araguaia	11	28%	–	85%	0.29
	Pantanal	11	19%	–	91%	0.25
	Paraná	11	66%	–	86%	0.19
Ciliates	Amazon	11	9%	–	77%	0.33
	Araguaia	11	_	–	83%	0.48
	Pantanal	11	_	–	63%	0.40
	Paraná	11	_	–	84%	0.12
Testate amoebae	Amazon	11	_	–	20%	0.20
	Araguaia	11	54%	64%	35%	0.16
	Pantanal	11	91%%	–	92%	0.09
	Paraná	11	_	92%	24%	0.04

Phytoplankton	Amazon	11	–	–	78%	0.05
	Araguaia	11	–	–	5%	0.06
	Pantanal	11	–	16%	5%	0.05
	Paraná	11	–	–	–	–
Rotifera	Amazon	11	–	_	78%	0.28
	Araguaia	11	96%	_	30%	0.10
	Pantanal	11	_	12%	5%	0.13
	Paraná	11	_	_	24%	0.04

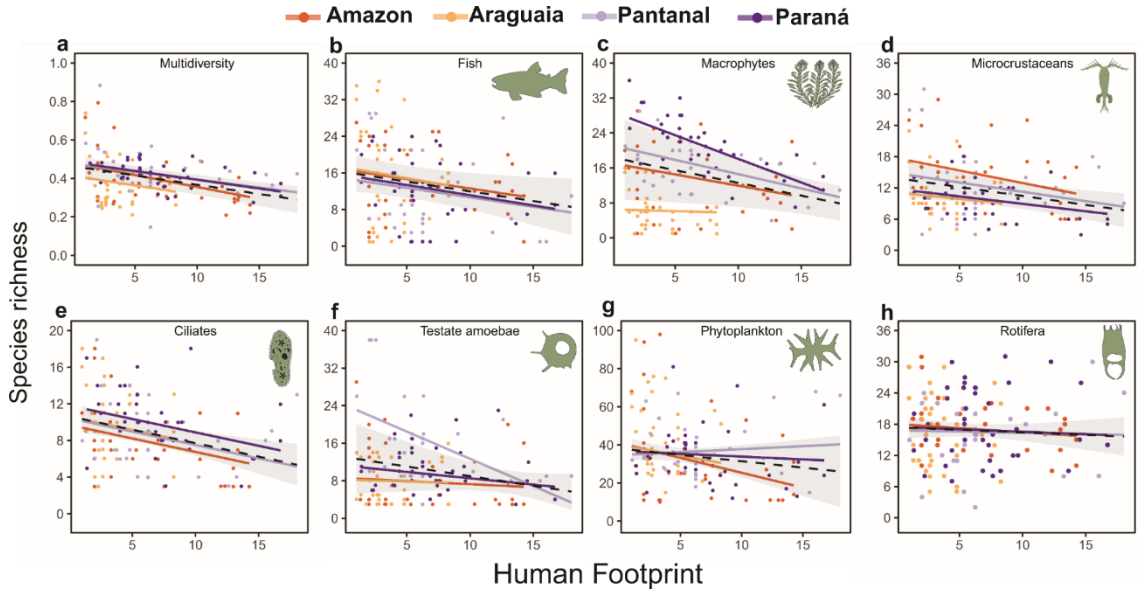
Supplementary Fig 3. The relationship between the species richness of aquatic organisms and multifunctionality using multithreshold approach. The effect of species richness (linear coefficient regressing the number of functions above the full range of thresholds against richness of each organismal group). Effect was plotted to a continuum of thresholds from 1 to 99% of the maximum observed levels of function. Multithreshold metrics are provide and above table represents: T_{min} (i.e., the lowest threshold where species richness begins to have an effect on multifunctionality); T_{max} (i.e., the threshold beyond which the slope first declines and becomes not significantly different from zero); T_{mde} (i.e., threshold where species richness has its strongest positive or negative effect on multifunctionality), and R_{mde} (i.e., the slope where species richness has its strongest positive and/or negative effect on multifunctionality). For a fuller description of the multithreshold approach, see Byrnes et al.[85].



Organismal groups	Wetland	N_{func}	T_{min}	T_{max}	T_{mde}	R_{mde}
Functional diversity						
Fish	Amazon	11	–	–	93%	4.18
	Araguaia	11	60%	–	98%	4.17
	Pantanal	11	–	98%	96%	4.60
	Paraná	11	8%	99%	97%	3.30
Macrophytes						
	Amazon	11	–	–	93%	3.79
	Araguaia	11	–	–	92%	1.42
	Pantanal	11	6%	–	96%	2.23
	Paraná	11	18%	88%	87%	1.10
Microcrustaceans	Amazon	11	37%	–	70%	4.00
	Araguaia	11	–	–	–	–
	Pantanal	11	12%	–	98%	2.52
	Paraná	11	50%	–	94%	1.58
Ciliates	Amazon	11	–	–	74%	3.56
	Araguaia	11	68%	–	97%	4.58
	Pantanal	11	9%	–	96%	6.61
	Paraná	11	68%	97%	96%	2.29
Testate amoebae	Amazon	11	5%	–	75%	4.02
	Araguaia	11	–	–	97%	1.18
	Pantanal	11	39%	–	98%	5.66
	Paraná	11	40%	77%	73%	0.71
	Amazon	11	23%	–	85%	4.17

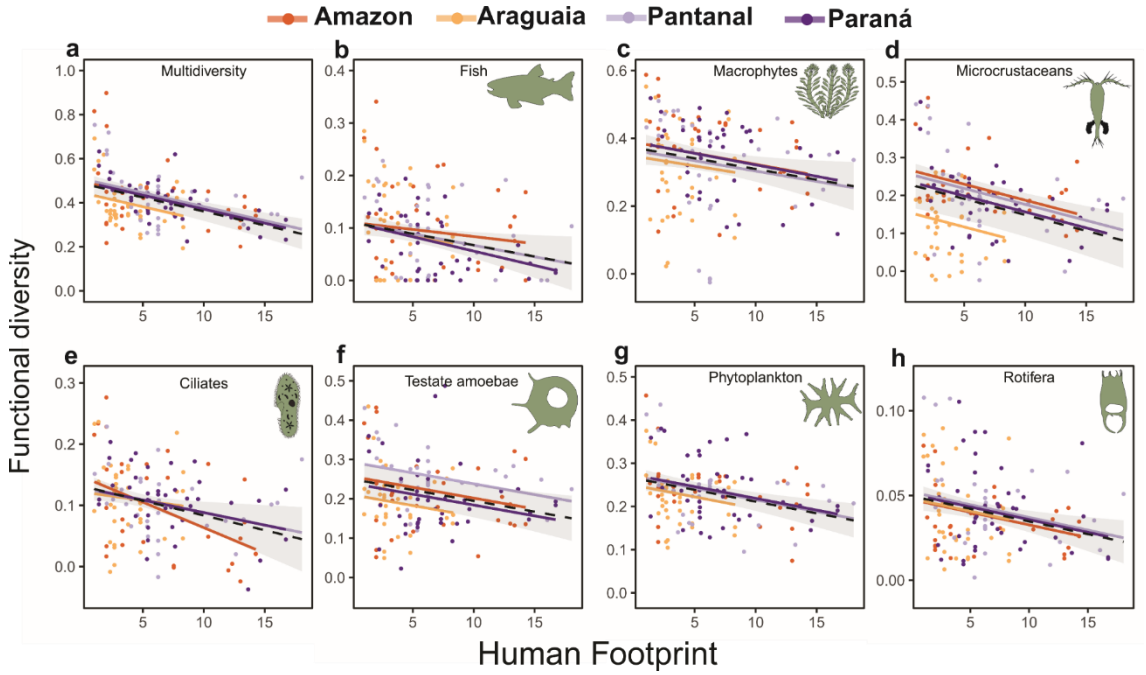
	Araguaia	11	61%	–	98%	3.28
Phytoplankton	Pantanal	11	–	–	97%	4.71
	Paraná	11	23%	–	92%	2.55
	Amazon	11	96%	–	99%	16.63
Rotifera	Araguaia	11	11%	–	98%	5.63
	Pantanal	11	10%	97%	96%	11.03
	Paraná	11	–	–	96%	5.58

Supplementary Fig 4. The relationship between the functional diversity of aquatic organisms and multifunctionality using multithreshold approach. The effect of functional diversity (linear coefficient regressing the number of functions above the full range of thresholds against functional diversity of each organismal group). Effect was plotted to a continuum of thresholds from 1 to 99% of the maximum observed levels of function. Multithreshold metrics are provide and above table represents: T_{min} (i.e., the lowest threshold where functional diversity begins to have an effect on multifunctionality); T_{max} (i.e., the threshold beyond which the slope first declines and becomes not significantly different from zero); T_{mde} (i.e., threshold where functional diversity has its strongest positive or negative effect on multifunctionality), and R_{mde} (i.e., the slope where functional diversity has its strongest positive and/or negative effect on multifunctionality). For a fuller description of the multithreshold approach, see[85].



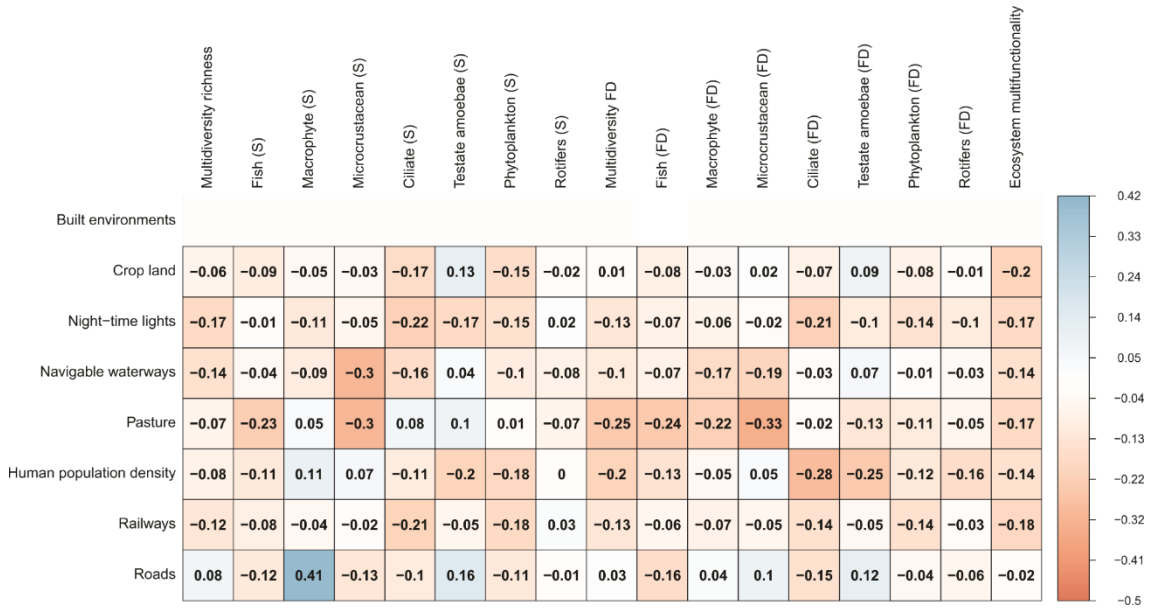
Response	Human Footprint						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Species richness							
Multidiversity	–0.009	–0.013	–0.005	0.002	132	–4.54	<0.001***
Fish	–0.423	–0.812	–0.034	0.197	132	–2.15	0.033*
Macrophytes	–0.583	–1.07	–0.094	0.247	132	–2.36	0.020*
Microcrustaceans	–0.342	–0.598	–0.086	0.129	132	–2.65	0.009**
Ciliates	–0.293	–0.451	–0.136	0.079	132	–3.68	<0.001***
Testate amoebae	–0.410	–0.987	0.167	0.292	132	–1.40	0.162
Phytoplankton	–0.688	–2.11	0.731	0.717	132	–0.959	0.339
Rotifera	–0.099	–0.392	0.194	0.148	132	0.670	0.504

Supplementary Fig 5. Effect of Human Footprint (HFP) on the species richness of seven organismal groups in Neotropical wetlands. a-g, Relationships between HFP and species richness of (a) fish, (b) macrophytes, (c) microcrustaceans, (d) ciliates, (e) testate amoebae, (f) phytoplankton, and (g) rotifera. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show 95% confidence interval for the overall trend.

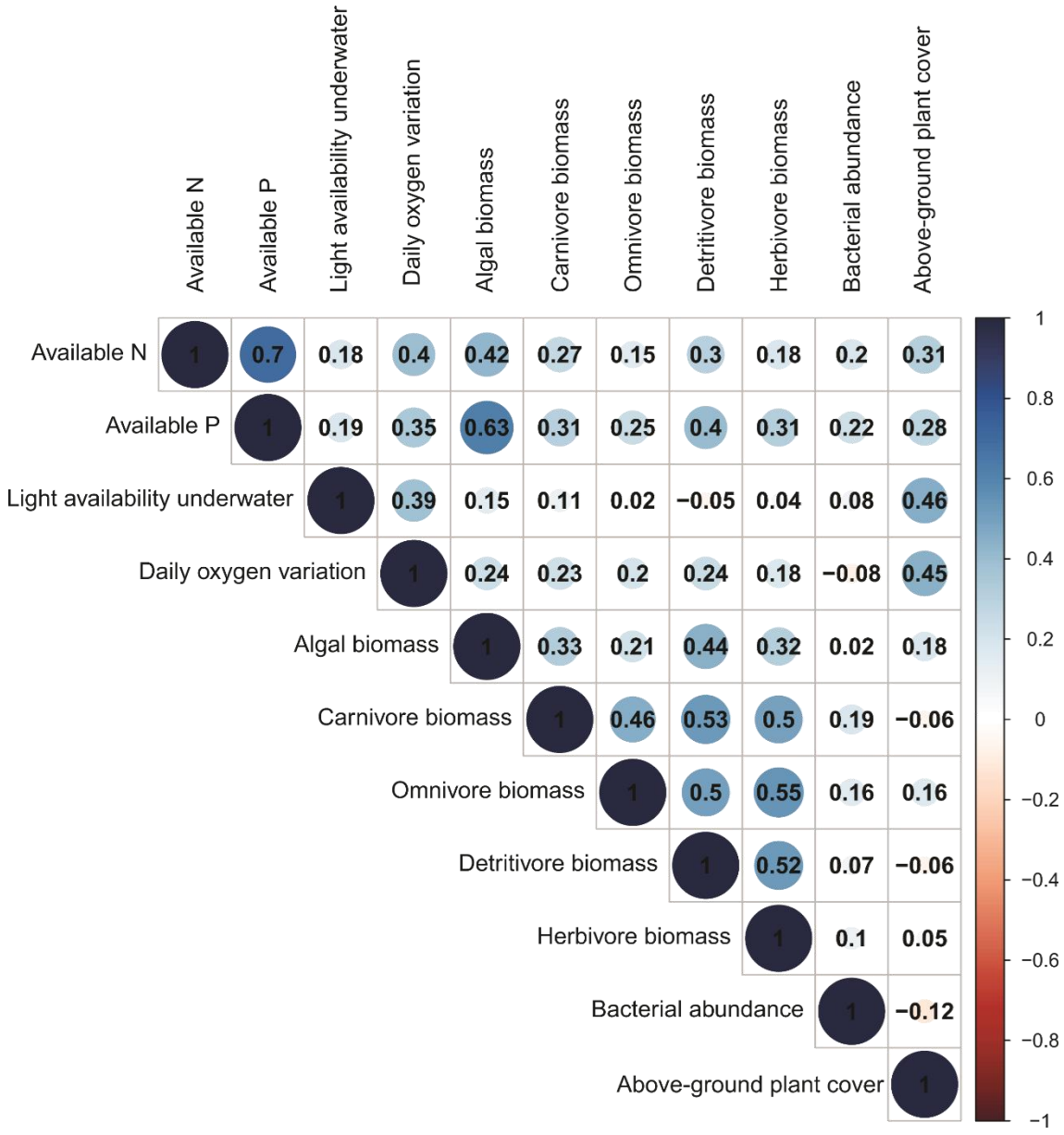


Response	Human Footprint						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Functional diversity							
Multidiversity	–0.012	–0.017	–0.007	0.002	132	–4.54	<0.001***
Fish	–0.004	–0.007	–0.0007	0.001	132	–2.40	0.018*
Macrophytes	–0.006	–0.011	–0.0008	0.002	132	–2.30	0.023*
Microcrustaceans	–0.008	–0.012	–0.004	0.001	132	–4.30	<0.001***
Ciliates	–0.004	–0.008	–0.0009	0.001	132	–2.46	0.015*
Testate amoebae	–0.005	–0.008	–0.002	0.001	132	–3.17	0.002**
Phytoplankton	–0.005	–0.008	–0.002	0.001	132	–3.74	<0.001***
Rotifera	–0.001	–0.002	–0.0005	0.0004	132	–3.09	0.002**

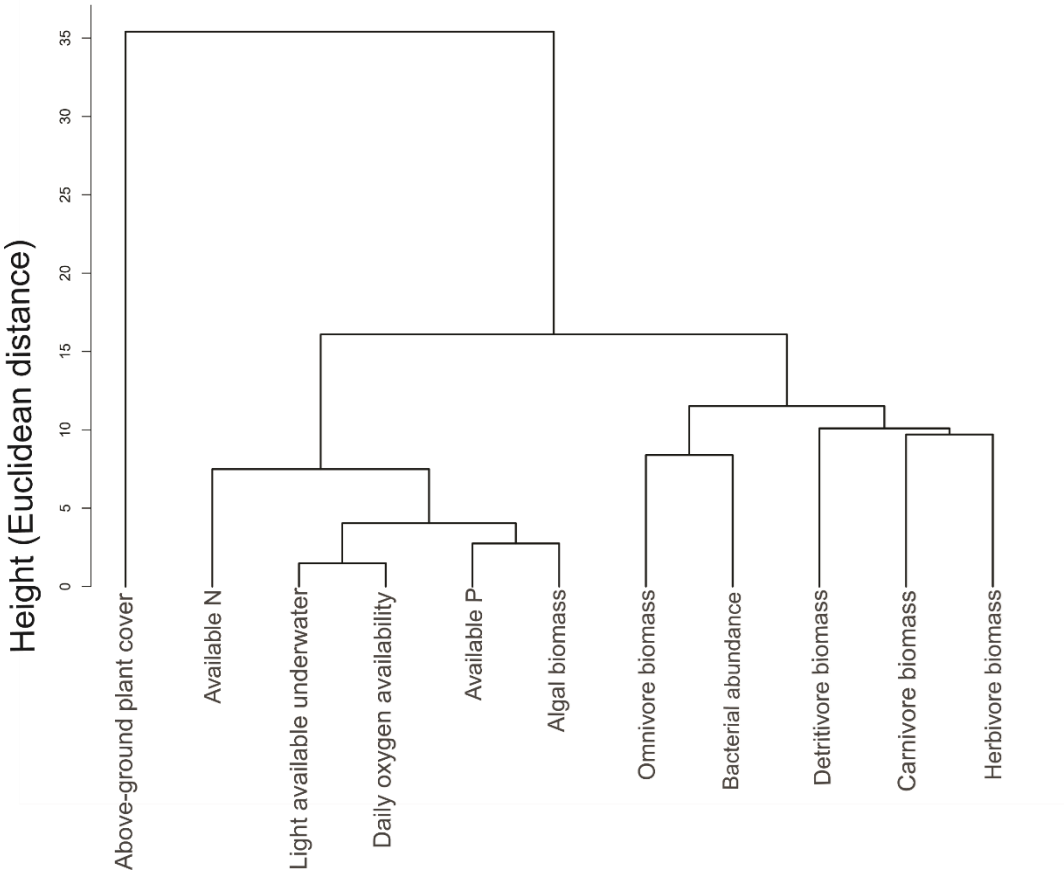
Supplementary Fig 6. Effect of Human Footprint (HFP) on the multidiversity functional and functional diversity of each of the seven organismal groups in neotropical wetlands. a-g, Relationships between HFP and functional diversity of (b) multidiversity, and functional diversity of (b) fish, (c) macrophyte, (d) microcrustacean, (e) ciliates, (f) testate amoebae, (g) phytoplankton, and (h) rotifera. Statistical analysis was performed using linear mixed-effect models. Dashed black and solid lines are predicted (fitted) values from LMMs for overall and local trends (for each wetland ecosystem), respectively. Shaded areas show 95% confidence interval for the overall trend.



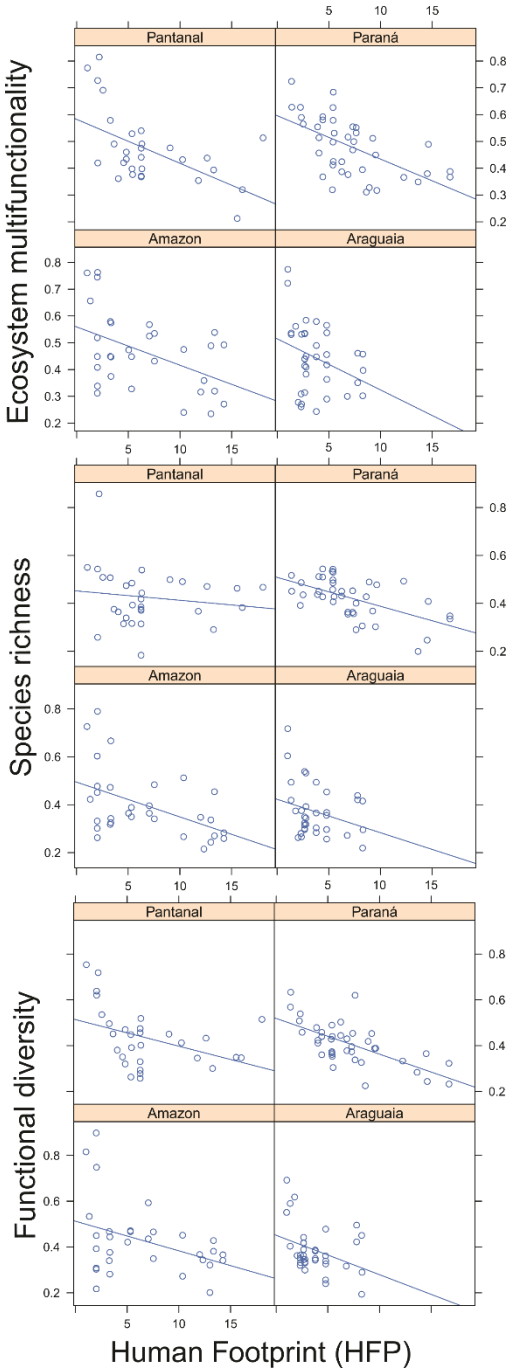
Supplementary Fig 7. Spearman correlations between the individual human pressures with species richness (S), functional diversity (FD) of individual groups of aquatic organisms, multidiversity, and ecosystem multifunctionality. Note that most correlations are negative (varying -0.5 ~ +0.2), suggesting that as these human pressures increase, biodiversity and multifunctionality decrease. Moreover, no correlations with "human built environments" were made because this pressure was barely present in our data (most of the data were 0).



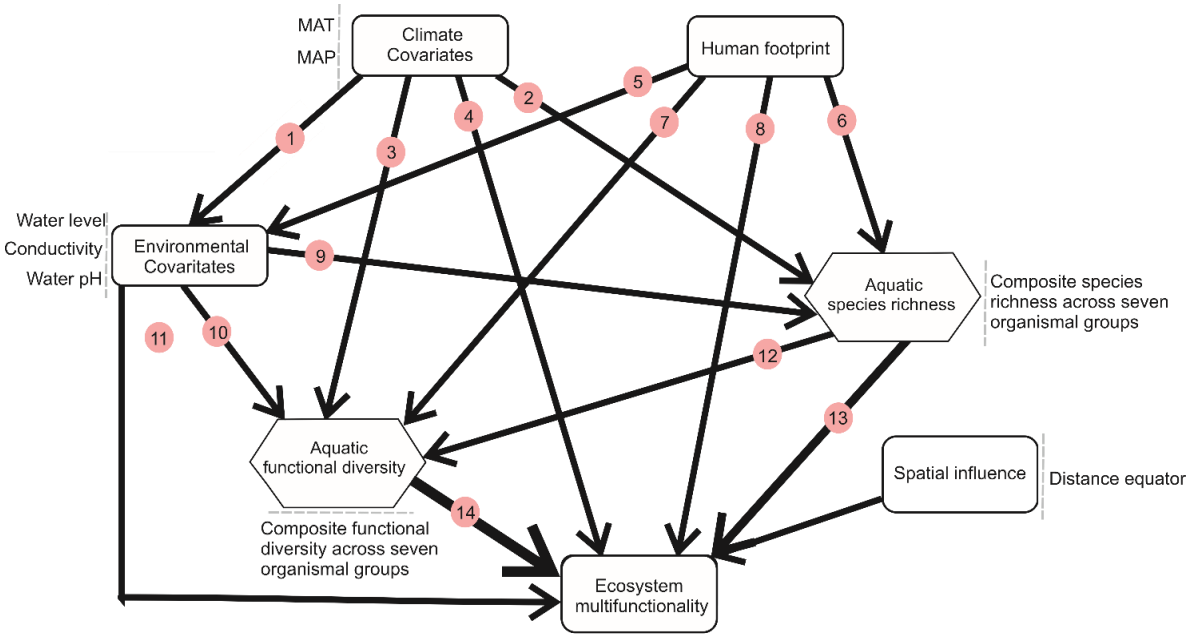
Supplementary Figure 8. Correlation matrix (Pearson) ecosystem variables used to create the multifunctionality.



Supplementary Figure 9. Dendrogram of ecosystem functions showing clusters. Variables within same cluster received similar weight following Manning et al. (2018). Weighted multifunctionality is calculated as the average of all these functions. Note that there are three clear clusters: (1) with an aboveground plant cover, (2) with available N and P, light availability underwater, daily oxygen variation, and algal biomass, and (3) with carnivore biomass, omnivore biomass, detritivore biomass, omnivore biomass, and bacterial abundance. The high value of aboveground plant cover in cluster 1 indicate lakes with high habitat heterogeneity, whereas high values of all variables in cluster 2 indicate lakes highly productive (high nutrient concentrations, resource availability and primary production). Finally, high values of all variables in cluster 2 illustrate lakes with high biomass production. Specifically, weighted multifunctionality was calculated as the average of all variables within in each cluster. For instance, the functions within cluster 2 (i.e., nitrogen and phosphorus available, light availability underwater, daily variation in dissolved oxygen underwater, and algae biomass) were weighted with weight of 0.2. These functions were then averaged into a standardized variable. Similarly, the variables within cluster 3 (i.e., carnivore biomass, omnivore biomass, detritivore biomass, herbivore biomass, and bacterial abundance) also were weighted with weight of 0.2 and subsequently averaged. Finally, the above-ground plant cover was assigned with weight 1.



Supplementary Figure 10. The slopes of each of the relationships between the responses (species richness, functional diversity, and ecosystem multifunctionality) and the covariate (HFP) appear similar among wetlands; thereby there is no evidence to range disparity or heterogeneity of slopes.



#	Links	*Rationale*	Ref.
1	**Climate → Environment**	It is well known that climate affects the environmental conditions in wetlands. For example, precipitation is expected to control water level fluctuations in lakes and with increasing precipitation there is an increase in water level.	86, 87
2	**Climate →Specie.rich**	Climate is also a key driver of species richness across multiple taxonomic groups. For example, precipitation has been reported to be positively related to the species richness of fish, macrophytes, zooplankton, and protists. Likewise, temperature is often positively related to the species richness of multiple aquatic organismal groups.	88–91
3	**Climate → Func.Div**	Climate is expected to control the functional traits of multiple aquatic organisms. Consequently, climate will affect the functional multidiversity of wetlands. For example, increased in temperatures may drive a decrease in the body size of aquatic organisms. Moreover, temperature and precipitation are key drivers that affect, positively or negatively, traits related to resource-acquisition and mobility.	88, 92–94
4	**Climate → EMF**	Climate is a key driver of ecosystem multifunctionality. For example, temperature and precipitation are expected to be positively related to ecosystem multifunctionality	83, 92.
5	**HFP → Environment**	Human pressures may control environmental conditions in aquatic ecosystems. For example, human activities such as agriculture, cities, and damming are expected to be negatively related to water level fluctuations in aquatic ecosystems.	95–97

6	**HFP →Specie.rich**	Human pressures are considered to be major drivers of global taxonomic biodiversity decline. Human activities are expected to decrease species richness across multiple taxonomic groups	98–102
7	**HFP → Func.Div**	Likewise, human pressures are key global drivers of functional biodiversity decline. Human activities are expected to decrease functional diversity across multiple taxonomic groups	56, 96, 100, 103, 104
8	**HFP → EMF**	Human pressures may also control ecosystem multifunctionality of aquatic ecosystems. For example, human activities are expected to decrease ecosystem multifunctionality mediated by a decline of the species richness and functional diversity across multiple taxonomic groups	100
9	**Environment → Specie.rich**	Environmental variables are known to affect the species richness of multiple organismal groups in aquatic ecosystems. For example, water level fluctuation in natural aquatic systems is expected to have a strong positive effect on species richness of fish, macrophytes, microcrustaceans, etc.	22, 105,106
10	**Environment → Func.Div**	Environmental variables are known to be largely associated with functional diversity of aquatic organisms. For example, water level fluctuations may affect specific traits related to mobility and resource-acquisition. This occurs because water level fluctuations control the connection and resources availability within wetlands.	56, 107, 108
11	**Environment → EMF**	Environmental variables may control multifunctionality in aquatic ecosystems. For example, water level fluctuations exercise strong negative or positive effects on multiple ecosystem functions, such as nutrient cycling, primary productivity, and decomposition, both directly and indirectly via impacts on aquatic biodiversity.	109, 110
12	**Specie.rich→ Func.Div**	Species richness and functional attributes are known to be strongly associated with each other. In communities with low functional redundancy, an increase in the number of species will lead to an increase in trait diversity.	104, 111
13	**Specie.rich→ EMF**	Species richness also controls wetlands functioning. Lakes with high standing biomass, nutrient cycling, and ecosystem metabolism often have a high species richness of multiple aquatic organisms. Aquatic organisms may support ecosystem multifunctionality through multiple pathways. For example, species richness of animals (fish, microcrustaceans, rotifers, and protists) may provide large amounts of feces, carcasses, biomass, and resuspended nutrients from the sediment, which favors primary productivity and standing biomass. Similarly, macrophyte	40, 111, 112

		richness controls nutrient recycling, primary production, animal biomass, and light availability of lakes.	
14	**Func.Div → EMF**	Finally, functional diversity is expected to be a key driver of ecosystem multifunctionality. Functional diversity may promote multifunctionality via either co-occurring species with contrasting traits that increase the overall resource pool utilization or the presence of species with key traits that enhance ecosystem functioning. Consequently, functional diversity is expected to be strongly related to ecosystem multifunctionality.	35, 100, 111, 113

Supplementary Figure 11. A priori structural equation modeling (SEM) aimed to evaluate how the human pressure (human footprint [HFP]) affects direct and biodiversity-mediated multifunctionality in wetlands. Also, we account for effects of key ecosystem factors spatial influence (latitude of the lakes), lake variables: water level, conductivity, and pH and climate: *in situ* temperature and mean annual precipitation [MAP]) drivers in the models. All variables in the model were measured at lake level, and represent the real conditions of the lake environments. Different categories of predictors were grouped into the same box in the model for graphical simplicity. However, they were considered individually in the model. From this prior model, we performed model selection to remove predictors unimportant for diversity and multifunctionality and retained only predictors with significant effects (see Supplementary Table 5). In the table, we provide the conceptual support for all the links within the model based on results from other studies. Therefore, all relationships within our model occur in nature and are not spurious. Aquatic species richness and functional diversity represented by a hexagon were obtained through a composite variable.

Supplementary Table 1: Functional traits used for the classification of all taxonomic groups including types of traits, their categories, and ecological importance.

Biological Group	Trait	Type	Category	Trait importance	References
Phytoplankton	Body size	Continuous	Average length (µm)	Dispersal ability, secondary productivity, and energy transfer	47, 48, 49
	Cell organization	Categorical	Coenobium Colony Chains Filament Unicellular	Resource acquisition and predation avoidance	47, 49
	Pigment color	Categorical	Green Brown	Resource acquisition	47, 49
	Mixotrophy	Categorical	Mixotrophic Non-mixotrophic	Resource acquisition	47, 49
	Cell motility	Categorical	Absence Aerotopes Flagellated	Resource acquisition and predation avoidance	47, 49
	Presence of silica	Binary	Presence Absence	Predation avoidance	47, 49
Ciliates	Body size	Continuous	Average length (µm)	Dispersal ability, secondary productivity, and energy transfer	19
	Feeding type	Categorical	Nano/Micro-interceptors Nano-filterers Pico/Nano-filterers Pico-filterers	Foraging strategy; detecting and capturing prey	50
	Life mode	Categorical	Benthic	Habitat use and nutrient cycling	19

			Planktonic Periphitic Epibiontic		
	Encystment ability	Binary	Presence Absence	Resistance and resilience to disturbances	19
Testate amoebae	Body size	Continuous	Average length (µm)	Dispersal ability, secondary productivity, and energy transfer	51, 52
	Gas vacuole	Binary	Presence Absence	Ability to actively float	53, 54
	Shell compression	Binary	Presence Absence	Minimizes resistance to water and flotation	51, 52
	Shell constitution	Categorical	Agglutinated Protein Siliceous	Adaptations to the environment	54, 55
Rotifers	Body size	Continuous	Average length (µm)	Dispersal ability secondary productivity, and energy transfer	24, 56
	Life mode	Categorical	Littoral Pelagic	Habitat use and nutrient cycling	24, 56
	Feeding type	Categorical	Filtration Sugador Predator	Foraging strategy; detecting and capturing prey	56, 57
	Lorica	Binary	Presence Absence	Defense against predation	24
Microcrustaceans	Body size	Continuous	Average length (µm)	Dispersal ability, secondary productivity, and energy transfer	48, 56, 58
	Life mode	Categorical	Littoral Pelagic	Habitat use and nutrient cycling	59, 60
	Feeding type	Categorical	Daph-filterers Sidi-filterers	Foraging strategy; detecting and capturing prey	30, 61, 62,

			Cop-filterers Scraper Raptorial		
	Trophic group	Categorical	Herbivorous Herb-Detritivorous Omnivorous	Resource sharing	63
	Predatory escape response	Categorical	Low Medium Big Maximum	Ability to repel predators; swimming agility, body shape and size, and visibility of a predator.	64
Fish	Body size	Continuous	Average length (mm)	Influence on dispersal ability, secondary productivity, and	65, 66
	Life mode	Categorical	Benthopelagic Pelagic Demersal	Habitat use	66
	Trophic group	Categorical	Benthivorous Omnivorous Piscivorous Invertivorous Planktivorous Detritivorous Herbivorous	Feeding and resource sharing	66, 67
	Trophic level	Continuous	Numeric variable	Feeding and resource sharing	66, 67, 68
	Migration	Binary	Presence Absence	Habitat use; long reproductive migrations (over 100 km)	69, 70
	Parental Care	Binary	Presence Absence	Energy investment, breeding success	69, 71

Macrophytes	Body size	Continuous	Average length (cm)	Dispersal ability, secondary productivity, and energy transfer	10–14
	Growth form	Categorical	Emergent Free-floating Rooted submerged Free submerged Submerged fixed Submerged fixed/emergent	Habitat use	10–14
	Propagule unit	Categorical	Epiphytic Fragment Fragment/ Epiphytic Fragment/ Epiphytic /Bulbe Nuculas Seed Seed/Seedling Seed/Fragment Seed/ Epiphytic Seed/Rhizome Spore Spore/ Epiphytic Stolon Stolon/Seedling Stolon/Epiphytic Spikes Spikes/Nuculas Rhizome	Dispersal ability, establishment success, and growth	10–14

Dispersion mode	Categorical	Autochory Autochory/Hydrochory Autochory/Zoochory Autochory/Hydrochory/Zoochory Hydrochory Hydrochory/Zoochory Zoochory	Dispersal ability	10–14
Seasonality	Categorical	Annual Annual/Perennial Perennial	Persistence and resource sharing	10–14

Supplementary Table 2: Theoretical information on all 11 functions and their importance.

Ecosystem functions	**Importance**
Nutrient cycling	
In situ N availability	Total phosphorus and nitrogen reflect all nutrient fractions underwater, and their availability often limit primary producers, and consequently primary production in aquatic ecosystems[72]
In situ P availability	
Ecosystem metabolism	
Daily variation in O_2 underwater	Daily variation in dissolved oxygen represents the balance between the processes of production and respiration, thereby reflecting the metabolic regime underwater[73]
Multitrophic standing biomass	
Algae	Multitrophic standing biomass is one of the most commonly used metrics of ecosystem function, and serves as a useful proxy for functions including energy flux and nutrient cycling[74-76]. For instance, algae are the main primary producer in aquatic systems, and their standing biomass provide the base resource for aquatic food web[77].
Carnivore biomass	
Omnivore biomass	
Herbivore biomass	
Detritivore biomass	
Photosynthetically active radiation	
Variation in light availability underwater	Light availability is the main driver of primary producers[78], and often limit primary production in aquatic ecosystems[79].
Microorganism abundance	
Bacterial abundance	Bacteria are among the most abundance microorganisms in aquatic ecosystems and play key roles in affecting nutrient cycling, primary production (via inorganic carbon fixation;[80], litter decomposition, and climate regulation[81-83].
Variation in habitat complexity underwater	
Variation in plant aboveground cover	Variation in above-ground plant cover increase ecosystem complexity, affecting predator-prey interactions and the energy flux of food webs[84]

Supplementary Table 3. Results of linear mixed-effects model showing effects of species richness and functional diversity of seven groups of aquatic organisms, and multidiversity (joint diversity of the seven organismal groups; standardized between 0 and 1) on ecosystem multifunctionality (n = 137). These results reflect the pattern observed in Fig. 2 and 3 of the main manuscript.

Predictors	**Ecosystem multifunctionality**						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Species richness							
Multidiversity	0.711	0.566	0.856	0.073	128	9.70	<0.001***
Fish	0.011	0.009	0.013	0.001	128	10.1	<0.001***
Macrophytes	0.012	0.007	0.017	0.002	128	4.53	<0.001***
Microcrustaceans	0.060	0.018	0.102	0.021	128	2.87	0.005** log relation
Ciliates	0.012	0.006	0.019	0.003	128	3.72	0.0001***
Testate amoebae	0.034	–0.013	0.081	0.023	128	1.43	0.154 log relation
Phytoplankton	0.075	0.001	0.150	0.037	128	2.03	0.045* log relation
Rotifers	0.004	–0.001	0.010	0.002	128	1.63	0.106
Functional diversity							
Multidiversity	0.721	0.594	0.848	0.064	128	11.2	<0.001***
Fish	1.15	0.944	1.36	0.106	128	10.9	<0.001***
Macrophytes	0.402	0.258	0.547	0.073	128	5.52	<0.001***
Microcrustaceans	0.487	0.289	0.685	0.099	128	4.88	<0.001***
Ciliates	0.834	0.500	1.17	0.169	128	4.94	<0.001***
Testate amoebae	0.560	0.198	0.922	0.183	128	3.06	0.003**
Phytoplankton	0.784	0.410	1.16	0.189	128	4.15	<0.001***
Rotifers	1.87	0.077	3.67	0.907	128	2.06	0.041*

$P < 0.05$*; $P < 0.01$**; $P < 0.001$***

Supplementary Table 4. Model averaging procedure (using AIC) showing the best set of predictors explaining ecosystem multifunctionality in neotropical wetlands. Full models account for effects of species richness and functional diversity of single organismal groups and multidiversity, as well as effects of other well-known drivers of multifunctionality such as space (distance from equator), climate (MAP and MAT), and aquatic properties (conductivity, pH, and water level). We performed a backward selection to each model of diversity organismal. Importantly, all model selection accounted for the influence of biodiversity on multifunctionality. This indicates that biodiversity is a strong driver of multifunctionality in neotropical wetlands even after account to other ecosystem drivers. MAP = mean annual of precipitation; MAT = mean annual de temperature.

	Ecosystem multifunctionality			
Models	df	AICc	ΔAIC	weight
Species richness models				
(i) Multidiversity				
multidiversity + MAP + water level	10	–249.5	0	0.420
multidiversity + MAP + MAT + water level	11	–249.5	0.05	0.408
multidiversity + MAP + MAT + water level + conductivity	12	–247.7	1.79	0.172
(ii) Fish				
fish rich + MAP + pH	10	–304.6	0	0.269
fish rich + MAP + pH + conductivity	11	–303.9	0.68	0.191
fish rich + MAP + MAT + pH	11	–303.9	0.76	0.184
fish rich + MAP + MAT + pH + conductivity	12	–303.1	1.57	0.123
fish rich + MAP + conductivity	10	–303.1	1.57	0.123
(iii) Macrophyte				
macrophyte rich + MAP + water level	10	–231.8	0	1
(iv) Microcrustacean (log)				
microcrustacean rich + MAP + water level	10	–190.8	0	0.325
microcrustacean rich + MAP + MAT + water level	11	–190.0	0.85	0.213
microcrustacean rich + MAP + pH + water level	11	–189.6	1.21	0.177
microcrustacean rich + MAP + conductivity + water level	11	–189.5	1.36	0.165
MAP + water level	9	–188.8	1.99	0.120
(v) Ciliates				

ciliates rich + MAP + water level	10	–203.4	0	0.436
ciliates rich + MAP + MAT + water level	11	–203.0	0.45	0.349
ciliates rich + MAP + pH + water level	11	–202.0	1.41	0.215
(vi) Testate amoebae (log)				
water level + MAP	9	–190.1	0	0.248
water level + MAP + MAT	10	–189.2	0.85	0.162
water level + MAP + conductivity	10	–189.1	1.01	0.149
testate rich + MAP + MAT + pH + water level	11	–189.0	1.05	0.147
testate rich + MAP + MAT + pH + conductivity + water level	12	–188.4	1.65	0.109
(vii) Phytoplankton (log)		-		
phytoplankton rich + MAP + MAT + water level	11	–192.1	0	0.152
phytoplankton rich + MAP + water level	10	–191.9	0.19	0.139
MAP + MAT + water level	10	–191.4	0.73	0.106
MAP + water level	9	–191.2	0.89	0.098
phytoplankton rich + MAP + conductivity + water level	11	–191.0	1.12	0.087
(viii) Rotifera				
rotifera rich + MAP + water level	10	–190.1	0	0.198
rotifera rich + MAP + conductivity + water level	11	–189.8	0.35	0.166
rotifera rich + MAP + MAT + water level	11	–189.4	0.75	0.136
MAP + water level	9	–189.0	1.18	0.110
MAP + conductivity + water level	10	–188.8	1.28	0.104

Functional diversity models				
(i) Multidiversity				
multidiversity + water level	9	–270.4	0	0.362
multidiversity + MAT + water level	10	–269.0	1.37	0.183
multidiversity + pH + water level	10	–268.8	1.63	0.161
multidiversity + conductivity + water level	10	–268.8	1.65	0.158

multidiversity + MAP + water level	10	–268.4	1.96	0.136
(ii) Fish FD				
fish FD + pH	9	–262.2	0	0.152
fish FD + MAT + pH	10	–261.8	0.46	0.121
fish FD + MAP + MAT + pH	11	–261.5	0.73	0.105
fish FD + pH + conductivity	10	–260.9	1.33	0.078
fish FD + MAP + conductivity + water level	11	–260.9	1.37	0.076
(iii) Macrophyte FD				
macrophyte FD + MAP + water level	10	–208.9	0	0.305
macrophyte FD + MAP	9	–207.8	1.11	0.176
macrophyte FD + MAP + MAT + water level	11	–207.6	1.31	0.158
macrophyte FD + MAP + pH + water level	11	–207.3	1.65	0.134
macrophyte FD + MAP + MAT	10	–206.9	1.97	0.114
(iv) Microcrustacean FD				
microcrustacean FD + MAP + water level	10	–202.2	0	0.428
microcrustacean FD + MAP + MAT + water level	11	–201.0	1.17	0.238
microcrustacean FD + MAP + pH + water level	11	–200.4	1.81	0.174
microcrustacean FD + Distance equator + MAP + water level	11	–200.2	1.97	0.160
(v) Ciliates FD				
ciliates FD + MAP + MAT + water level	11	–205.1	0	0.484
ciliates FD + MAP + water level	10	–204.3	0.76	0.332
ciliates FD + MAP + MAT + Ph + water level	12	–203.1	1.93	0.184
(vi) Testate amoebae FD				
testate FD + MAP + conductivity + water level	11	–201.6	0	0.308
testate FD + MAP + water level	10	–201.3	0.23	0.275
testate FD + MAP + pH + water level	11	–200.2	1.35	0.157
testate FD + MAP + MAT + water level	11	–199.9	1.65	0.135
testate FD + MAP + MAT + conductivity + water level	12	–199.8	1.79	0.126

(vii) Phytoplankton FD				
phytoplankton FD + MAP + conductivity + water level	11	–211.5	0	0.284
phytoplankton FD + MAP + water level	10	–211.0	0.51	0.220
phytoplankton FD + MAP + MAT + conductivity + water level	12	–210.7	0.77	0.193
phytoplankton FD + MAP + MAT + water level	11	–210.7	0.78	0.192
phytoplankton FD + MAP + pH + water level	11	–209.6	1.86	0.112
(viii) Rotifera FD				
rotifera FD + MAP + MAT + water level	11	–197.9	0	0.158
rotifera FD + MAP + water level	10	–197.7	0.22	0.141
rotifera FD + MAP + MAT + conductivity + water level	12	–197.5	0.39	0.129
rotifera FD + MAP + conductivity + water level	11	–197.2	0.74	0.109
rotifera FD + MAP + MAT + pH + conductivity + water level	12	–196.8	1.11	0.090

Supplementary Table 5. Results of linear mixed-effects model showing effects of species richness of aquatic organisms on 11 single ecosystem functions (n = 137; above-ground cover, algae biomass, bacterial abundance, carnivorous biomass, detritivorous biomass, herbivorous biomass, light availability under-water, nitrogen available, omnivorous biomass, daily oxygen variation, and phosphorus available). These results reflect the pattern observed in Supplementary Fig. 5. Ecosystem functions were scaled to interpret parameter estimates on a comparable scale. For some BEF relationships an exponential term was added. P < 0.05*; P < 0.01**; P < 0.001***.

Ecosystem functions	**Species richness**						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Multidiversity effect							
Above-ground cover	3.19	1.72	4.65	0.740	128	4.31	<0.001***
Algae biomass	4.82	2.00	7.63	1.42	128	3.39	0.001**
Bacterial abundance	0.273	–1.18	1.72	0.732	128	0.373	0.710
Carnivorous biomass	2.63	1.28	3.98	0.682	128	3.86	0.0001***
Detritivorous biomass	3.09	1.61	4.58	0.752	128	4.11	<0.001***
Herbivorous biomass	4.42	3.04	5.80	0.696	128	6.35	<0.001***
Light availability	2.97	1.57	4.38	0.711	128	4.18	<0.001***
Nitrogen available	2.87	1.23	4.52	0.831	127	3.46	0.001**
Omnivorous biomass	2.73	0.529	4.92	1.11	128	2.46	0.015*
Daily oxygen variation	2.32	0.445	4.19	0.947	128	2.45	0.016*
Phosphorus available	3.77	2.58	4.96	0.603	128	6.25	<0.001***
Fish effect							
Above-ground cover	0.018	–0.003	0.040	0.011	128	1.65	0.100
Algae biomass	0.023	0.009	0.037	0.007	128	3.24	0.002**
Bacterial abundance	0.015	–0.003	0.034	0.009	128	1.60	0.112
Carnivorous biomass	0.080	0.066	0.094	0.006	128	11.5	0.0001***
Detritivorous biomass	0.078	0.054	0.102	0.011	128	6.54	<0.001***
Herbivorous biomass	0.086	0.072	0.100	0.006	128	12.3	<0.001***
Light availability	0.026	–0.001	0.054	0.014	128	1.84	0.068
Nitrogen available	1.33	–0.316	2.97	0.831	127	1.60	0.112
Omnivorous biomass	0.094	0.070	0.118	0.011	128	7.95	<0.001***

Daily oxygen variation	0.029	–0.003	0.062	0.016	128	1.78	0.078
Phosphorus available	0.026	0.009	0.043	0.008	128	3.09	0.002**
Macrophyte effect							
Above-ground cover	0.109	0.087	0.130	0.010	128	10.2	<0.001***
Algae biomass	0.050	0.028	0.072	0.010	128	4.63	<0.001***
Bacterial abundance	–0.000	–0.033	0.033	0.016	128	–0.005	0.996
Carnivorous biomass	0.023	–0.001	0.047	0.012	128	1.89	0.060
Detritivorous biomass	0.023	0.001	0.045	0.011	128	2.12	0.036*
Herbivorous biomass	0.047	0.022	0.073	0.012	128	3.70	<0.001***
Light availability	0.097	0.065	0.130	0.016	128	6.07	<0.001***
Nitrogen available	0.0521	0.028	0.075	0.011	128	4.45	<0.001***
Omnivorous biomass	0.023	-0.015	0.063	0.020	128	1.19	0.235
Daily oxygen variation	0.062	0.005	0.118	0.028	128	2.19	0.030*
Phosphorus available	0.061	0.030	0.092	0.015	128	3.90	<0.001***
Microcrustacean effect							
Above-ground cover	0.044	0.010	0.078	0.017	128	2.58	0.011*
Algae biomass	0.052	0.026	0.077	0.012	128	4.10	<0.001***
Bacterial abundance	0.020	–0.003	0.044	0.012	128	1.72	0.087
Carnivorous biomass	0.015	–0.021	0.051	0.018	128	0.824	0.412
Detritivorous biomass	0.033	–0.007	0.075	0.020	128	1.61	0.109
Herbivorous biomass	0.042	0.010	0.075	0.016	128	2.63	0.010*
Light availability	3.32	1.50	5.13	0.918	127	3.61	<0.001*** Polynomial
Nitrogen available	2.74	0.135	5.34	1.32	127	2.08	0.039* Polynomial
Omnivorous biomass	2.99	1.27	4.70	0.868	127	3.44	0.001** Polynomial
Daily oxygen variation	2.36	0.803	3.91	0.785	127	3.00	0.003** Polynomial
Phosphorus available	0.071	0.045	0.097	0.013	128	5.44	<0.001***
Ciliates effect							
Above-ground cover	0.054	0.014	0.093	0.019	128	2.73	0.007**
Algae biomass	0.105	0.059	0.150	0.023	128	4.56	<0.001***

Bacterial abundance	0.002	–0.056	0.062	0.030	128	0.095	0.924
Carnivorous biomass	0.050	0.001	0.098	0.024	128	2.03	0.044*
Detritivorous biomass	0.036	–0.013	0.087	0.025	128	1.44	0.152
Herbivorous biomass	0.071	0.027	0.115	0.022	128	3.19	0.002**
Light availability	0.042	–0.002	0.087	0.022	128	1.85	0.066
Nitrogen available	1.09	–0.416	2.60	0.761	127	1.43	0.155
Omnivorous biomass	0.054	–0.032	0.141	0.043	128	1.25	0.215
Daily oxygen variation	2.49	0.679	4.30	0.915	127	2.72	0.007**
Phosphorus available	0.075	0.036	0.115	0.019	128	3.86	<0.001***
Testate amoebae effect							
Above-ground cover	0.147	–0.080	0.376	0.115	128	1.28	0.204
Algae biomass	0.033	–0000	0.067	0.017	128	1.97	0.051
Bacterial abundance	–0.002	–0.034	0.030	0.016	128	–0.136	0.892
Carnivorous biomass	0.011	–0.025	0.049	0.018	128	0.635	0.526
Detritivorous biomass	0.018	–0.008	0.045	0.013	128	1.38	0.170
Herbivorous biomass	1.10	–1.01	3.20	1.06	127	1.03	0.304
Light availability	2.47	0.393	4.55	1.05	127	2.35	0.020*
Nitrogen available	1.53	–1.94	2.53	1.13	127	0.261	0.794
Omnivorous biomass	0.003	–0.042	0.049	0.023	128	0.164	0.870
Daily oxygen variation	2.23	0.358	4.11	0.948	127	2.36	0.020* Polynomial
Phosphorus available	0.018	-0.005	0.043	0.012	128	1.53	0.128
Phytoplankton effect							
Above-ground cover	0.003	–0.005	0.011	0.004	128	0.677	0.500
Algae biomass	0.027	0.014	0.040	0.006	128	4.17	<0.001***
Bacterial abundance	–0.000	–0.010	0.008	0.004	128	–0.164	0.870
Carnivorous biomass	0.005	–0.007	0.019	0.006	128	0.885	0.378
Detritivorous biomass	0.004	–0.008	0.017	0.006	128	0.613	0.541
Herbivorous biomass	0.010	–0.003	0.024	0.006	128	1.49	0.140
Light availability	0.321	–1.88	2.52	1.11	127	0.289	0.773

Nitrogen available	–1.92	–3.75	-0.087	0.927	127	–2.07	0.040* Polynomial
Omnivorous biomass	1.56	–1.88	5.00	1.74	127	0.897	0.371
Daily oxygen variation	0.705	–1.53	2.94	1.13	127	0.623	0.534
Phosphorus available	0.013	0.004	0.021	0.004	128	3.07	0.003**
Rotifera effect							
Above-ground cover	0.022	–0.013	0.057	0.017	128	1.24	0.219
Algae biomass	0.062	0.004	0.120	0.029	128	2.13	0.035*
Bacterial abundance	–0.007	–0.040	0.024	0.016	128	–0.476	0.635
Carnivorous biomass	–0.767	–2.76	1.22	1.00	127	–0.763	0.447
Detritivorous biomass	0.017	–0.006	0.042	0.012	128	1.44	0.153
Herbivorous biomass	2.39	–0.513	5.30	1.47	127	1.63	0.106
Light availability	2.10	–1.97	6.17	2.06	127	1.02	0.310
Nitrogen available	1.28	–1.45	4.02	1.38	127	0.931	0.354
Omnivorous biomass	0.388	–2.20	2.97	1.31	127	0.297	0.767
Daily oxygen variation	1.32	–0.733	3.36	1.04	127	1.27	0.206
Phosphorus available	0.035	–0.000	0.070	0.017	128	1.98	0.050*

Supplementary Table 6. Results of linear mixed-effects model showing effects of functional diversity of aquatic organisms on 11 single ecosystem functions (n = 137; above-ground cover, algae biomass, bacterial abundance, carnivorous biomass, detritivorous biomass, herbivorous biomass, light availability under-water, nitrogen available, omnivorous biomass, daily oxygen variation, and phosphorus available). These results reflect the pattern observed in Supplementary Fig. 6. Ecosystem functions were scaled to interpret parameter estimates on a comparable scale. For some BEF relationships an exponential term was added. P < 0.05*; P < 0.01**; P < 0.001***

Ecosystem functions	**Functional diversity**						
	Estimate	CI (lower)	CI (Upper)	Std. Error	DF	t-value	p-value
Multidiversity effect							
Above-ground cover	2.92	1.65	4.20	0.644	128	4.54	<0.001***
Algae biomass	3.53	1.23	5.83	1.16	128	3.03	0.003**
Bacterial abundance	2.07	–1.07	5.22	1.59	127	1.31	0.194
Carnivorous biomass	3.33	2.14	4.53	0.605	128	5.51	<0.001***
Detritivorous biomass	3.23	1.83	4.63	0.709	128	4.55	<0.001***
Herbivorous biomass	4.32	3.17	5.47	0.580	128	7.44	<0.001***
Light availability	3.26	1.98	4.55	0.649	128	5.03	<0.001***
Nitrogen available	3.94	1.91	5.97	1.02	127	3.84	<0.001***
Omnivorous biomass	3.04	1.50	4.58	0.779	128	3.90	<0.001***
Daily oxygen variation	3.89	–0.207	8.0	2.07	127	1.88	0.063
Phosphorus available	3.74	2.63	4.86	0.562	128	6.67	<0.001***
Fish effect							
Above-ground cover	2.33	–0.104	4.76	1.23	128	1.89	0.061
Algae biomass	3.11	1.38	4.85	0.878	128	3.55	0.001**
Bacterial abundance	2.42	–0.227	5.07	1.34	127	1.81	0.073C
Carnivorous biomass	7.67	5.36	9.98	1.17	128	6.57	0.0001***
Detritivorous biomass	6.50	3.42	9.57	1.56	128	4.18	<0.001***
Herbivorous biomass	8.32	6.08	10.6	1.13	128	7.34	<0.001***
Light availability	3.02	0.771	5.27	1.14	128	2.66	0.009**
Nitrogen available	3.21	1.53	4.89	0.850	127	3.77	<0.001***

Omnivorous biomass	7.01	4.88	9.14	1.08	128	6.52	<0.001***
Daily oxygen variation	2.42	–0.215	5.06	1.33	128	1.82	0.071
Phosphorus available	3.87	1.93	5.81	0.981	128	3.95	<0.001***
Macrophyte effect							
Above-ground cover	1.35	0.260	2.45	0.553	128	2.45	0.016*
Algae biomass	1.76	0.916	2.60	0.426	128	4.13	<0.001***
Bacterial abundance	1.16	–0.366	2.69	0.773	127	1.51	0.135
Carnivorous biomass	3.16	1.43	4.90	0.877	127	3.61	<0.001***
Detritivorous biomass	1.94	0.856	3.02	0.546	128	3.55	0.001**
Herbivorous biomass	2.41	1.21	3.62	0.610	128	3.96	<0.001***
Light availability	3.51	1.86	5.16	0.833	127	4.21	<0.001*** polynomial
Nitrogen available	2.03	0.562	3.50	0.742	127	2.74	0.007** polynomial
Omnivorous biomass	2.18	0.426	3.94	0.888	127	2.46	0.015* polynomial
Daily oxygen variation	1.69	0.401	2.97	0.650	127	2.60	0.011* polynomial
Phosphorus available	1.64	0.580	2.70	0.536	128	3.06	0.003**
Microcrustacean effect							
Above-ground cover	2.58	0.804	4.35	0.897	128	2.88	0.005**
Algae biomass	2.63	–1.43	6.68	2.05	128	1.28	0.202
Bacterial abundance	0.505	–1.97	2.98	1.25	127	0.404	0.687
Carnivorous biomass	2.01	0.107	3.91	0.961	127	2.09	0.039*
Detritivorous biomass	1.75	0.262	3.23	0.750	128	2.33	0.021*
Herbivorous biomass	3.32	1.62	5.03	0.860	12	3.86	<0.001*** Polynomial
Light availability	3.41	1.68	5.14	0.876	127	3.89	<0.001*** Polynomial
Nitrogen available	2.38	0.206	4.56	1.10	127	2.17	0.032
Omnivorous biomass	1.83	–0.123	3.79	0.989	127	1.85	0.066
Daily oxygen variation	3.03	1.61	4.45	0.719	127	4.22	0.003** Polynomial
Phosphorus available	3.17	1.73	4.61	0.727	128	4.36	<0.001***
Ciliates effect							
Above-ground cover	2.28	0.057	4.50	1.12	128	2.03	0.044*

Algae biomass	6.96	3.73	10.2	1.63	127	4.27	<0.001***
Bacterial abundance	1.41	–0.244	3.07	0.836	127	1.69	0.094
Carnivorous biomass	2.20	0.400	4.0	0.910	127	2.42	0.017*
Detritivorous biomass	3.14	0.731	5.54	1.22	128	2.58	0.011*
Herbivorous biomass	3.72	0.873	6.56	1.44	128	2.59	0.011*
Light availability	1.17	–1.59	3.92	1.39	127	0.837	0.404
Nitrogen available	3.05	–0.251	6.36	1.67	127	1.43	0.070
Omnivorous biomass	2.48	0.090	4.87	1.21	127	2.05	0.042*
Daily oxygen variation	1.58	–1.00	4.15	1.30	127	1.21	0.229
Phosphorus available	6.28	3.84	8.72	1.24	128	5.08	<0.001***
Testate amoebae effect							
Above-ground cover	0.119	–0.240	0.478	0.181	128	0.657	0.512
Algae biomass	2.70	–1.98	7.39	2.37	128	1.14	0.255
Bacterial abundance	–0.164	–2.18	1.86	1.02	128	-0.161	0.873
Carnivorous biomass	2.72	0.917	4.53	0.913	128	2.98	0.003**
Detritivorous biomass	1.91	0.226	3.59	0.850	128	2.24	0.027*
Herbivorous biomass	2.41	0.094	4.72	1.17	127	2.06	0.042*
Light availability	4.56	1.55	7.57	1.52	127	2.99	0.003**
Nitrogen available	1.00	–0.728	2.73	0.873	127	1.15	0.254
Omnivorous biomass	2.13	0.272	3.99	0.939	127	2.27	0.025* Polynomial
Daily oxygen variation	2.96	–0.883	6.80	1.94	127	1.52	0.130
Phosphorus available	1.76	–0.417	3.93	1.10	127	1.60	0.112
Phytoplankton effect							
Above-ground cover	3.88	2.08	5.69	0.913	128	4.25	<0.001***
Algae biomass	4.62	2.02	7.22	1.31	128	3.51	0.001**
Bacterial abundance	1.57	–0.052	3.20	0.822	127.	1.91	0.058
Carnivorous biomass	3.40	1.25	5.55	1.09	128	3.13	0.002**
Detritivorous biomass	3.62	1.21	6.03	1.22	128	2.97	0.004**
Herbivorous biomass	3.96	0.737	7.19	1.63	128	2.43	0.016*

Light availability	2.06	0.328	3.79	0.875	127	2.35	0.020* Polynomial
Nitrogen available	2.07	0.490	3.65	0.799	127	2.59	0.011*
Omnivorous biomass	2.51	0.876	4.15	0.828	127	3.04	0.003** Polynomial
Daily oxygen variation	1.52	–0.859	3.89	1.20	127	1.26	0.209
Phosphorus available	4.70	2.08	7.32	1.33	128	3.54	0.001**
Rotifera effect							
Above-ground cover	6.58	–0.515	13.7	3.58	128	1.83	0.069
Algae biomass	9.35	–9.16	12.9	5.57	128	0.332	0.74
Bacterial abundance	1.85	–0.040	0.024	0.016	128	–0.476	0.635
Carnivorous biomass	1.30	–2.83	5.43	2.09	127	0.622	0.535
Detritivorous biomass	8.77	2.07	15.5	3.38	128	2.59	0.011*
Herbivorous biomass	3.48	0.929	6.02	1.29	127	2.70	0.008**
Light availability	2.48	0.660	4.31	0.922	127	2.70	0.008**
Nitrogen available	1.90	0.401	3.41	0.759	127	2.51	0.013*
Omnivorous biomass	2.12	–0.539	4.78	1.34	127	1.58	0.117
Daily oxygen variation	1.37	–0.877	3.61	1.13	127	1.21	0.230
Phosphorus available	8.86	3.20	14.5	2.86	128	3.09	0.002**

Supplementary Table 7. Coefficient estimates and confidence intervals from mixed-effects models that include interaction terms to test whether the human pressure (human footprint [HFP]) influences the relationship between biodiversity (multi-dimensional species richness and functional diversity) and ecosystem multifunctionality in neotropical wetlands (n = 137). Note that no intervals that overlap zero, indicating high significance in the models. Both species richness and functional diversity were quantified using a multi-dimensional synthetic index reflecting total ecosystem biodiversity; multidiversity according to[114]. Results are presented in the main manuscript, Fig. 4a and Fig 5a of the main manuscript. P < 0.05*; P < 0.01**; P < 0.001***

Predictor	**Ecosystem multifunctionality**					
	Estimate	**CI (lower)**	**CI (Upper)**	**Std. Error**	**t-value**	**p-value**
Species richness						
Multidiversity	0.403	0.267	0.538	0.068	5.89	<0.001***
Multidiversity*HFP	–0.248	–0.363	–0.132	0.058	–4.25	<0.001***
Fish	0.645	0.538	0.752	0.054	11.9	<0.001***
Fish*HFP	–0.102	–0.201	–0.003	0.049	–2.05	0.042*
Macrophytes	0.467	0.291	0.643	0.088	5.25	<0.001***
Macrophytes*HFP	–0.077	–0.235	0.080	0.079	–0.972	0.333
Microcrustaceans	0.117	–0.019	0.252	0.068	1.70	0.092
Microcrustaceans*HFP	–0.319	–0.437	–0.201	0.059	–5.35	<0.001***
Ciliates	0.170	0.016	0.324	0.077	2.19	0.030*
Ciliates *HFP	–0.179	–0.309	–0.048	0.065	–2.71	0.008**
Testate amoebae	0.021	–0.144	0.188	0.083	0.259	0.796
Testate amoebae*HFP	–0.228	–0.373	–0.083	0.073	–3.12	0.002**
Phytoplankton	0.132	–0.028	0.293	0.081	1.62	0.107
Phytoplankton*HFP	–0.052	–0.173	0.068	0.060	–0.858	0.393
Rotifera	0.122	–0.021	0.264	0.072	1.69	0.094
Rotifera*HFP	–0.172	–0.298	–0.045	0.063	–2.69	0.008**
Functional diversity						
Multidiversity	0.489	0.345	0.633	0.072	6.71	<0.001***
Multidiversity*HFP	–0.125	–0.244	–0.006	0.060	–2.09	0.039*
Fish	0.543	0.416	0.669	0.063	8.49	<0.001***
Fish*HFP	–0.135	–0.249	–0.021	0.057	–2.35	0.020*

Macrophytes	0.276	0.144	0.407	0.066	4.15	<0.001***
Macrophytes*HFP	–0.156	–0.300	–0.011	0.073	–2.13	0.035*
Microcrustaceans	0.125	–0.028	0.278	0.077	1.61	0.111
Microcrustaceans*HFP	–0.259	–0.401	–0.117	0.071	–3.60	<0.001***
Ciliates	0.185	0.038	0.330	0.073	2.51	0.014*
Ciliates *HFP	–0.193	–0.322	–0.062	0.065	–2.94	0.004**
Testate amoebae	0.179	0.040	0.070	0.066	2.55	0.012*
Testate amoebae*HFP	–0.268	–0.413	–0.123	0.073	–3.66	<0.001***
Phytoplankton	0.211	0.072	0.349	0.069	3.02	0.003**
Phytoplankton*HFP	–0.206	–0.337	–0.075	0.066	–3.12	0.002**
Rotifera	0.143	–0.001	0.288	0.073	1.96	0.053
Rotifera*HFP	–0.110	–0.248	0.027	0.069	–1.58	0.117

Supplementary Table 8. Standardized and unstandardized direct paths of all ecosystem drivers, including: climate (temperature and precipitation), space (distance from equator), local aquatic properties (conductivity, pH, and water level), besides human pressure (human footprint index), and aquatic biodiversity on ecosystem multifunctionality from species richness model (Fig. 6A). This table includes all significant and nonsignificant path considered by our model, and also includes those variables which were allow to covary. Information on variables included in BOXA-D in Fig. 6B is highlighted in this table in the column "BOX". *= $P < 0.05$, **= $P < 0.01$, and ***= $P < 0.001$. This table show only results from model with species richness. Double-headed arrows (◄►) indicate the variables that covary (n = 137). MAP = mean annual of precipitation; MAT = mean annual de temperature.

Box	Predictors		Response	Standardized coefficients	Regression weights	P-value
	Distance equator	⟶	Multifunctionality	–0.071	–0.002	0.089
	Human footprint	⟶	Multifunctionality	–0.173	–0.093	<0.001***
	MAT	⟶	Multifunctionality	0.038	0.043	0.338
	MAP	⟶	Multifunctionality	0.022	0.015	0.623
	Water level	⟶	Multifunctionality	–0.099	–0.040	0.031*
	pH	⟶	Multifunctionality	–0.020	–0.003	0.673
	Conductivity	⟶	Multifunctionality	0.013	0.007	0.776
	Species richness	⟶	Multifunctionality	0.792	0.914	<0.001***
	Distance equator	⟶	Water level	–0.137	–0.012	0.075
	Distance equator	⟶	pH	–0.040	–0.009	0.627
	Distance equator	⟶	Conductivity	–0.249	–0.017	0.003**
	Distance equator	⟶	MAT	–0.005	–0.000	0.949
	Distance equator	⟶	MAP	–0.204	–0.010	0.011*
A	Distance equator	⟶	Fish rich	–0.034	–0.002	0.689
A	Distance equator	⟶	Macrophyte rich	–0.240	–0.016	0.005**
A	Distance equator	⟶	Microcrustacean rich	–0.402	–0.023	<0.001***
A	Distance equator	⟶	Ciliate rich	0.097	0.006	0.247
A	Distance equator	⟶	Testate amoebae rich	–0.253	–0.014	0.002**
A	Distance equator	⟶	Phytoplankton rich	0.100	0.006	0.255
A	Distance equator	⟶	Rotifera rich	–0.074	–0.005	0.387

	Human footprint	→	Water level	–0.036	–0.048	0.642
	Human footprint	→	pH	0.206	0.714	0.013*
	Human footprint	→	Conductivity	0.067	0.067	0.451
B	Human footprint	→	Fish rich	–0.271	–0.279	0.002 **
B	Human footprint	→	Macrophyte rich	–0.151	–0.153	0.078
B	Human footprint	→	Microcrustacean rich	–0.260	–0.227	0.001**
B	Human footprint	→	Ciliate rich	–0.319	–0.318	<0.001***
B	Human footprint	→	Testate amoebae rich	–0.269	–0.237	0.001**
B	Human footprint	→	Phytoplankton rich	–0.168	–0.154	0.056
B	Human footprint	→	Rotifera rich	–0.056	–0.053	0.514
	Human footprint	↔	MAT	–0.141	–0.004	0.103
	Human footprint	↔	MAP	–0.266	–0.011	0.003**
	MAT	→	pH	–0.189	–.389	0.018*
	MAT	→	Conductivity	–0.135	–0.301	0.099
C	MAT	→	Fish rich	0.175	0.381	0.035*
C	MAT	→	Macrophyte rich	–0.258	–0.553	0.002**
C	MAT	→	Microcrustacean rich	0.116	0.214	0.125
C	MAT	→	Ciliate rich	0.018	0.038	0.827
C	MAT	→	Testate amoebae rich	–0.229	–0.426	0.004**
C	MAT	→	Phytoplankton rich	0.073	0.140	0.393
C	MAT	→	Rotifera rich	–0.289	0.096	0.002**
	MAT	↔	MAP	–0.087	–0.002	0.308
	MAP	→	Water level	0.422	0.720	<0.001***
	MAP	→	pH	0.011	0.051	0.902
	MAP	→	Conductivity	–0.054	–0.072	0.571
C	MAP	→	Fish rich	0.003	0.005	0.970
C	MAP	→	Macrophyte rich	–0.127	–0.166	0.173

C	MAP	→	Microcrustacean rich	0.124	0.139	0.147
C	MAP	→	Ciliate rich	–0.109	–0.141	0.232
C	MAP	→	Testate amoebae rich	–0.162	–0.183	0.072
C	MAP	→	Phytoplankton rich	0.120	0.142	0.209
C	MAP	→	Rotifera rich	–0.289	–0.348	0.002**
	Water level	→	pH	0.262	0.685	0.003**
	Water level	→	Conductivity	0.128	0.102	0.162
D	Water level	→	Fish rich	–0.231	–0.180	0.013*
D	Water level	→	Macrophyte rich	0.102	0.078	0.275
D	Water level	→	Microcrustacean rich	–0.080	–0.053	0.346
D	Water level	→	Ciliate rich	–0.163	–0.123	0.074
D	Water level	→	Testate amoebae rich	–0.138	–0.091	0.127
D	Water level	→	Phytoplankton rich	–0.189	–0.130	0.049*
D	Water level	→	Rotifera rich	0.295	0.208	0.002**
D	pH	→	Fish rich	0.086	0.026	0.369
D	pH	→	Macrophyte rich	–0.075	–0.022	0.437
D	pH	→	Microcrustacean rich	0.057	0.014	0.516
D	pH	→	Ciliate rich	0.030	0.009	0.747
D	pH	→	Testate amoebae rich	–0.025	–0.006	0.792
D	pH	→	Phytoplankton rich	–0.059	–0.015	0.553
D	pH	→	Rotifera rich	–0.153	–0.041	0.116
	pH	↔	Conductivity	0.441	0.079	<0.001***
D	pH	→	Fish rich	–0.023	–0.023	0.805
D	pH	→	Macrophyte rich	–0.131	–0.126	0.162
D	pH	→	Microcrustacean rich	0.002	0.001	0.984
D	pH	→	Ciliate rich	–0.123	–0.118	0.178
D	pH	→	Testate amoebae rich	0.120	0.101	0.184

D	pH	→	Phytoplankton rich	0.129	0.112	0.181
D	pH	→	Rotifera rich	0.048	0.043	0.612
	Fish rich	↔	Macrophyte rich	–0.069	–0.004	0.417
	Fish rich	↔	Microcrustacean rich	0.252	0.010	0.004**
	Fish rich	↔	Ciliate rich	0.269	0.013	0.002**
	Fish rich	↔	Testate amoebae rich	0.034	0.001	0.688
	Fish rich	↔	Phytoplankton	0.300	0.014	0.001**
	Fish rich	↔	Rotifera rich	0.147	0.007	0.089
	Macrophyte rich	↔	Microcrustacean rich	0.024	0.001	0.777
	Macrophyte rich	↔	Ciliate rich	0.172	0.008	0.047*
	Macrophyte rich	↔	Testate amoebae rich	0.088	0.004	0.305
	Macrophyte rich	↔	Phytoplankton	–0.057	–0.003	0.505
	Macrophyte rich	↔	Rotifera rich	0.071	0.003	0.409
	Microcrustacean rich	↔	Ciliate rich	0.254	0.010	0.004**
	Microcrustacean rich	↔	Testate amoebae rich	0.250	0.008	0.005**
	Microcrustacean rich	↔	Phytoplankton	0.343	0.013	<0.001***
	Microcrustacean rich	↔	Rotifera rich	0.386	0.014	<0.001***
	Ciliate rich	↔	Testate amoebae rich	0.175	0.007	0.044*
	Ciliate rich	↔	Phytoplankton rich	0.580	0.026	<0.001***
	Ciliate rich	↔	Rotifera rich	0.153	0.007	0.076
	Testate amoebae rich	↔	Testate amoebae rich	0.189	0.007	0.029*
	Testate amoebae rich	↔	Rotifera rich	0.275	0.011	0.002**
	Phytoplankton rich	↔	Rotifera rich	0.201	0.009	0.021*

Supplementary Table 9. Standardized and unstandardized direct paths of all ecosystem drivers, including: climate (temperature and precipitation), space (distance from equator), local aquatic properties (conductivity, pH, and water level), besides human pressure (human footprint index), and aquatic biodiversity on ecosystem multifunctionality from functional diversity model (Fig. 6C). This table includes all significant and nonsignificant path considered by our model, and also includes those variables which were allow to covary. Information on variables included in BOX A-D in Fig. 6C is highlighted in this table in the column "BOX". *= $P < 0.05$, **= $P < 0.01$, and ***= $P < 0.001$. This table show only results from model with functional diversity. Double-headed arrows (↔) indicate the variables that covary (n = 137). MAP = mean annual of precipitation; MAT = mean annual de temperature.

Box	Predictors		Response	Standardized coefficients	Regression weights	P-value
	Distance equator	→	Multifunctionality	–0.054	–0.002	0.308
	Human footprint	→	Multifunctionality	–0.166	–0.089	0.004**
	MAT	→	Multifunctionality	0.001	0.002	0.978
	MAP	→	Multifunctionality	–0.044	–0.030	0.438
	Water level	→	Multifunctionality	–0.152	–0.061	0.008**
	pH	→	Multifunctionality	0.008	0.001	0.893
	Conductivity	→	Multifunctionality	–0.064	–0.033	0.259
	Functional diversity	→	Multifunctionality	0.721	0.911	<0.001***
	Distance equator	→	Water level	–0.036	–0.048	0.642
	Distance equator	→	pH	–0.040	–0.009	0.627
	Distance equator	→	Conductivity	–0.249	–0.017	0.003**
	Distance equator	→	MAT	–0.005	–0.000	0.949
	Distance equator	→	MAP	–0.204	–0.010	0.011*
A	Distance equator	→	Fish rich	–0.163	–0.010	0.051
A	Distance equator	→	Macrophyte FD	–0.161	–0.010	0.064
A	Distance equator	→	Microcrustacean FD	–0.446	–0.025	<0.001***
A	Distance equator	→	Ciliate FD	0.012	0.001	0.891
A	Distance equator	→	Testate amoebae FD	–0.176	–0.009	0.039*
A	Distance equator	→	Phytoplankton FD	–0.130	–0.007	0.129
A	Distance equator	→	Rotifera FD	0.067	0.004	0.448

	Human footprint	→	Water level	–0.036	–0.048	0.642
	Human footprint	→	pH	0.206	0.714	0.013*
	Human footprint	→	Conductivity	0.067	0.067	0.451
B	Human footprint	→	Fish FD	–0.327	–0.313	<0.001***
B	Human footprint	→	Macrophyte FD	–0.156	–0.151	0.075
B	Human footprint	→	Microcrustacean FD	–0.264	–0.226	0.001**
B	Human footprint	→	Ciliate FD	–0.362	–0.288	<0.001***
B	Human footprint	→	Testate amoebae FD	–0.227	–0.176	0.008**
B	Human footprint	→	Phytoplankton FD	–0.304	–0.235	<0.001***
B	Human footprint	→	Rotifera rich	–0.237	–0.219	0.007**
	Human footprint	↔	MAT	–0.141	–0.004	0.103
	Human footprint	↔	MAP	–0.266	–0.011	0.003**
	MAT	→	pH	–0.189	–1.389	0.018*
	MAT	→	Conductivity	–0.136	–0.301	0.099
C	MAT	→	Fish FD	0.145	0.293	0.075
C	MAT	→	Macrophyte FD	0.110	0.225	0.194
C	MAT	→	Microcrustacean FD	–0.042	–0.075	0.584
C	MAT	→	Ciliate FD	–0.090	–0.151	0.273
C	MAT	→	Testate amoebae FD	–0.078	–0.128	0.346
C	MAT	→	Phytoplankton FD	–0.028	–0.046	0.735
C	MAT	→	Rotifera FD	–0.006	–0.012	0.942
	MAT	↔	MAP	–0.087	–0.002	0.308
	MAP	→	Water level	0.422	0.720	<0.001***
	MAP	→	pH	0.011	0.051	0.902
	MAP	→	Conductivity	–0.054	–0.072	0.571
C	MAP	→	Fish FD	0.046	0.056	0.618
C	MAP	→	Macrophyte FD	–0.003	–0.003	0.977

C	MAP	⟶	Microcrustacean FD	–0.005	–0.005	0.958
C	MAP	⟶	Ciliate FD	0.015	0.015	0.870
C	MAP	⟶	Testate amoebae FD	0.032	0.032	0.729
C	MAP	⟶	Phytoplankton FD	0.033	0.032	0.729
C	MAP	⟶	Rotifera FD	–0.088	–0.105	0.360
	Water level	⟶	pH	0.262	0.685	0.003**
	Water level	⟶	Conductivity	0.128	0.102	0.162
D	Water level	⟶	Fish FD	–0.169	–0.122	0.065
D	Water level	⟶	Macrophyte FD	–0.078	–0.057	0.411
D	Water level	⟶	Microcrustacean FD	0.022	0.015	0.793
D	Water level	⟶	Ciliate FD	–0.026	–0.015	0.783
D	Water level	⟶	Testate amoebae FD	–0.074	–0.043	0.430
D	Water level	⟶	Phytoplankton FD	–0.084	–0.049	0.373
D	Water level	⟶	Rotifera FD	0.125	0.087	0.196
D	pH	⟶	Fish FD	0.093	0.026	0.323
D	pH	⟶	Macrophyte FD	–0.053	–0.015	0.592
D	pH	⟶	Microcrustacean FD	–0.082	–0.020	0.351
D	pH	⟶	Ciliate FD	–0.067	–0.015	0.484
D	pH	⟶	Testate amoebae FD	–0.084	–0.019	0.383
D	pH	⟶	Phytoplankton FD	–0.033	–0.007	0.737
D	pH	⟶	Rotifera FD	–0.044	–0.012	0.659
	pH	⟷	Conductivity	0.441	0.079	<0.001***
D	Conductivity	⟶	Fish FD	0.061	0.055	0.510
D	Conductivity	⟶	Macrophyte FD	–0.136	–0.125	0.156
D	Conductivity	⟶	Microcrustacean FD	–0.056	–0.046	0.515
D	Conductivity	⟶	Ciliate FD	–0.016	–0.012	0.867
D	Conductivity	⟶	Testate amoebae FD	0.215	0.158	0.022*

D	Conductivity	→	Phytoplankton FD	0.094	0.069	0.318
D	Conductivity	→	Rotifera FD	0.108	0.095	0.264
	Fish FD	↔	Macrophyte FD	0.238	0.011	0.007**
	Fish FD	↔	Microcrustacean FD	0.133	0.005	0.121
	Fish FD	↔	Ciliate FD	0.245	0.009	0.005**
	Fish FD	↔	Testate amoebae FD	0.153	0.005	0.075
	Fish FD	↔	Phytoplankton FD	0.200	0.007	0.020*
	Fish FD	↔	Rotifera FD	0.162	0.007	0.057
	Macrophyte FD	↔	Microcrustacean FD	0.247	0.009	0.005 **
	Macrophyte FD	↔	Ciliate FD	0.153	0.006	0.076
	Macrophyte FD	↔	Testate amoebae FD	0.222	0.008	0.011*
	Macrophyte FD	↔	Phytoplankton FD	0.230	0.009	0.007**
	Macrophyte FD	↔	Rotifera FD	0.225	0.010	0.008**
	Microcrustacean FD	↔	Ciliate FD	0.228	0.007	0.009**
	Microcrustacean FD	↔	Testate amoebae FD	0.418	0.012	<0.001***
	Microcrustacean FD	↔	Testate amoebae FD	0.320	0.010	<0.001***
	Microcrustacean FD	↔	Rotifera FD	0.243	0.009	0.004**
	Ciliate FD	↔	Testate amoebae FD	0.171	0.005	0.047*
	Ciliate FD	↔	Phytoplankton FD	0.265	0.008	0.002**
	Ciliate FD	↔	Rotifera FD	0.166	0.006	0.047*
	Testate amoebae FD	↔	Phytoplankton FD	0.320	0.009	<0.001***
	Testate amoebae FD	↔	Rotifera FD	0.173	0.006	0.036*

Supplementary Table 10. Results of multigroup analysis in the SEM model. Importantly, the multigroup analysis revealed significant differences between the free model (in which all parameters are free to differ between wetlands) and the constrained model (where parameters are fixed and constrained to a single value in all wetlands). Significant differences between the free and constrained models were observed for both models concerning species richness ($P < 0.001$; diff = 255) and functional diversity ($P < 0.001$; diff = 699.61). The below table shows paths comparison between the free and the constrained model. Since our focus was only to assess (i) whether the impacts of ecosystem drivers on multifunctionality varied from wetland to wetland; and (ii) where impacts of human footprint on species richness and functional diversity of organismal groups also differed by wetland, we only tested for differences for the paths involving multifunctionality and the diversity of organismal groups. Differences for the other paths were not analyzed. In particular, if a given path was significantly different between the free and constrained model (ANOVA: $P < 0.05$), the path varied significantly among the wetlands. In contrast, if the path was not significantly different between the free and the constrained path, it did not vary by wetland.

Paths within model	**Path comparison between free and constrained model**	
	Chi-square difference	*P*-value
Species richness – model; Fig SA		
Distance equator →Multifunctionality	0.2968	0.9606
Human footprint → Multifunctionality	0.99266	0.803
MAT → Multifunctionality	0.58818	0.8991
MAP → Multifunctionality	9.7924	0.02042*
Water level → Multifunctionality	2.2972	0.5131
pH → Multifunctionality	1.1882	0.7558
Conductivity → Multifunctionality	1.6559	0.6468
Composite richness → Multifunctionality	3.4548	0.3267
Human footprint → Fish richness	1.9713	0.5784
Human footprint → Macrophyte richness	16.848	0.0007***
Human footprint → Microcrustacean richness	1.8586	0.6023
Human footprint → Ciliate richness	0.2381	0.9712
Human footprint → Testate amoebae richness	5.9363	0.1147
Human footprint → Phytoplankton richness	7.3637	0.06117
Human footprint → Rotifera richness	3.6428	0.3027

Functional diversity – model; Fig. 5C		
Distance equator →Multifunctionality	3.4553	0.3266
Human footprint → Multifunctionality	0.77652	0.8551
MAT → Multifunctionality	2.154	0.5411
MAP → Multifunctionality	0.60454	0.8954
Water level → Multifunctionality	5.1126	0.1637
pH → Multifunctionality	8.4803	0.03706*
Conductivity → Multifunctionality	1.5078	0.6805
Composite FD → Multifunctionality	0.74066	0.8636
Human footprint → Fish FD	4.917	0.178
Human footprint → Macrophyte FD	3.6444	0.3025
Human footprint → Microcrustacean FD	4.4098	0.2205
Human footprint → Ciliate FD	6.4495	0.09168
Human footprint → Testate amoebae FD	2.2486	0.5224
Human footprint → Phytoplankton FD	4.4067	0.2208
Human footprint → Rotifera FD	3.6211	0.3054

Supplementary Table 11. Indirect effects of human footprint on multifunctionality mediated by human footprint effects on diversity of each organismal group used to created composite diversity. Indirect effects were calculated by multiplying the effects of HFP on diversity of a given organismal group plus the standardized loading of this organism group on composite plus coefficient of composite on multifunctionality. The table below shows how these effects were multiplied and the total indirect effect of HFP mediated by species richness and functional diversity of each organismal group.

Diversity	HFP effect		Standardized loading on composite diversity		Composite effect on multifunctionality		Indirect effect of HFP on multifunctionality
Species richness							
Fish	–0.271	*	0.815	*	0.792	=	–0.1749
Macrophyte	–0.151	*	0.413	*	0.792	=	–0.0493
Microcrustacean	–0.260	*	0.085	*	0.792	=	–0.0175
Ciliates	–0.319	*	0.092	*	0.792	=	–0.0232
Testate amoebae	–0.269	*	0.099	*	0.792	=	–0.0210
Phytoplankton	–0.168	*	0.077	*	0.792	=	–0.0103
Rotifera	–0.056	*	–0.039	*	0.792	=	0.0017
Functional diversity							
Fish	–0.327	*	0.630	*	0.721	=	–0.1485
Macrophyte	–0.156	*	0.244	*	0.721	=	–0.0274
Microcrustacean	–0.264	*	0.052	*	0.721	=	–0.0098
Ciliates	–0.362	*	0.227	*	0.721	=	–0.0592
Testate amoebae	–0.227	*	0.088	*	0.721	=	–0.0144
Phytoplankton	–0.304	*	0.215	*	0.721	=	–0.0471
Rotifera	–0.237	*	–0.015	*	0.721	=	0.0025

Supplementary Table 12. Proportion of variance explained by each endogenous variables in SEM. This table showed a R^2 of all endogenous variables (i.e., multifunctionality, diversity of single organismal groups, MAT, MAP, pH, water level, and conductivity). R^2 is represented for both species richness and functional diversity models. MAP = mean annual of precipitation; MAT = mean annual de temperature.

Endogenous variables	**R^2 - explained variance**
Species richness model – Fig 5A	
Multifunctionality	0.800
MAP	0.042
MAT	0.000
Water level	0.229
Conductivity	0.107
pH	0.153
Fish richness	0.137
Macrophyte richness	0.135
Microcrustacean richness	0.278
Ciliate richness	0.171
Testate amoebae richness	0.188
Phytoplankton richness	0.085
Rotifera richness	0.116
Functional diversity model – Fig 5C	
Multifunctionality	0.681
MAP	0.042
MAT	0.000
Water level	0.229
Conductivity	0.107
pH	0.153
Fish FD	0.167
Macrophyte FD	0.098

Microcrustacean FD	0.273
Ciliate FD	0.145
Testate amoebae FD	0.136
Phytoplankton FD	0.120
Rotifera FD	0.077

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4 HUMAN LAND-USES HOMOGENIZE STREAM ASSEMBLAGES AND REDUCE ANIMAL BIOMASS PRODUCTION

ABSTRACT

1. Human land-use change is a major threat to natural ecosystems worldwide. Nonetheless, the effects of human land-uses on the structure of plant and animal assemblages and their functional characteristics are poorly understood. Furthermore, the pathways by which human land uses affect ecosystem functions, such as biomass production, remain unclear.

2. We compiled a unique dataset of fish, arthropod and macrophyte assemblages from 61 stream ecosystems in two Neotropical biomes (Amazonian rainforest, Uruguayan grasslands). We then tested how the cover of agriculture, pasture, urbanization and afforestation affected the taxonomic richness and functional diversity of those three assemblages, and the consequences of this for animal biomass production. Single trait categories and functional diversity combining recruitment and life-history, resource and habitat-use, and body size, were evaluated.

3.The effects of intensive human land-uses on taxonomic and functional diversities were as strong as other drivers known to affect biodiversity, such as climate, and local environmental factors. In both biomes, the taxonomic richness and functional diversity of animal and plant assemblages decreased with increasing cover of agriculture, pasture, and urbanization. Human land-uses were associated with functional homogenization of both animal and plant assemblages. Human land-uses reduced animal biomass through direct and indirect pathways mediated by declines in taxonomic and functional diversities.

4. Our findings indicate that converting natural ecosystems to supply human demands results in species loss and trait homogenization across multiple biotic assemblages, which ultimately reduces animal biomass production in streams.

Keywords: biodiversity loss, ecosystem functioning, grasslands, human pressures, land-use changes, rainforest, streams, trait diversity.

4.1 Introduction

Global biodiversity is declining in the Anthropocene, which has been widely attributed to conversion of natural landscapes to resource extraction, agriculture and urban settlements

(Newbold et al., 2015; Moi et al., 2022a). Recent research has revealed that human land-uses cause species losses and filter sets of functional traits out of ecosystems (Gámez-Virués et al., 2015; Gossner et al., 2016; Leitão et al., 2018; Le Provost et al., 2021). This is predicted to affect ecosystem functioning, which relies on multiple dimensions of biodiversity (Barnes et al., 2017). Although empirical evidence documents the negative effects of agriculture, pasture, and urbanization on biodiversity and ecosystem functions (Gossner et al., 2016; Le Provost et al., 2021), it remains poorly understood whether the effect of those land-uses differ in magnitude (but see, Ligeiro et al., 2014). Also, the degree to which impacts from these land-uses are comparable to other drivers of diversity, including climate and local environmental condition, remains unclear (but see Marzin et al., 2012; Macedo et al., 2014).

Taxonomic richness responses to land-uses differ among animal and plant assemblages (Marzin et al., 2012; Le Provost et al., 2021), suggesting that using taxonomic richness alone hinders generalizing overall diversity responses of communities to land-use changes. Such problems can be overcome by using trait-based approaches, because different assemblages sharing similar traits usually respond similarly to human land-use intensification (Gámez-Virués et al., 2015; Moi et al., 2022a). Also, land-use impacts are stronger for larger animals, because tend to be more vulnerable to habitat loss (Estes et al., 2011; Enquist et al., 2020). Because natural assemblages are constantly interacting through multitrophic linkages, changes in one assemblage may cascade up and down across organismal groups (García et al., 2011). For example, changes in plant diversity have strong cascading effects on arthropod diversity (Scherber et al., 2010). Similarly, subtle shifts in animal diversity may exert cascading effects on plant diversity (Duffy et al., 2007). It has been proposed that human land-uses exert cascading impacts on the structure of entire communities (Barnes et al., 2017).

Human land-uses have altered the relationship between diversity and ecosystem functions (biomass production) through direct and diversity-mediated indirect pathways (Barnes et al., 2014, 2017). Direct effects involve changes in environmental conditions, such as reductions in riparian vegetation, depths, and dissolved oxygen, all of which can decrease biomass production (Walker & Walters, 2019). Indirect effects are more difficult to predict and may manifest via effects on multiple diversity facets (e.g., taxonomic and functional) and across many animal and plant assemblages (Barnes et al., 2017). Given that both taxonomic richness and functional diversity affect ecosystem capacities to maintain their functions (Moi et al., 2022b), human land-uses negatively affecting those facets of diversity would indirectly reduce

biomass production. Moreover, animal and plant assemblages are interwoven through network of biotic interactions, which mediates the capacity of biodiversity to maintain biomass production (Moi et al., 2022a). Human land-uses decreasing the diversity of individual assemblages could disrupt relationships between assemblages, with negative consequences for biomass production (Manning et al., 2015). Understanding direct- and diversity-mediated indirect effects of human land-uses on biomass production can provide new insights for ecological science and its applications.

Using a spatially extensive dataset from Neotropical stream sites, we investigated how four land-uses affect taxonomic richness, multivariate functional diversity, and trait category diversity of fish, arthropod and macrophyte assemblages. Additionally, we analyzed how land-use intensification affects the relationship of fish and arthropod diversity with their respective biomass production. Agriculture, pasture, urbanization, and afforestation are common human pressures in most aquatic and terrestrial ecosystems worldwide (Grimm et al., 2008; Gossner et al., 2016). Extensive agricultural and pasture activities continue to expand in the Neotropics, mostly at the expense of forest cover (Nessimian et al., 2008; Bonanomi et al., 2019). Neotropical landscapes have experienced expansion of urban settlements (United Nations, 2018), and many native rainforests and grasslands have been replaced by eucalyptus plantations (Cantanhêde et al., 2021).

We tested four predictions. (1) The effects of human land-use on multifaceted animal and plant diversity are as strong as other drivers of diversity, such as climate (precipitation and temperature) and environmental features (sediment heterogeneity, water quality and, stream depth). (2) Taxonomic richness and functional diversity would decrease with increasing cover of agriculture, pasture, urbanization and afforestation. (3) Fishes and arthropods would be more strongly affected by land-uses than macrophytes. (4) Negative effects of land-uses on macrophyte diversity would have cascading effects on arthropod and fish diversities, indirectly reducing animal biomass.

4.2 Material and methods

4.2.1 Study area

We conducted this study in 61 stream sites distributed across two different neotropical biomes (31 sites in the Amazonian rainforest and 30 in the Uruguayan grasslands; Fig. 1A). The rainforest in eastern Amazonia (Pará state, Brazil), which has a tropical climate with a

mean annual temperature of 26.8°C and mean annual precipitation of 2140 mm. The grassland sites in Uruguay have a subtropical climate, with a mean annual temperature of 17.4ºC and mean annual precipitation of 1200 mm. In both biomes, sites were randomly selected and followed a gradient of human land-use intensity (Fig. 1B,C). Each site was sampled twice – during a dry and rainy season, totaling 122 sampling events carried out between 2017 and 2019. The sampling included fish, arthropod (insects), and macrophyte (see Appendix S1). The sampling efforts and methods for fishes, arthropods, and macrophytes differed between biomes, hindering rigorous inter-biome comparisons of their diversities and responses to human land-use (Roswell et al., 2021). Therefore, we focused on showing how diversity and biomass in rainforest and grassland stream sites respond to human land-use individually.

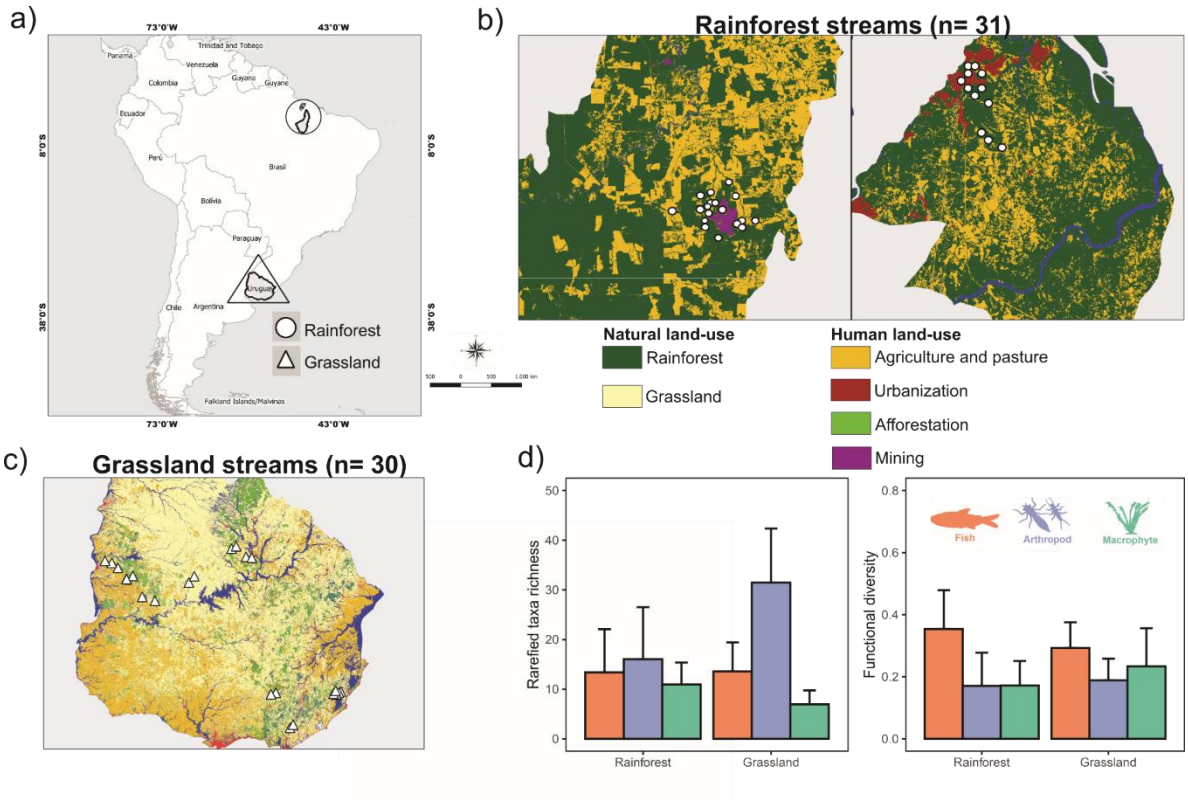

FIGURE 1. Study site locations in the eastern Brazilian Amazon and Uruguayan grasslands (a); land-use cover in rainforest (b) and grassland biomes (c). Land-use covers: agriculture and pasture (yellow) urbanization (red), and afforestation (green). Taxonomic and functional diversities of fishes (orange), arthropods (blue) and macrophytes (green) in rainforest and grassland biomes (d). Study sites included two river basins in the rainforest biome and almost the entire nation of Uruguay in the grassland biome.

4.2.2 Quantifying human land-use cover

The selected site catchments included those with little (0.29%) to predominantly (92%) forested cover or natural grasslands (Fig. 1). The former sites have undergone extensive land conversion, from forest and grasslands to agriculture, pasture, cities, and silviculture of non-native vegetation (Fig. 1). We estimated for each site catchment the cover of four human land-uses: (i) agriculture (soybean and corn crops), (ii) pasture (cattle and sheep farming), (iii) urbanization (urban centers), and (iv) afforestation (*Eucalyptus sp* silviculture). To estimate these land use covers, we first calculated the catchment area upstream from each site. Next, the catchment and hydrography were delimited using the topographic data present in the Shuttle Radar Topography Mission (SRTM) with TauDem 5.3 (Tarboton, 2005). We calculated the cover of the four land-uses for each catchment area through the supervised classification of Landsat 8 images, using the Semi-Automatic Classification plugin using QGis 3.6 (Macedo et al., 2014). Land cover images in 2017 and 2019 (coinciding with the organisms sampling) were obtained from the United States Geological Survey's Earth Explorer project. These images provide information on the shape and texture of landscape elements, identifying different land use types at the site catchment level. We submitted these image sets to atmospheric correction to reduce reflectance effects (Antunes et al., 2012). After the semi-automatic classification, each category was validated by using high-resolution Google Earth images. We calculated the percentages of agriculture, pasture, urbanization and afforestation at a resolution of 30-m pixel in each catchment.

4.2.3 Taxonomic richness and functional traits of animal and plant assemblages

We accounted for differences in the number of sampled organisms by estimating taxonomic richness as the Chao index with abundance-based data using the R package iNEXT (Hsieh et al., 2016). The Chao index is based on rarefaction and extrapolation of Hill numbers. It provides an unbiased estimate of asymptotic taxonomic richness, thus enabling comparisons among areas within same biomes. We selected traits that reflect the functional diversity responses of animal and plant assemblages to land-uses (see Appendix S1). Functional traits were related to three major categories: (1) recruitment and life-history, (2) resource and habitat use, and (3) body size. We calculated eight traits for each fish, six for each arthropod, and nine for each macrophyte taxon (Table 1). We either directly measured the traits (e.g., body length) or carefully retrieved and curated them from published literature sources (Table S1). For body size estimates, we used maximum length of fishes and arthropods and plant height. For

recruitment and life-history traits, we used traits related to persistence and reproduction. For resource and habitat use, we selected traits that are closely related to animal feeding modes and macrophyte growth form and nutrient acquisition (Table 1).

TABLE 1. Traits considered in this study were grouped into three broad categories: (i) recruitment and life-history traits, (ii) resource and habitat use traits, and (iii) body size traits.

Assemblage	Trait diversity category	Traits	Units
Fish	Body size	maximum length	cm
	Recruitment and life-history	eggs parental care	(i) yes, (ii) no
		larval parental care	(i) yes, (ii) no
		reproduction mode	(i) viviparous, (ii) oviparous
		fecundity mode	(i) internal, (ii) external
	Resource and habitat use	feeding modes	(i) piscivore, (ii) omnivore, (iii) detritivore, (iv) herbivore, (v) insectivore, (vi) invertivore
		mouth position	(i) subterminal, (ii) terminal, (iii) superior, (iv) low
		position in water	(i) pelagic, (ii) benthopelagic, (iv) benthic
Arthropod	Body size	maximum length	cm
	Recruitment and life-history	respiration mode	(i) air, (ii) branchial, (iii) integumentary, (iv) plastron, (v) stigmata
		reproduction cycle	(i) univoltine, (ii) semivoltine, (iii) plurivoltine
		refuge use	(i) networks, (ii) sand and debris, (iii) wood, (iv) builders, (v) no refuge
	Resource and habitat use	feeding mode	(i) shredders, (ii) predators, (iii) scrapers, (iv) collector-gatherers, (v) collector-filtering, (vi) piercers
		habitat use	(i) burrowers, (ii) climbers, (iii) skaters, (iv) skaters, (v) sprawlers, (vi) swimmers
	Body size	plant vegetative height	m
	Recruitment and life-history	seed dry mass	mg
		propagation mode	(i) seed/ spore, (ii) mostly by seed/spore, also vegetatively, (iii) by seed/spore and vegetatively, (iv) mostly vegetatively, also by seed/spore, (v) vegetatively

Macrophyte		main dispersal agent	(i) passive, (ii) wind, (iii) water, (iv) animals (v) wind+water, (vi) wind+animals, (vii) water+animals, (viii) wind+water+animals
		plant phenology	(i) perennial, (ii) annual/short-lived perennial
	Resource and habitat use	growth form	(i) submerged, (ii) emergent, (iii) free-floating, (iv) rooted-floating
		leaf compoundness	(i) simple, (ii) compound
		leaf area	$mm^2\ mg^{-1}$
		specific leaf area	$mm^2\ mg^{-1}$

4.2.3.1 Functional diversity indices

To assess the functional diversities of fish, arthropod, and macrophyte assemblages, we calculated three complementary indices: (i) multivariate functional diversity (FDis), (ii) standardized community-weighted variance (CWV), and (iii) community-weighted means (CWM). We computed the multivariate index of functional diversity (FDis) based on trait dispersions for each assemblage, i.e., functional dispersion (Laliberté & Legendre, 2010). FDis represents the mean distance of individual taxa in PCoA space from a distance measure. We calculated FDis from a square root-corrected Gower dissimilarity matrix. Because biomass was estimated for some fish and arthropod using length–weight relationships, we computed the FDis for fishes and arthropods without body size.

We calculated community-weighted means (CWMs) and community abundance-weighted variances (CWVs) (Bernard-Verdier et al., 2012) for each assemblage and each trait separately:

$$CWM_{jk} = \sum_{i=1}^{R} p_{ik} \times t_{ij}$$

$$CWV_{jk} = \sum_{i=1}^{R} P_{ik} \times \left(t_{ij}\right)^2 - \left(CWM_{jk}\right)^2$$

Where p_{ik} is the relative abundance of taxa i in assemblage k, and t_{ij} is the value of trait j for taxon i. CWM represents the assemblage composition with respect to one species-specific trait, while CWV is a measure of trait dispersion within a given assemblage weighted by the abundance of each taxon. We calculate an averaged index of community abundance-weighted

variance for each trait: (i) recruitment and life-history, (2) resource and habitat-use and (3) body size.

We analyzed the correlation between CWV and CWM of each trait category for each assemblage. If CWM is positively correlated with CWV, this means that the variation in the mean results from an increased relative abundance in the assemblage of taxa with unique traits (Gaüzère et al., 2019). Conversely, if CWM is negatively correlated with CWV, the variation in the mean is driven by losses in taxa with original traits. Lastly, we analyzed the response of both CWV and CWM to the increasing cover of human land-uses.

4.2.4 Environmental and climatic covariates

We assessed physical habitat structure quantitatively for 50-m in each site. A line was drawn perpendicular to the channel every 10-m and the substrates were identified every 25 cm according to grain size. We identified four sediment classes: (i) mud (< 0.00006 mm), (ii) silt (>0.0039 mm and <0.0625 mm), (iii) sand (>0.0625 mm and <2 mm), and (iv) gravel (>2 mm). We measured the percentage of each sediment class per site and used those percentages to estimate sediment heterogeneity. We calculated sediment heterogeneity by using the coefficient of variation (CV, the ratio between the standard deviation SD and the mean μ [SD/μ]) of the percentage of the sediment. We measured depths with a ruler at the same points where we sampled the substrate.

To determine water quality, we measured dissolved oxygen (DO, mg L^{-1}), total phosphorus (TP, μg L^{-1}), total nitrogen (TN, μg L^{-1}), and conductivity (uS/cm) with standard methods. The sampling and analytical protocols for each variable are detailed in Appendix S1. To evaluate patterns of water quality variation, we used principal component analysis (PCA). The first PCA axis synthesized the major source of variation in the original four variables (55.8%), and this axis was negatively correlated with DO (Spearman correlation; r = –0.560), and positively correlated with TP (r = 0.510), TN (r = 0.531), and conductivity (r = 0.377; Fig. S2). Therefore, we used PCA axis-one scores as a proxy for water quality deterioration.

We measured climatic predictors including mean annual temperature [MAT] and mean annual precipitation [MAP]. Both MAT and MAP data were obtained from the WorldClim 2.0 database (http://www.worldclim.org) at a 1- km^2 spatial resolution. MAT and MAP are known to be correlated with biodiversity variation (Patrick et al., 2019).

4.2.5 Animal biomass production

We measured the biomasses of fishes and arthropods, which are common proxies for ecosystem production (Benkwitt et al., 2020). To estimate fish biomass, individuals were weighed on a microbalance (0.01 g precision). Because of sampling issues, we calculated biomass for some species from published species-specific length–weight relationships (e.g., Froese & Pauly, 2018). To estimate arthropod biomass, we used regression analyzes of length–weight relationships. The regression used was: $Y = aX^b$, where Y = dry biomass (mg) of an individual; X = total body length (mm) of that individual; a and b are coefficients of regression, where a = the intercept and b = the slope.

4.2.6 Data analysis

We investigated the effects of land-uses, climate, environmental characteristics, and stream mean depth on multifaceted biodiversity. This included: (i) taxonomic richness, (ii) multivariate functional diversity, and (iii) diversity of trait categories of fish, arthropod and macrophyte assemblages. For this, we employed linear mixed-effects models (LMEs) in the R 'nlme' package (Pinheiro et al., 2013). To account for differences in sampling methods, we treated biomes as a fixed effect. Contingent and consistent responses across biomes were tested by including their interactions with land-use types, climate and environmental variables. The random effect consisted of the two seasonal sampling periods (2017 and 2019) nested within each stream. To obtain the model with better predictors, we applied a stepwise (backward) regression procedure using Akaike's Information Criterion corrected for small sample size (AICc). With the best selected models (i.e., lowest AICc), we performed a model-averaging procedure based on AICc selection ($\Delta AICc < 2$) to determine parameter coefficients for the final subset of predictors for each response variable. We performed this procedure by using the dredge function in the 'MuMIn' package (Bartoń, 2014). Visual inspection of residuals using graphical diagnostics (Q-Q plots and residual plots) revealed that the assumptions of normality and homoscedasticity were met. We assessed the multicollinearity between variables using the variance inflation factor (VIF), and we removed all variables with VIF > 3. Variables not strongly correlated were retained (Fig. S3). We standardized all predictors (z-scored: centered to mean and divided by the SD) to interpret slope estimates at a comparable resolution (Schielzeth, 2010).

We expressed the importance of predictors as the percentage of variance they explained (Le Provost et al., 2020). We compared the absolute value of the standardized coefficient of

each predictor with the sum of all standardized regression coefficients from all predictors considered in the best models (Tables S4 and S5). The following variance fractions were examined: (i) biomes, (ii) land-uses, (iii) climate covariates, (iv) local environmental covariates, (v) stream morphology, and (vi) taxonomic richness (only for functional diversity metrics).

We analyzed the relationship of taxonomic and functional diversity of fishes and arthropods with their corresponding biomass by applying LMEs. To account for dominance effects (i.e., dominant traits are more important than diversity) we included the CWM of the three trait categories. To determine whether land-use types altered the diversity-biomass relationships, we added interaction terms of diversity components with agriculture, pasture, urbanization, and afforestation to the mixed-effects models and measured the estimated coefficients for these interactions. We conducted separate analyses for rainforest and grassland sites.

We evaluated the direct- and diversity-mediated indirect pathways through which land-uses influence fish and arthropod biomasses by using structural equation modeling (SEM) in the R package 'lavaan' (Rosseel 2015). Considering that the four land-uses worked in combination to predict animal and plant diversity, we summarized their effects into a composite variable. We combined the four land-uses to build a composite index that would reflect the joint influence of these human land-uses on biomass. We created the composite variable by summing the weighted effect (coefficients) of the four land uses on animal biomass. Importantly, we assigned distinct weights to each land-use (according to its standardized effect on animal biomass) (see Grace & Bollen, 2008). In this vein, the composite variable represents the combined influence (weighted) of four land uses on animal biomass. We fitted separate SEMs to taxonomic richness and functional diversity and accounted for climate and environmental covariates in the SEM (Fig. S1). To evaluate pathway consistency between biomes, we applied a multigroup analysis in the SEM. We considered the two biomes as the grouping variables. In the multigroup approach, we constructed a SEM model in which all parameters were free to differ between biomes and a SEM in which all parameters were fixed. We compared the free and fixed models; a detectable difference indicating that pathway coefficients vary between biomes. Because there were differences between free and fixed models for taxonomic richness ($\chi^2 = 54.84$, $P = 0.013$) and functional diversity ($\chi^2 = 71.39$, $P < 0.001$), we investigated which pathways differed.

We calculated the standardized direct coefficients for each pathway within the models. We estimated the indirect effect of land-uses on biomass mediated by the diversity of fishes, arthropods, and macrophytes. To do so, we multiplied the coefficient of composite land-uses on taxonomic and functional diversity of a given assemblage by the standardized loading of this assemblage on biomass. For example, if the direct effect of composite land-uses on fish richness = 0.50 and the direct effect of fish richness on standing biomass = 0.60, then the indirect effect of composite land-use on standing biomass via fish richness = 0.50 × 0.60 = 0.30. We evaluated the SEM fits using a relative (comparative fit index, CFI) and an absolute (standardized root mean residual, SRMR) fit index (Hu & Bentler, 1999). For taxonomic richness models, the CFI was 0.978 and the SRMR was 0.047, and for the functional diversity model, the CFI was 0.983 and the SRMR was 0.042. Both CFI and SRMR indexes were beyond the thresholds for good model fits (CFI ≥ 0.95; SRMR < 0.08; Hu & Bentler, 1999). All analyses were performed in R version 4.1.1. (R Core Team 2021).

4.3 Results

Both rainforest and grassland sites were dominated by intensive land-uses, especially agriculture and pasture, which covered ~90% of the catchments of some sites (Fig. 1B.C). Several of sites were near cities and silviculture (*Eucalyptus sp.*) covered ~60% of several catchments (Fig. 1C). We recorded 141 fish species, 321 arthropod taxa, and 43 macrophyte taxa across the two biomes (Fig. 1D).

Land-use was a key driver of animal and plant diversity, accounting for 41%, 32% and 82% of the explained variance in taxonomic richness for fish, arthropod, and macrophyte assemblages, respectively (Fig. S4). Land-use accounted for 28%, 33%, and 20% of the explained variance in functional diversity (FDis) for fish, arthropod, and macrophyte assemblages (Fig. S4). No detectable interactions between land-use and biomes were observed (Table S4), implying that the relationship between land-uses and diversity were consistent between biomes. The taxonomic richness and FDis of fish, arthropod and macrophyte assemblages decreased linearly with increasing agriculture, pasture, and urbanization cover (Fig. 2A, Table S6). Fish richness was associated with agriculture and urbanization, arthropod richness was associated with pasture and urbanization, and macrophyte richness was with agriculture, pasture, and urbanization (Fig. 2A, Fig. S5). Fish FDis was associated with

agriculture, pasture, and urbanization, arthropod FDis was associated with urbanization, and macrophyte FDis was associated with agriculture and urbanization (Fig. 2A, Fig. S5).

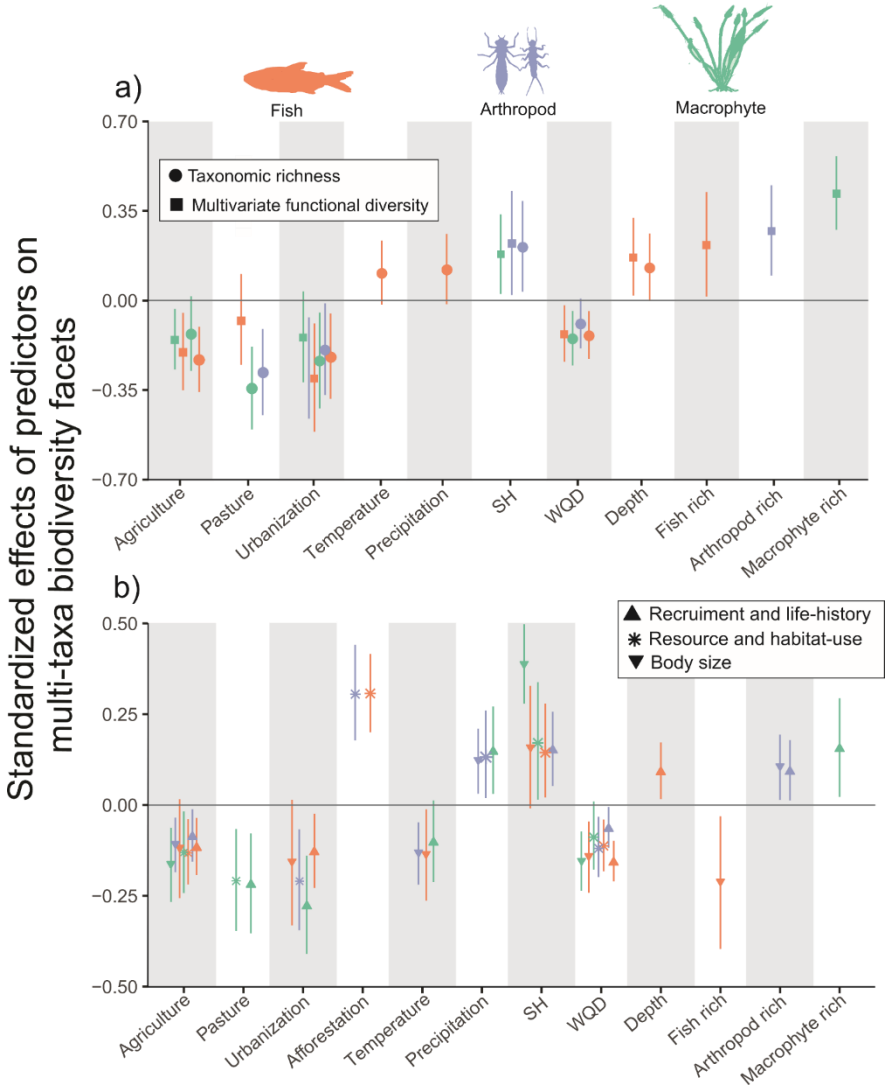

FIGURE 2. Effects of land-uses (agriculture, pasture, urbanization, and afforestation), climate (precipitation and temperature), environmental variables (sediment heterogeneity [SH] and water quality deterioration[WQD]), stream morphology (depth), and species pool (taxa richness) on (a) taxonomic richness (circles) and multivariate functional diversity (FDis - squares), and (b) diversity of trait categories (CWV of recruitment and life-history - triangles, resource and habitat-use - asterisks, and body size - inverted triangles) of fishes, arthropods, and macrophytes. Effect sizes were adjusted using linear mixed-effects models. Colors represent different assemblages (Tables S6 and S70). Points represent estimates, thick lines represent 75% CIs, and thin lines represent 95% CIs.

Land-uses were important predictors of trait categories (Fig. 2B, Table S7), and accounted for 53%, 24%, and 56% of the explained variance for recruitment and life-history

traits of fish, arthropod, and macrophyte, respectively (Fig. S6). Likewise, land-use explained 61%, 63%, and 55% of the variance in diversity of resource and habitat-use traits for fish, arthropod, and macrophyte, respectively (Fig. S6). Land-use explained 34%, 34% and 30% of the variance in diversity of body size traits for fish, arthropod, and macrophyte, respectively (Fig. S6). The diversity of recruitment and life-history, resource and habitat-use, and body size traits of the three assemblages decreased with increasing agriculture, pasture and urbanization cover (Fig. 2B, Fig. S7). There was a decrease in the community-weighted mean traits (CWM) of the three traits categories with increasing agriculture, pasture, and urbanization cover (Table S8). We also found positive correlations between CWM and CWV values (Table S9).

We found positive relationships between the diversity of fish and arthropod assemblages and their corresponding biomasses (Table S10). This finding was consistent for taxonomic richness and functional diversity in both biomes (Fig. 3A-D). Functional diversity (FDis) was associated with biomass production even after accounting for the influence of CWM of trait categories, indicating that biodiversity (*per se*) is more important than functional composition in promoting biomass production. Moreover, as land-use covers increased, the slope of the relationship between diversity and biomass declined, and in some cases, even changed from positive to negative. The changes in the relationship between fish and arthropod diversity with their biomass occurred consistently in rainforest and grassland sites, and were driven by increasing cover of agriculture, pasture, urbanization and afforestation (Fig. 3E-H). Nonetheless, there were differences between biomes and assemblages. For instance, pasture contributed to fish diversity-biomass relationships more strongly in rainforest sites, but agriculture was more important in grassland sites (Fig. 3E-H). Afforestation cover reduced the slope of the relationship between arthropod functional diversity and biomass only in grassland sites and urban cover decreased the slope of the relationship of fish and arthropod diversities with their biomass in both biomes (Fig. 3).

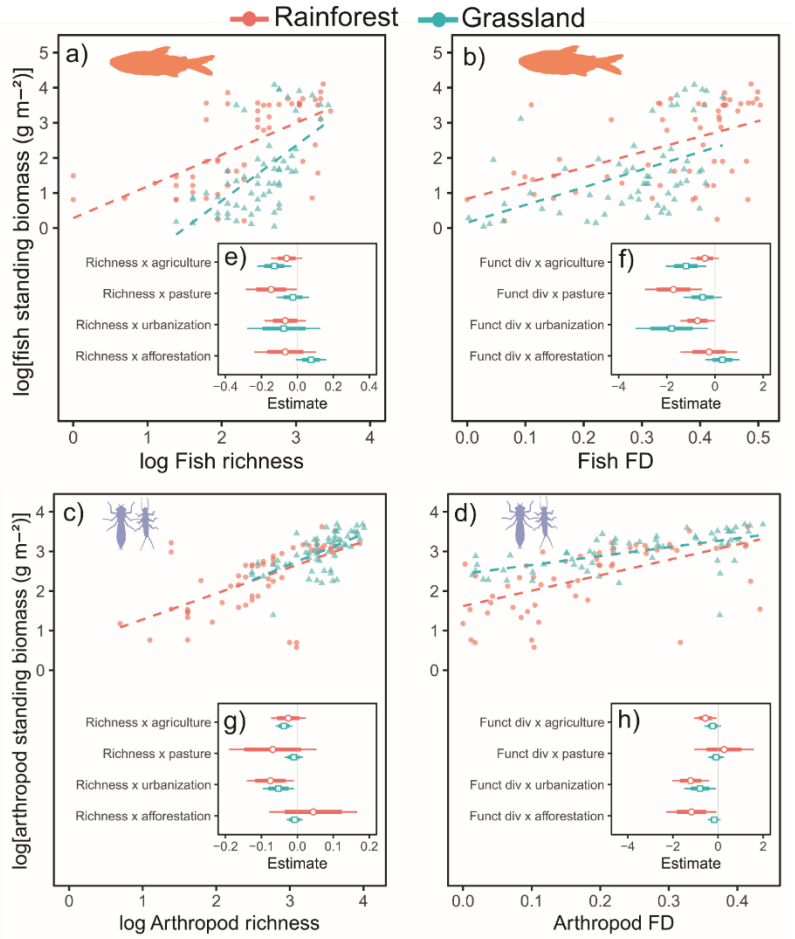

FIGURE 3. Land-use effects on the relationships of fish and arthropod assemblage diversity with their corresponding biomasses in rainforests (red dashed line) and grasslands (green dashed line). Relationships of fish biomass with their (a) richness and (b) functional diversity; Relationships of arthropod biomass with their (c) richness and (d) functional diversity. Dashed colored lines are extracted from LMM to display trends specific to each biome. LMM were performed for each biome separately (Table S9). Estimated coefficients for the interaction terms between land-use with (e) fish richness, (f) fish functional diversity, (g) arthropod richness, and (h) arthropod functional diversity in rainforest (red) and grassland (green) biomes. Points represent estimates, thick lines represent 75% CIs, and thin lines represent 95% CIs.

Land-uses negatively influenced biomass through direct and biodiversity-mediated, indirect pathways (Tables S11 and 12). In the rainforest sites, land-uses indirectly reduced fish biomass by decreasing fish and macrophyte richness (Fig. 4C; –0.161 and–0.123, respectively), fish functional diversity (Fig. 5C; –0.175), and macrophyte functional diversity (Fig. 5C; –0.144). Land-uses indirectly reduced arthropod biomass by decreasing macrophyte richness (Fig. 4C; –0.124) and arthropod and macrophyte functional diversity (Fig. 5C;–0.034 and –0.074, respectively).

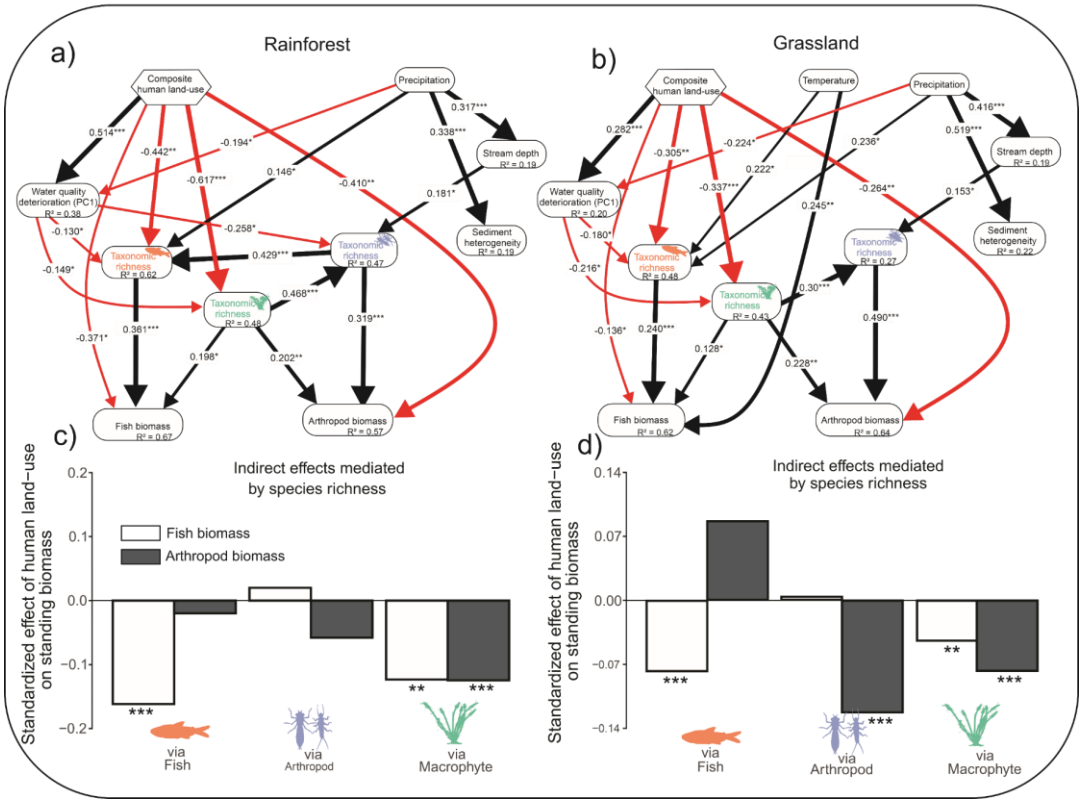

FIGURE 4. Structural equation models (SEMs) examining direct and indirect effects of composite land-uses on standing biomasses of fishes and arthropods mediated by taxonomic diversity. The results are displayed for (a) rainforest and (b) grassland, individually. The combined intensification of land-uses, represented by hexagons, were obtained through composite variables (see Materials and Methods). Models accounted for climate, environmental and stream morphology covariates. Results for the multi-group analysis (i.e., rainforest and grassland) are provided in Table S10. For simplicity, only significant paths were plotted. Solid black and red arrows represent significant positive and negative pathways, respectively ($P \leq 0.05$), whereas the thickness of the arrows represent the magnitudes of the standardized regression coefficients. Numbers in the arrows are the standardized path coefficients of the relationship, and R^2 values for each model are given in the boxes of the variables. Significance levels are $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Figures c and d show the standardized indirect effects of human land-uses on standing biomass mediated by the taxonomic richness of fishes, arthropods, and macrophytes in rainforest (c) and grassland (d).

Land-uses also negatively influenced biomass through direct and biodiversity-mediated, indirect pathways in the grassland sites. Land-uses indirectly decreased fish biomass mediated by decreasing fish and macrophyte richness (Fig. 4D; –0.074 and –0.043, respectively) and reducing fish functional diversity (Fig. 5D; –0.107). Land-uses indirectly reduced arthropod biomass by decreasing arthropod and macrophyte richness (Fig. 4D;–0.122 and–0.076,

respectively) and arthropod and macrophyte functional diversity (Fig. 5D;–0.066 and–0.054, respectively). Land-uses indirectly reduced animal and plant diversity through increased water quality deterioration (Figs. 4 and 5). Macrophyte diversity was positively associated with arthropod diversity, and arthropod diversity was positively associated with fish diversity (Figs. 4 and 5).

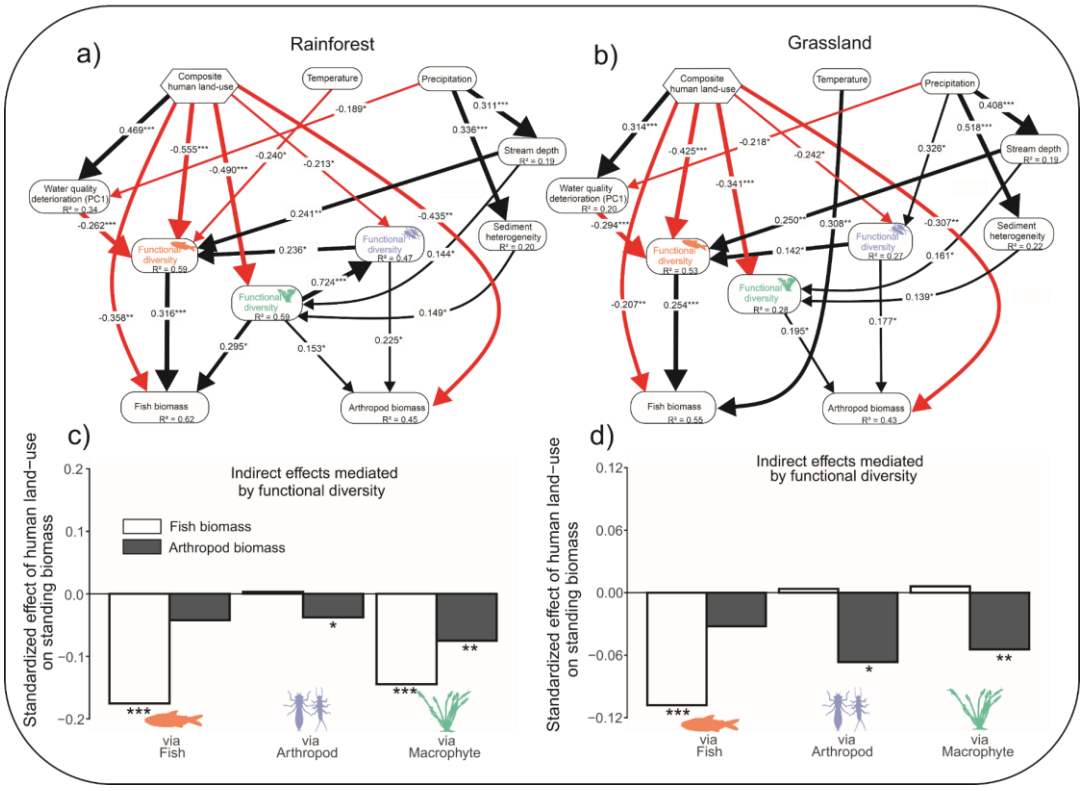

FIGURE 5. Structural equation models (SEMs) examining direct and indirect effects of composite land-uses on standing biomasses of fish and arthropod assemblages mediated by functional diversity (FDis). The combined intensification of land-uses, represented by hexagons, were obtained through composite variables (see Materials and Methods). Results for the multi-group analysis (i.e., rainforest and grassland) are provided in Table S11. For simplicity, only significant paths were plotted. Solid black and red arrows represent significant positive and negative pathways, respectively ($P \leq 0.05$), whereas the thickness of the arrows represent the magnitudes of the standardized regression coefficients. Numbers in the arrows are the standardized path coefficients of the relationship, and R^2 values for each model are given in the boxes of the variables. Significance levels of each predictor are $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Figures c and d show the standardized indirect effects of land-uses on standing biomass mediated by the functional diversity of fish, arthropod, and macrophyte assemblages in rainforest (c) and grassland (d) streams.

4.4 Discussion

Our analyses revealed multiple negative associations of land-uses with the taxonomic and functional diversities of animal and plant assemblages in rainforest and grassland biomes. Decomposing multi-trait diversity into 3 independent trait categories (recruitment and life-history, habitat-use and body size) allowed us to show that land uses jointly triggered multi-taxa homogenization of stream ecosystems. The diversity of fish and arthropod assemblages were positively associated with their respective biomasses in sites with low land-use intensity. However, such relationships shifted to negative with increasing land-use intensity, suggesting that land-uses impair the ability of diversity to promote biomass production. The impacts of land-uses on diversity were as strong as the impacts of other well-known drivers of diversity, such as climate and local environmental features. Our results expand those of geographically more restricted studies (Weijters et al., 2008; Maloney & Weller, 2011; Cantanhêde et al., 2021), suggesting that land-use impacts propagate across large spatial extents and across different biomes. We showed that land-uses cause losses of functional diversity across multiple assemblages, indicating multi-trait homogenization of stream ecosystems. Thus, focusing research on single land-uses types and individual traits of single assemblages hinders our ability to understand biodiversity responses to increasing human pressures in streams.

We detected declines in both CWV and CWM of traits related to recruitment and life-history, resource and habitat-use and body size of fish, arthropod and macrophyte assemblages with land-use intensification. Combined with the evidence that CWV and CWM were closely associated, this indicates that increasing land-use impacts cause local extirpations of species with unique trait combinations (Gaüzère et al., 2019). The decrease in multivariate functional diversity with increasing land-use cover illustrates a widespread multi-trait homogenization of stream ecosystems (Le Provost et al., 2020). Such multi-trait homogenization was general to the animal and plant assemblages in our study, thereby highlighting a spatially extensive functional restructuring of multiple assemblages. There are several potential explanations for this multi-trait homogenization. For example, increased land-use homogenizes stream habitats, reducing the availability of resources (Walker & Walters, 2019; Marques et al., 2021). Decreasing resource availability favors generalist consumers, whereas specialized consumers are filtered out (Walker & Walters, 2019; Cantanhêde et al. 2021). Such resource homogenization causes trophic simplification of stream ecosystem, largely through the elimination of specialized consumers (Moi & Teixeira de Mello, 2022). As we have shown, the

diversity of resource and habitat-use traits decreased under human-dominated streams. Considering that the diversity of resource and habitat-use traits drive overall resource use and habitat exploitation (Naeem et al., 1994), the multi-taxa homogenization of resource and habitat-use traits following land-use intensification threatens the provision of stream ecosystem services (Hanna et al., 2017).

We found declines in diversity of recruitment and life-history and body size traits with land-uses intensification. This suggests that land-uses have homogenized the functional characteristics of animal and plant assemblages. Land-uses degrade the environmental quality of streams by decreasing sediment heterogeneity, stream depth and water quality (Walker & Walters, 2019; Marques et al., 2021; Moi & Teixeira de Mello, 2022). However, there also were positive effects of land-uses on water quality deterioration (Figs. 4 and 5). Furthermore, the diversity of recruitment and life-history and body size traits increased with increasing sediment heterogeneity, but decreased with increasing water quality deterioration. This illustrates that human land use is likely reshaping functional features of animal and plant assemblages through declines in environmental quality. The degradation of environmental quality restricts the phenology of organisms though influencing seasonal reproduction patterns. For instance, environmental quality affects species life history traits such as voltinism, life spans and growth rates (Firmiano et al., 2021). Moreover, larger animals are more sensitive to environmental quality degradation (Marzin et al., 2022), and their diversities tend to decline in degraded streams (Townsend & Thompson, 2007). The diversity of life history and body size traits is closely associated with many aspects of species physiology and ecology (e.g., metabolism and growth rate; Brown et al., 2004) that are modulators of the ability of biodiversity to provide stream ecosystem services (Atkinson et al., 2017). Therefore, homogenization of these trait categories will likely reduce the ability of stream to provide ecosystem services.

Our findings demonstrated a broad-scale positive association between the taxonomic richness and functional diversity of fish and arthropod assemblages with their corresponding biomass in rainforest and grassland biomes. These results underline the important role of multifaceted animal biodiversity for biomass production of animals across neotropical streams, as shown in marine systems (e.g., Benkwitt et al., 2020). This close association of taxonomic and functional animal diversity with biomass suggests that a decline in these facets of biodiversity could result in direct and immediate reduction in animal biomass (Cardinale et al., 2012). As expected, increasing land-use intensity weakens and changes diversity-biomass

relationships for both fish and arthropod assemblages from positive to negative. Although in different magnitudes, agriculture, pasture, urbanization and afforestation negatively affected the diversity-biomass relationships, suggesting that these human land-uses combine to weaken diversity-biomass relationships in streams. The changes in the magnitude and direction of the relations between diversity and biomass suggest that such relationships are context-dependent (Catford et al., 2022). Therefore, the ability of animal assemblages for biomass production increases at low levels of human land-use, but decreases at high levels We demonstrated that land-uses affect animal biomass through a direct negative pathway and indirect negative pathways mediated by losses in taxonomic and functional diversities. This agrees with our prediction that the decrease of the strength of the relationship between diversity and biomass is driven by reducing taxonomic and functional diversities. Whereas both direct and indirect pathways were consistent across rainforest and grassland sites, those pathways were also not mutually exclusive and likely worked in tandem, driving declines in animal biomass. This highlights how challenging it can be to manage anthropogenic pressures in an increasingly human-dominated world (Reid et al., 2019).

We found positive multi-taxa associations in the rainforest biome. The positive associations between arthropod and fish diversities can be partly explained by the high rainforest macrophyte diversity, which facilitate coexistence between fish and arthropod taxa via increasing habitat heterogeneity (García-Girón et al., 2020; Monato et al., 2021). The positive association between rainforest macrophyte and arthropod assemblages also favored positive relationships between fish and arthropod assemblages. Beyond the positive effect of macrophytes on animal diversity, there were positive associations between macrophyte diversity and fish and arthropod biomasses in both rainforest and grassland biomes. This suggests that increased macrophyte diversity leads to increased animal diversity and biomass production (Moi et al., 2022b). Our findings suggest that macrophyte diversity has bottom-up effects on animal biomass, which are mostly indirect and mediated by increasing fish and arthropod diversity. Because macrophytes increase both diversity and biomass of fishes and arthropods, this indicates that losses in macrophyte biodiversity will cascade up to reduced animal diversity, with negative consequences for stream biomass.

Functional diversity increased with taxonomic richness across different assemblages in both biomes, indicating relatively low functional redundancy in these ecosystems. This low functional redundancy combined with the positive association between CWV and CWM

suggests that fish, arthropod, and macrophyte assemblages are composed of taxa with many unique traits (Gaüzère et al., 2019). This result agrees with the low functional redundancy predicted for the neotropics (Leitão et al., 2016; Rodrigues-Filho et al., 2018). It also implies that neotropical ecosystems are vulnerable to human pressures, and that a decline in taxonomic diversity will be closely accompanied by changes in functional community composition. Moreover, the positive multi-taxa associations suggest that loss of diversity in one assemblage would result in cascading impacts on other assemblages.

We used two independent datasets for rainforest and grassland biomes and sampled assemblages with different methods. Therefore, comparisons of results between biomes should be made with caution (Roswell et al., 2021). Despite that, we did not find divergent results between biomes and we did not observe a land-use vs. biome interaction. Whereas our results suggest that multiple land-uses can combine to affect the diversity of different biotic assemblages, the effects of land-use occurred consistently in both rainforest and grassland stream sites. Our results raise new questions about whether the observed effects of land-uses on multifaceted animal and plant diversity changes over time. Although our data prevent temporal analyses at this time, we encourage future studies using long-term (>10 years) land-use datasets to test if temporal variation in different land uses also contribute to observed diversity patterns. This is important because human land-uses can increase or change gradually over time, and current diversity patterns may be the result of region-specific land-use legacies from the past (Maloney & Weller, 2011; Le Provost et al., 2020).

4.5 Conclusions

Our study provides new insights to understanding human impacts by revealing that taxonomic richness and functional diversity of fish, arthropod and macrophyte assemblages declined with increasing cover of agriculture, pasture and urbanization. The strong positive correlations between CWM and CWV of the three trait categories of fish, arthropod and macrophyte assemblages indicate that these three assemblages are formed of taxa with unique sets of traits. However, both CWM and CWV of trait categories decreased with increasing land-use intensification, indicating that human land-uses are causing functional homogenization of these assemblages. The relationships between the diversities of fish and arthropod assemblages and their biomass production changed from highly positive to neutral or negative with increasing land-use cover. Our findings also underscore the important role of macrophytes in promoting diversity and biomass production of fish and arthropod assemblages, re-emphasizing

their roles as keystone taxa in streams. Given the projected increases in human population and land-uses (United Nations, 2018), we emphasize the urgent need to consider joint management of multiple human land-uses at catchment and ecoregional levels. Also, we have shown that different land-uses affect different assemblages somewhat differently; therefore, the same management programs are unlikely to be applicable across assemblages and will require multiple research and management perspectives applied locally and regionally.

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APPENDIX C – Details of the study area and results

Abiotic sampling procedure

For in-stream abiotic sampling in the rainforest biome, we measured dissolved oxygen (DO, mg/L - polarographic method) and conductivity (mS/cm - 4 AC electrode method) in situ using a U- 50 HORIBA multiparameter sonde (www.horiba.com). At each site, we took water samples for subsequent nutrient analysis in laboratory. We measured N, NO_3-N; NH_4-N, and total phosphorus. Nitrogen was estimated according to the method of Müller and Weidemann (1955); total phosphorus was estimated following Standard Method 4500-P-E.

In the grassland biome, we measured dissolved oxygen (DO, mg/4L) and conductivity (uS/cm) in situ with a YSI-600OMS-V2 multiparameter sonde (Xylem Analytics, Yellow Springs, OH, USA.). At each site, we took water samples for subsequent nutrient analysis in the laboratory. We measured N, NO_3-N; NH_4-N and total phosphorus. Nitrogen was estimated according to the method of Müller and Weidemann (1955); total phosphorus was estimated following Valderrama (1981).

Assemblage sampling

Fish

Fish were sampled for 150 m in the rainforest sites, as used in other works in Amazonian streams (Cantanhêde et al. 2022; Colares et al. 2022; Montag et al. 2019), through use of 55 cm diameter siene nets (3 mm metallic mesh). A standardized sampling effort of six hours was established for each site, divided between collectors and longitudinal sections. The fish sampling was approved by the UFPA Ethics Committee approved (CEUA no. 8293020418). Fish assemblages were sampled for 50 m in the grassland sites, without block nets, using a generator type FEG 1000 backpack electrofisher, as recommended by Teixeira de Mello et al. (2014). We estimated the surface area influenced by electric pulses in the field according to Teixeira de Mello et al. (2014). Fifty pulses (diameter approx. $1m^2$) of current were conducted. The fish sampling was approved by the Ethics Committee (CHEA N°603 (101)- CEUA CURE). In both biomes, the collected fish were euthanized with an overdose of 2-phenoxy- ethanol solution and then fixed in 10% formalin. Prior to species identification, the fish were soaked in tap water. All fish specimens were identified to species in both biomes.

Arthropods

Arthropods were also sampled for 150 m in the rainforest sites, subdivided into 10 equidistant longitudinal sections. In each section, we used a round dipnet (18 cm in diameter, 0.05 mm mesh) to obtain two portions of substrates (e.g., leaf, wood debris, gravel, and roots) for a total of 20 subsamples. Arthropods were sampled for 50 m in the grassland sites through use of a modified Surber net (50 cm long × 25 cm wide). This net was then placed against the water flow where a manual removal of the substrate was carried out to promote movement of macroinvertebrates towards the net. At each of the 30 sites, we obtained a total of 6 subsamples (3 stations/site, 2 seasons). In both biomes, the arthropods collected were preserved in jars with ethanol and identified to the lowest practical taxonomic level (species or genus). It is important to note that in our study we use only insects to represent arthropods.

Macrophytes

Macrophyte taxa were identified for 50 m in the rainforest sites through use of quadrats, as employed by Fares et al. (2020) and Nonato et al. (2021). In this method, a PVC (polyvinyl chloride) square of 1 m2 (1 m × 1 m) was placed on a macrophyte bed and the cover percentage was measured from the dominant to the least abundant taxa. The sampling was randomly established on riffles with dense macrophyte cover and relatively easy access to achieve greater precision in our estimates. In the grassland sites, macrophyte taxa were identified every 25 cm along six perpendicular transects to the channel (at 0, 10, 20, 30, 40, 50 m). Because the streams studied were shallow, most macrophytes were identified in the field to genus (Alonso 1998).

Functional traits

A core set of organismal traits were measured across the three assemblages (i.e., fishes, arthropods, and macrophytes). We selected traits linked to three sets of major functional categories: (i) recruitment and life-history strategies, (ii) resource and habitat use, and (iii) body size. These three trait categories were selected because they are expected to exert strong effects on ecosystem functioning (Allan et al. 2015, Bagousse-Pinguet et al. 2019). Most importantly, those trait categories are strongly affected by land use changes (Birkhofer et al. 2015, Leitão et al. 2018, Price et al. 2019, Provost et al. 2020). Consequently, if land use affects this set of traits, these changes could have indirect effects on functioning of streams. Body size is a key trait for important functions, such as metabolism (Pianka 1970), reproduction rates (Martin et

al. 1993) and biotic interactions (Woodward et al. 2005, Pawar 2015, Atkinson et al. 2017, García-Girón et al. 2020b). Resource and habitat use (growth forms in plants, feeding groups and mouth position in fish, or feeding groups in arthropods) may well reflect the diversity of the resource pool and feeding diets across co-occurring taxonomic groups, which affects food webs (Vanni et al. 2002, McIntyre et al. 2008). Life-history is an evolutionary trait that reflects the adaptation of organisms to environmental changes and it influences ecosystem functioning (Violle et al. 2007; Mcclanahan and Humphries 2012). Likewise, recruitment reflects organism reproductive success in different environments and the ecological role of each assemblage in ecosystems. Thus, high recruitment rates may indicate more ecosystem functions performed by organisms and greater biomass in ecosystems (Symstad andTilman 2001, Massol et al. 2017).

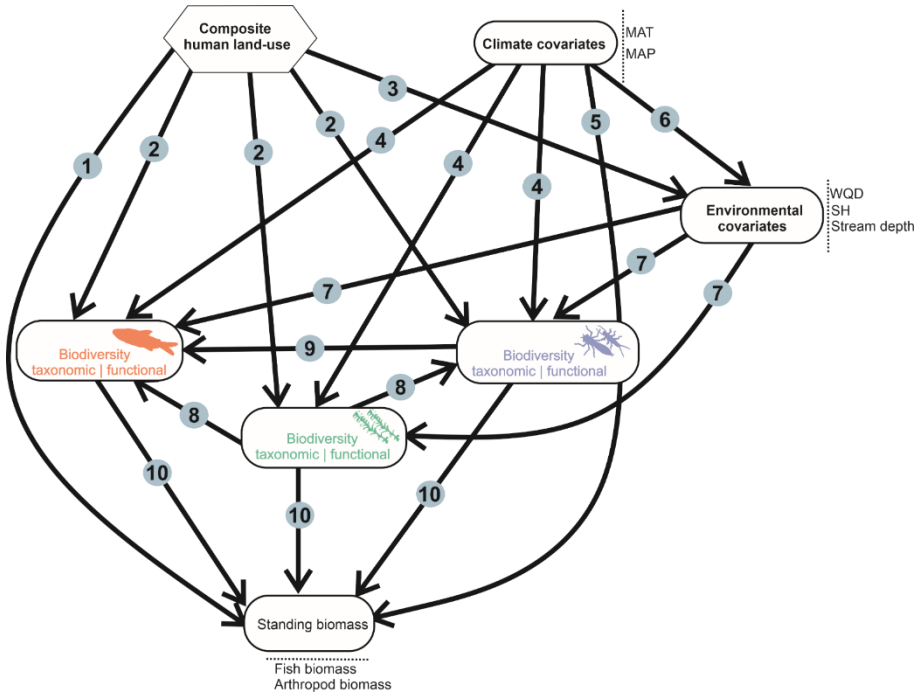


Figure S1. A priori structural equation modelling (SEM) aimed to evaluate how combined intensification of multiple land uses affects different facets of biodiversity (taxonomic and functional diversity), and their cascading effects on animal biomass in rainforest and grassland streams. We accounted for the effects of key environmental (water quality deterioration [WQD], and sediment heterogeneity [SH]) and climate (mean annual temperature [MAT] and precipitation [MAP]), and stream depth predictors in models. All variables in the model were measured at stream level, and represent the real conditions of these environments. For illustration purposes, different categories of predictors were grouped in the same box. However, these predictors were considered individually in the models. In the following table, we provide the conceptual supports for all the links within the model, based on results found in other studies. Therefore, all relationships within our model have theoretical support. Land-use is represented by a composite index (hexagon), which collapses the combined effect of four individual land-use (agriculture, pasture, urbanization and afforestation) into a unique effect.

	Links	*Rationale*	Ref.
1	**LUI → Standing biomass**	Land use intensification (LUI) may control ecosystem functions. For example, LUI is expected to decrease biomass stock in streams.	Allan et al., 2015; Fugère et al., 2018; Moi & Teixeira de Mello, 2021;
2	**LUI → Biodiversity**	Land use intensification is a key driver of biodiversity across multiple taxonomic groups. For example, species richness and functional diversity of fish, arthropod and macrophytes strongly decline with increasing LUI.	Newbold et al., 2015; Leitão et al., 2018; Price et al., 2019; Provost et al., 2020;
3	**LUI → Environmental covariates**	Land use intensity is expected to control environmental covariates in streams. For example, sediment is dominated by fine particles in streams with high levels of human-induced land use cover. Likewise, water quality decreases in streams experiencing high levels of upstream land-use degradation.	Teufl et al., 2013; Baumgartner & Robinson, 2015
4	**Climate → Biodiversity**	Climate is a key driver of species richness and functional diversity across multiple assemblages. For example, precipitation has been reported to be positively related to species richness and functional diversity of fish and macrophytes. Likewise, temperature tends to be positively correlated with species richness in multiple aquatic organisms.	Rasconi et al., 2015; Guo et al., 2015; Fu et al., 2021; Pereira et al., 2021; Boonman et l., 2021.
5	**Climate → Standing biomass**	Climate is a key driver of ecosystem functions. For example, temperature and precipitation are positively related to the standing biomass of ecosystems	Kratzer and Warren 2013; Levenstein et al. 2017;
6	**Climate → Environmental covariates**	Climate is considered as a major driver of local environmental covariates in streams	Olsen & Twonsend, 2003; Schmidt et al., 2006.
7	**Environmental covariates → Biodiversity**	Local environmental covariates are key drivers of species richness and functional diversity in streams. For example, sediment heterogeneity induces positive changes in species richness and functional diversity across multiple taxonomic groups.	Meuhlbauer & Doyle, 2012; Leitão et al.,

			2018; Moi & Teixeira de Mello, 2021
8	**Macrophyte → Fish and arthropod**	Macrophyte are expected to be strongly associated with the species richness and functional diversity of fishes and arthropods. In general, there is an increased in fish and arthropod diversity with increasing macrophyte diversity. This occurs because macrophyte provide habitat and refuge these two groups of organisms.	Warfe & Barmuta, 2006; Moi & Teixeira de Mello, 2021; Moi et al., 2021;
9	**Arthropod → Fish**	Arthropods are major food resource for fishes. Consequently, increased arthropod diversity leads to concomitant changes in fish diversity.	Small et al., 2012; Correa & Winemiller, 2018
10	**Biodiversity → Standing biomass**	Species richness and functional diversity are major drivers of ecosystem functioning, including standing biomass. Specifically, high levels of diversity cause an increase in standing biomass of ecosystems.	Lefcheck & Duffy, 2015; Moi & Teixeira de Mello, 2021; Ceulemans et al., 2021;

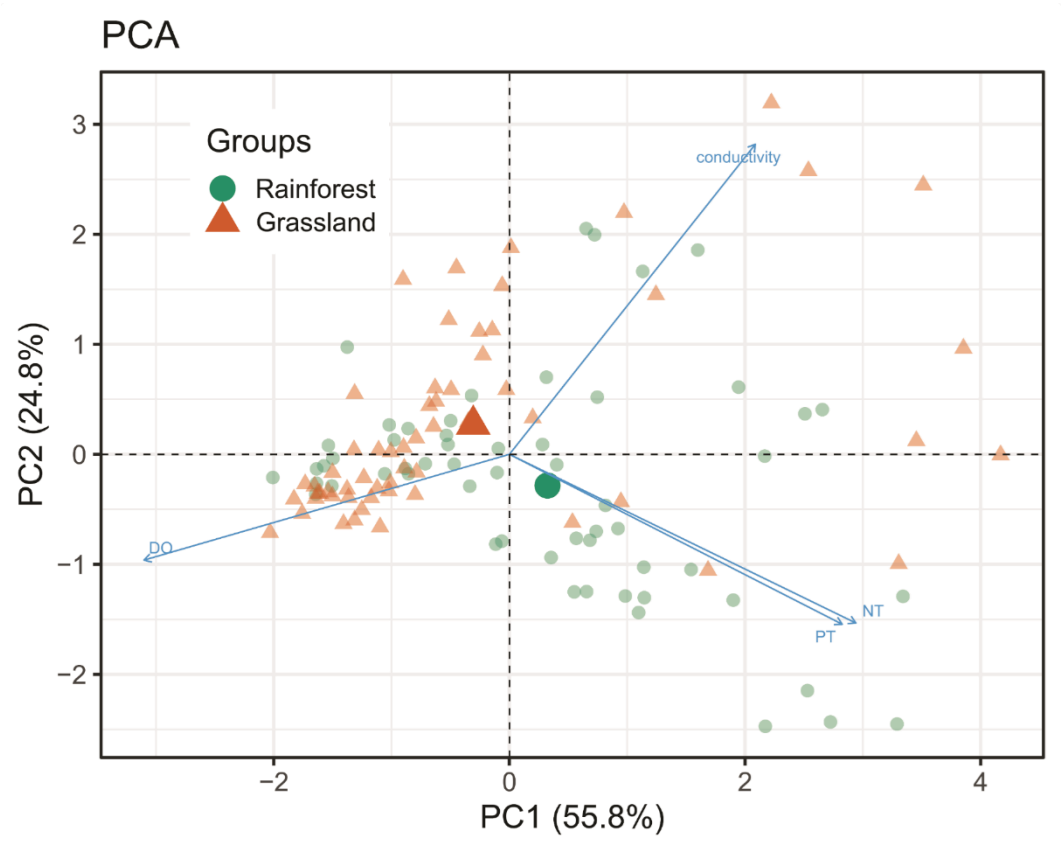


Figure S2. Relationships among water quality predictors. PC1 is positively correlated with total nitrogen (r = 0.53), total phosphorus (r = 0.51), and conductivity (r = 0.37), and negatively correlated with dissolved oxygen (r = -0.56). Thus, this axis represents a water quality deterioration index.

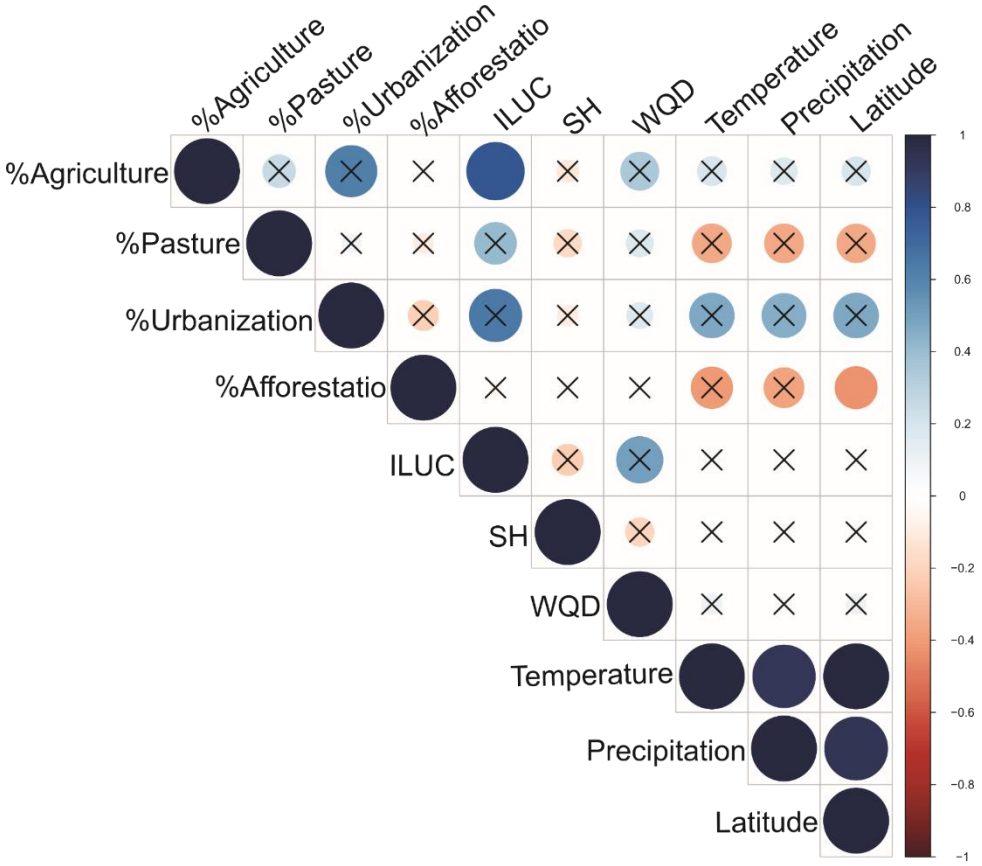


Figure S3. Correlations among predictors used in structural equation modeling (SEMs). Because of the high correlation between latitude and temperature (VIF > 2), we removed latitude from final models. Colors and color intensities represent correlation signs and levels. Non-significant correlations (P > 0.05) are indicated by an 'X'. ILUC = intensive human land-use, SH = sediment heterogeneity, and WQD = water quality deterioration.

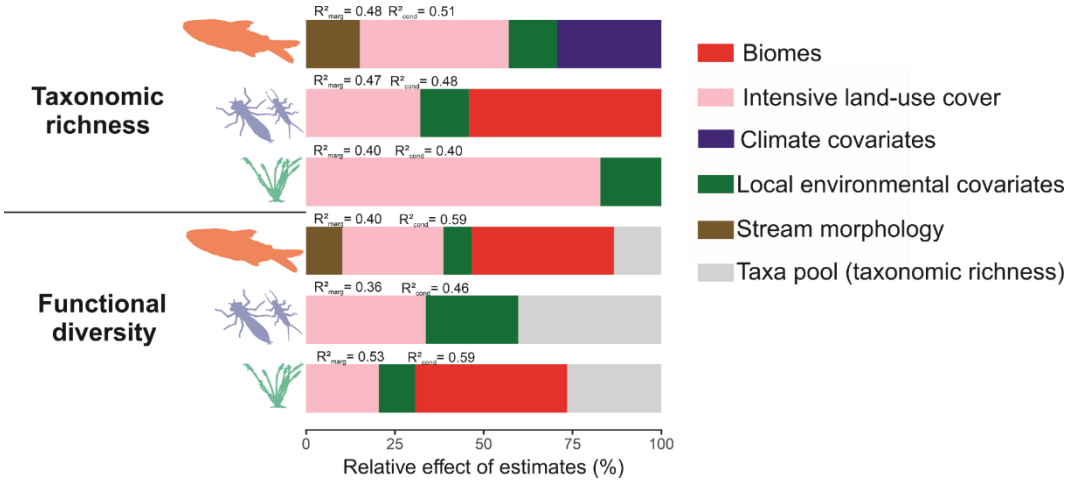


Figure S4. Relative importance of multiple predictors (biomes, human land-uses, climatic variables, environmental and, stream morphology, and the regional taxa pool) in explaining variation in taxonomic and functional diversities of fish, arthropods and macrophytes. The importance of predictors is expressed as the percentage of variance that they explain and is based on the absolute values of their standardized regression coefficients. All predictors were z-standardized to facilitate interpretation of parameter estimates on a comparable scale. Above the bars are the $R^2_{marginal}$ (variance explained only by fixed effects) and the $R^2_{conditional}$ (variance explained by fixed plus random effects) of the LMM model.

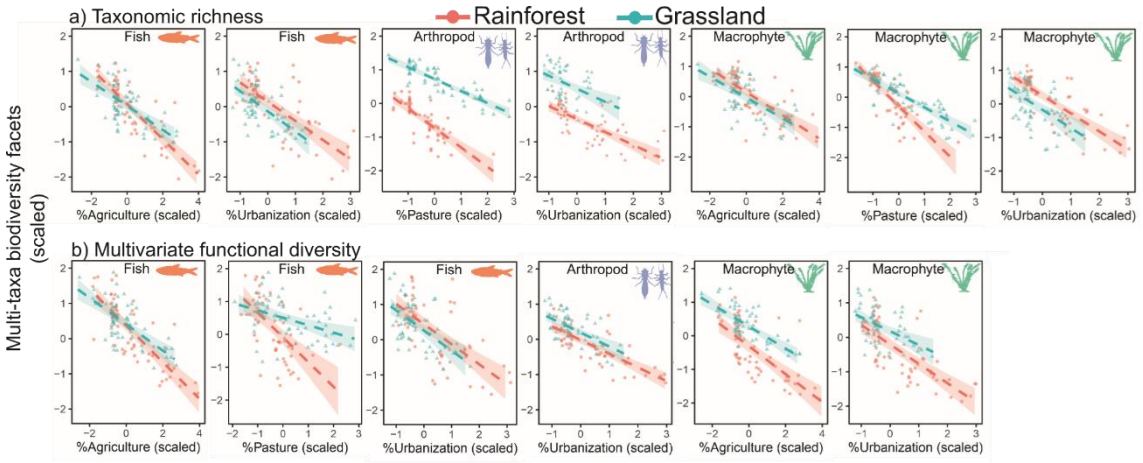
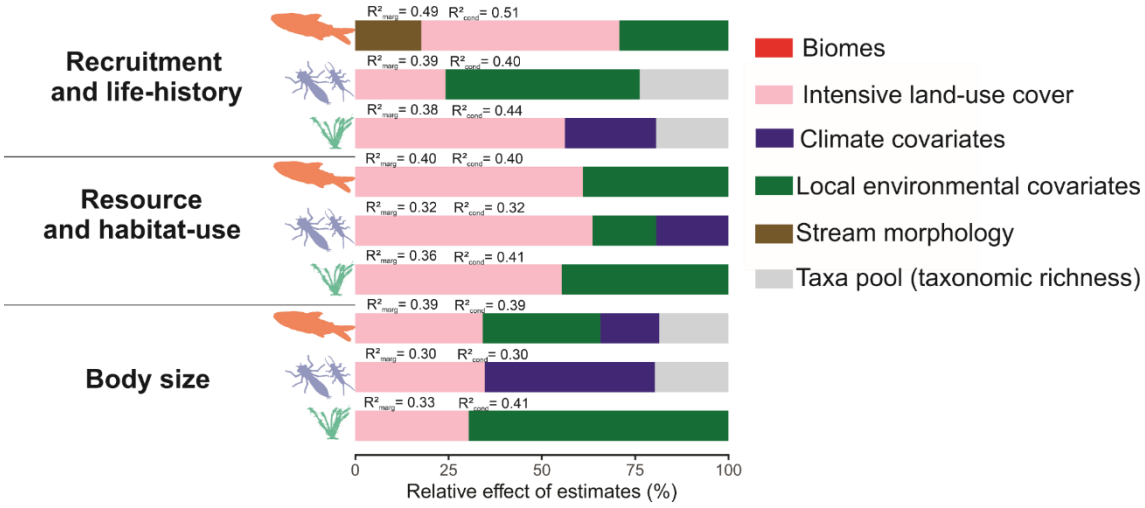


Figure S5. Relationships of the land-use types selected during backward selection for (a) taxonomic richness and (b) multivariate functional diversity of the assemblages. Dashed lines show model fits and colored shaded areas correspond to the 95% confidence interval from linear mixed effect models (LMM) for each biome. Model predictions were calculated using a model averaging procedure (see Methods). Land-use types were scaled to interpret parameter estimate on a comparable scale. *P*-values of the best predictors for each model are displayed. Symbols (n = 122) are the number of site visits and their shapes and colors indicate each biome: red circle (rainforest) and green triangle (grassland).



FigureS6. Relative importance of multiple predictors (biomes, human land-uses, environmental and climatic variables, stream morphology, and the regional taxa pool) in explaining the diversity of trait categories (i.e., CWV of recruitment and life-history, resource and habitat use, and body size) of fishes, arthropods, and macrophytes. The importance of predictors is expressed as the percentage of variance that they explain and is based on the absolute values of their standardized regression coefficients. All predictors were z-standardized to facilitate interpretation of parameter estimates on a comparable scale. Above the bars is shown the $R^2_{marginal}$ (variance explained only by fixed effects) and the $R^2_{conditional}$ (variance explained by fixed plus random effects) of the LMM model.

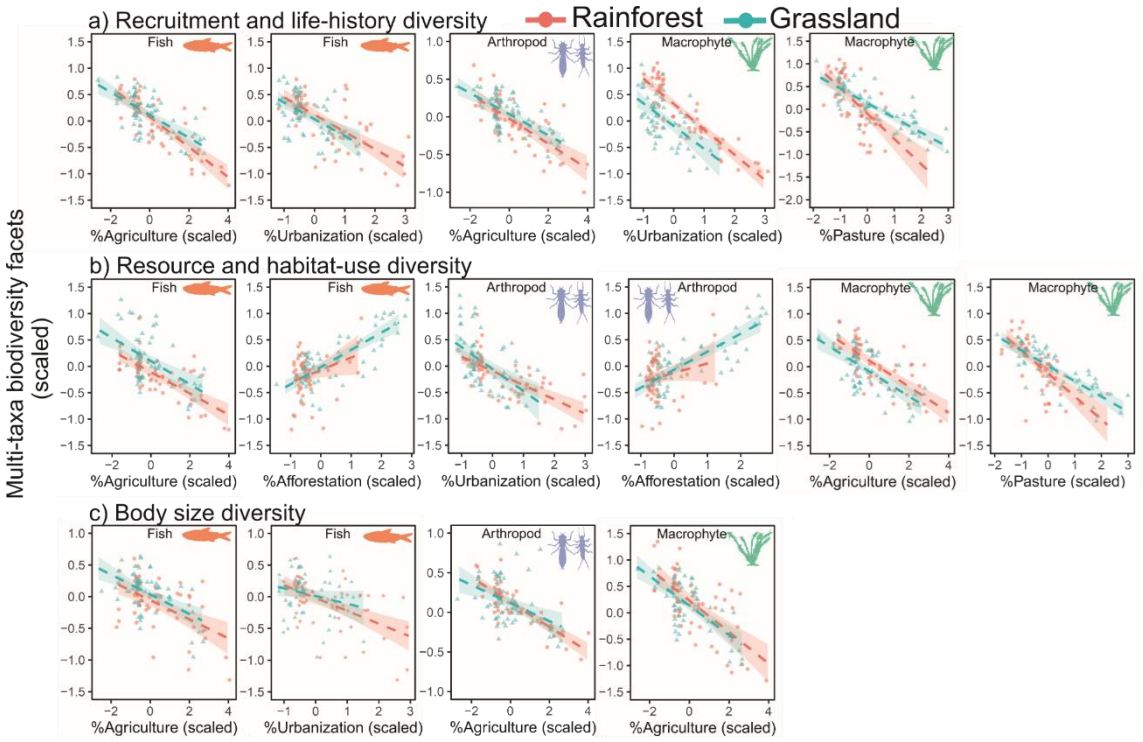


Figure S7. Relationships of the land-use types selected during backward selection with CWV of (a) recruitment and life-history, (b) resource and habitat-use, and (c) body size of fishes, arthropods and macrophytes. Dashed lines show the best model fits and colored shaded areas correspond to the 95% confidence interval from linear mixed effect models (LMM) for each biome. Model predictions were calculated using a model averaging procedure (see Materials and Methods). Land-use types were scaled to interpret parameter estimate on a comparable scale. P-values of the best predictors for each model are displayed. Symbols (n = 122) correspond to observed data and their shapes and colors indicate each biome: red circle (rainforest) and green triangle (grassland).

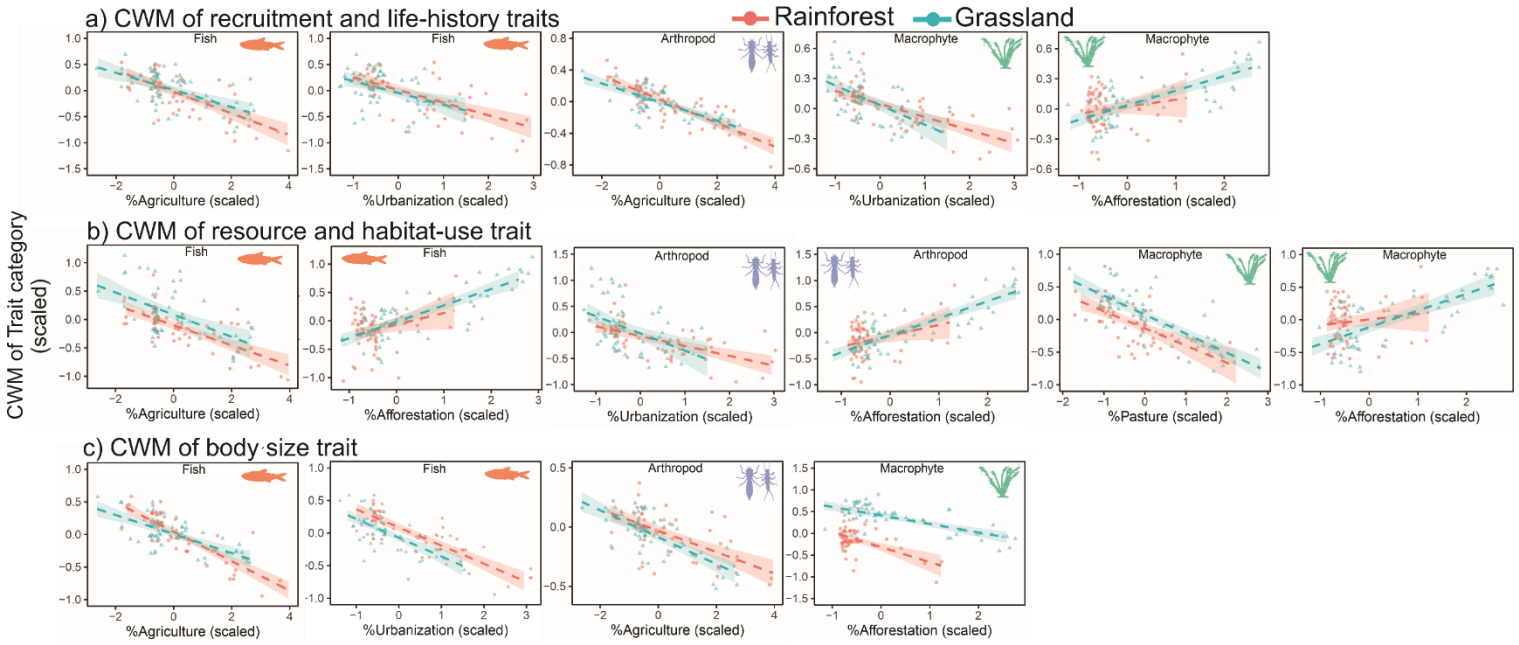


Figure S8. Relationships of the land-use types selected during backward selection with CWM of (a) recruitment and life-history, (b) resource and habitat-use, and (c) body size of fish, arthropod and macrophyte assemblages. Lines show the best model fits and colored shaded areas correspond to the 95% confidence interval from linear mixed effect models (LMM) for each biome. Model predictions were calculated using a model averaging procedure (see Methods). Land-use types were scaled to interpret parameter estimate on a comparable scale. P-values of the best predictors for each model are displayed. Symbols (n = 122) correspond to the number of site visits and their shapes and colors indicate the biome: red circles (rainforest) and green triangles (grassland).

Table S1. Functional traits used for classifying assemblages including types of traits, their categories and references used to identify each trait.

Taxonomic	Trait category	Trait	Type	Category	References
	Body size	Body size	Continuous	Average Length (cm)	Giam & Olden, 2018; Dos Santos et al., 2017; Oliveira et al.,
	Resource and habitat use	Position water		Pelagic Benthopelagic Benthic	Dos Santos et al., 2017; Oliveira et al., 2018.
		Trophic groups		Piscivore Omnivore Detritivore Herbivore Insectivore Invertivore	
				Subterminal Terminal Superior Low	
	Recruitment and	Eggs parental care	Dummy	Presence/absence	
		Larval parental care	Dummy	Presence/absence	Oliveira et al., 2018
				Viviparous Oviparous	
				Internal External	
Arthropods					
	Body size	Body size	Continuous	Average Length (mm)	Poff et al., 2006; Tomanova et al., 2007
				Shredders Predators Scrapers Collector-filters Collector-gatherers Piercers	
				Burrowers Climbers	

			Skaters Sprawlers Swimmers	
			Air Branchial Integumentary Plastron Stigmata	
	Recruitment and		Univoltine Semivoltine Plurivoltine	
		Refuge use	Categorical	Networkds Sand and debris Wood Builders No refuge	Poff et al., 2006; Tomanova et al., 2007; Hamada et al., 2014
Macrophytes					
	Body size	Plant vegetative height	Continuous	m	Kattge et al., 2011; García-Girón et al., 2020b
	Resource and habitat use			Submerged Emergent Free-floating Rooted-floating	Castroviejo, 2012; Campostrini-Forzza et al., 2010; Cirujano et al., 2014; García-Girón et al., 2020a
		Specific leaf area	Continuous	mm^2/mg^{-1}	Kattge et al., 2011; Fu et al., 2014; García-Girón et al.,
		Leaf area	Continuous	mm^2	Kattge et al., 2011
		leaf compoundness		simple compound	
		Seed dry mass	Continuous	mg	Kattge et al., 2011
		Propagation mode	Categorical	By seed/by spore Mostly by seed/spore, also By seed/spore and	

		Mostly vegetatively, also Vegetatively
Main dispersal agent	Categorical	Passive Wind Water Animals Wind+Water Wind+Animals Water+Animals Wind+Water+Animals
Plant phenology	Categorical	Perennial Annual/short-lived perennial

Table S2. Full linear mixed-effect models (i.e., LMM with all predictors) that were used for both taxonomic richness and functional diversity of each of the three assemblages. These full models were then reduced by using AICc selection to select only those best predictor of each biodiversity attribute (see Table S4 and S5).

Response	**Predictors**
Fish richness	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes
Arthropod richness	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes
Macrophyte richness	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes
Fish functional diversity	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Fish richness Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Arthropod functional diversity	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Arthropod richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Macrophyte functional diversity	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Macrophyte richness +

	Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Macrophyte richness*Biomes
Fish recruitment and life-history	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Fish richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Arthropod recruitment life-history	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Arthropod richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Macrophyte recruitment life-history	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Macrophyte richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Macrophyte richness*Biomes
Fish resource-habitat-use	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Fish richness Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Arthropod resource-habitat-use	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Arthropod richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Macrophyte resource-habitat-use	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Macrophyte richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Macrophyte richness*Biomes

Fish body size	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Fish richness Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Arthropod body size	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Arthropod richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Fish richness*Biomes
Macrophyte body size	Biomes + Agricul + Past + Urban + affor + Temp + Precip + SH + WQD + Depth + Macrophyte richness + Agricul*Biomes + Past*Biomes + Urban*Biomes + affor*Biomes + Temp*Biomes + Precip*Biomes + SH*Biomes + WQD*Biomes + Depth*Biomes + Macrophyte richness*Biomes

Table S3. Results of the variance inflation factor (VIF) to evaluate the risk of multicollinearity among predictors in the best selected models. VIF values exceeding 2 suggest multicollinearity in the model. MAP = mean annual precipitation; MAT = mean annual temperature; SH = sediment heterogeneity; WQD + water quality deterioration.

Predictor	VIF
Fish richness ~ %Agriculture + %urbanization + MAP + MAT + WQD + Stream depth	1.9
Arthropod richness ~ Biomes + %pasture + %urbanization + SH + WQD	1.5
Macrophyte richness ~ %Agriculture + %pasture + %urbanization + WQD	1.2
Fish FD ~ Biomes + %agriculture + %pasture + %urbanization + WQD + Stream depth + fish richness	1.4
Arthropod FD ~ %urbanization + SH + arthropod richness	1.4
Macrophyte FD ~ Biomes + %agriculture + %urbanization + SH + plant richness	1.2
Fish Recruitment and life-history ~ %Agriculture + %urbanization + WQD + stream depth	1.3
Arthropod Recruitment and life-history ~ %Agriculture + %afforestation + MAP + WQD	1.1
Macrophyte Recruitment and life-history ~ %Pasture + %urbanization + MAP + MAT + plant richness	1.7
Fish Resource and habitat use ~ %Agriculture + %afforestation + WQD + SH	1.0
Arthropod Resource and habitat use ~ %Urbanization + %afforestation + precipitation + WQD	1.0
Macrophyte Resource and habitat use ~ %Agriculture + %pasture + SH + WQD	1.2
Fish Body size ~ %Agriculture + %urbanization + MAT + SH + WQD + fish richness	1.2
Arthropod Body size ~ %Agriculture + MAP + MAT + arthropod richness	1.3
Macrophyte Body size ~ %Agriculture + SH + WQD	1.0

Table S4. Model selection using a backward selection procedure based on AICc of the best set of predictors explaining (a) taxonomic richness and (b) multivariate functional diversity of fish, arthropod, and macrophyte assemblages. ΔAICc = difference between the model with the lowest score and subsequent model. Only the best subset models (ΔAICc < 2) are presented. SH = sediment heterogeneity; WQD = water quality deterioration; MAT = mean annual temperature; MAP = mean annual precipitation. Colors indicate which predictors were included in the best models. Red represents biomes (rainforest and grasslands), pink represents land-use variables (agriculture, pasture, urbanization and afforestation), green represents local environmental covariates, deep blue represents climate covariates, gray represents species (taxonomic richness), and brown represents stream morphology (stream depth). White cells represent predictors not included in the best models.

A)

Taxa richness

		Fishes				Arthropods				Macrophytes	
	Models	1	2	3	4	1	2	3	4	1	2
	AICc	289.2	290.4	290.6	290.7	302.5	302.7	303.9	304.7	312.6	315.2
	ΔAICc	0	1.20	1.38	1.49	0	0.28	1.48	2.00	0	2.65
	Weight	0.184	0.101	0.092	0.088	0.276	0.240	0.132	0.090	0.540	0.143
Biome	Rainforest/grassland										
Human land-use	%Agric										
	%Pasture										
	%Urban										
	%Afforest										
	%Agric*Biome										
	%Pasture*Biome										
	%Urban*Biome										
	%Afforest*Biome										
Environment covariates	WQD										
	SH										
	WQD*Biome										
	SH *Biome										
Climate covariates	MAT										
	MAP										
	MAT*Biome										
	MAP*Biome										
Stream morphology	Depth										
	Depth*Biome										

B)

Multivariate functional diversity

		Fishes				Arthropods			Macrophytes		
	Models	1	2	3	4	1	2	3	1	2	3
	AICc	317.0	317.6	317.6	317.6	327.3	327.6	328.8	273.8	274.2	276.7
	ΔAICc	0	0.55	0.56	0.60	0	0.29	1.49	0	0.49	1.93
	Weight	0.170	0.129	0.128	0.125	0.338	0.293	0.161	0.362	0.283	0.084
Biome	Rainforest/grassland										
Human land-use	%Agric										
	%Pasture										
	%Urban										
	%Afforest										
	%Agric*Biome										
	%Pasture*Biome										
	%Urban*Biome										
	%Afforest*Biome										
Environment covariates	WQD										
	SH										
	WQD*Biome										
	SH*Biome										
Climate covariates	MAT										
	MAP										
	MAT*Biome										
	MAP*Biome										
Stream morphology	Depth										
	Depth*Biome										
Taxa pool	Taxa richness										
	Taxa richness*Biome										

Table S5. Model selection using a backward selection procedure based on AICc of the best set of predictors explaining trait categories, (a) recruitment and life-history, (b) resource and habitat-use, and (c) body size of fishes, arthropods, and macrophytes. ΔAICc = difference between the model with the lowest score and subsequent model. Only the best subset models (ΔAICc < 2) are presented. SH = sediment heterogeneity; WQD = water quality deterioration; MAT = mean annual temperature; MAP = mean annual precipitation. Predictors included in the selected best models are indicated with colors. Red represents biomes (rainforest and grasslands), pink represents human land-uses variables (agriculture, pasture, urbanization and afforestation), green represents local environmental covariates, deep blue represents climate covariates, gray represents species (taxonomic richness), and brown represents stream morphology (stream depth). White cells represent predictors not included in the best models.

A) **Recruitment and life-history**

		Fishes					Arthropods				Macrophytes		
	Models	1	2	3	4	5	1	2	3	4	1	2	3
	AICc	174.7	175.2	175.3	175.9	176.1	173.6	174.0	175.1	175.3	256.1	256.9	257.2
	ΔAICc	0	0.52	0.58	1.26	1.45	0	0.34	1.46	1.70	0	0.83	1.13
	Weight	0.261	0.201	0.195	0.139	0.126	0.258	0.218	0.124	0.110	0.311	0.206	0.177
Biome	Rainforest/grassland												
Human land-use	%Agric												
	%Pasture												
	%Urban												
	%Afforest												
	%Agric*Biome												
	%Pasture*Biome												
	%Urban*Biome												
	%Afforest*Biome												
Environment covariates	WQD												
	SH												
	WQD*Biome												
	SH*Biome												
Climate covariates	MAT												
	MAP												
	MAT*Biome												
	MAP*Biome												
Stream morphology	Depth												
	Depth*Biome												
Taxa pool	Taxa richness												
	*Biome												

B)

Resource and habitat-use

		Fishes				Arthropods			Macrophytes		
	Models	1	2	3	4	1	2	3	1	2	3
	AICc	232.3	233.0	234.1	234.2	271.5	272.4	272.9	280.0	280.2	282.0
	ΔAICc	0	0.76	1.84	1.89	0	0.91	1.40	0	0.19	1.99
	Weight	0.344	0.236	0.137	0.134	0.361	0.229	0.180	0.272	0.247	0.100
Biome	Rainforest/grassland										
Human land-use	%Agric										
	%Pasture										
	%Urban										
	%Afforest										
	%Agric*Biome										
	%Pasture*Biome										
	%Urban*Biome										
	%Afforest*Biome										
Environment covariates	WQD										
	SH										
	WQD*Biome										
	SH*Biome										
Climate covariates	MAT										
	MAP										
	MAT*Biome										
	MAP*Biome										
Stream morphology	Depth										
	Depth*Biome										
Taxa pool	Taxa richness										
	Taxa richness*Biome										

		Fishes						Arthropods		Macrophytes			
		Models	1	2	3	4	5	6	1	2	1	2	3	
		AICc	289.	289.1	289.9	290.5	290.7	291.0	191.8	194.0	345.1	346.2	347.1	347.1
		ΔAICc	0	0.04	0.84	1.45	1.67	1.94	0	2.00	0	1.11	2.07	
		Weight	0.101	0.099	0.066	0.049	0.044	0.038	0.373	0.125	0.505	0.290	0.179	
Biome	Rainforest/grassland													
Human land-use	%Agric													
	%Pasture													
	%Urban													
	%Afforest													
	%Agric*Biome													
	%Pasture*Biome													
	%Urban*Biome													
	%Afforest*Biome													
Environment covariates	WQD													
	SH													
	WQD*Biome													
	SH*Biome													
Climate covariates	MAT													
	MAP													
	MAT*Biome													
	MAP*Biome													
Stream morphology	Depth													
	Depth*Biome													
Taxa pool	Taxa richness													
	Taxa richness*Biome													

Table S6. Best models from the model averaging procedure in linear mixed effect models (Appendix S1: Table 4; LMM). SH = Sediment heterogeneity; and WQD = water quality deterioration. *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$. Note that interactive effects (predictors vs. biomes) were never selected, suggesting a consistency of relationships across biomes.

		Estimate	CI(lower)	CI(upper)	Std error	t-value	*P*-value
Fish taxonomic richness	Intercept	–0.035	–0.402	0.194	0.136	–0.556	0.657
	%Agriculture	–0.251	–0.347	–0.093	0.064	–3.52	< 0.001***
	%Urbanization	–0.243	–0.403	–0.069	0.084	–2.67	0.009**
	Precipitation	0.182	–0.001	0.245	0.063	1.97	0.052
	Temperature	0.163	–0.014	0.255	0.069	1.79	0.076
	WQD	–0.159	–0.235	–0.050	0.047	–2.93	0.004**
	Stream depth	0.177	0.009	0.271	0.066	2.0	0.048*
Arthropod taxonomic richness	Intercept	–0.361	–1.68	0.957	0.665	–0.542	0.589
	Rainforest	–0.786	–1.10	–0.472	0.163	–4.82	< 0.001***
	Grassland	0.503	0.118	0.711	0.154	2.69	0.008**
	%Pasture	–0.347	–0.445	–0.114	0.085	–3.26	0.001**
	%Urbanization	–0.217	–0.367	–0.014	0.091	–2.08	0.040*
	WQD	–0.107	–0.185	0.005	0.049	–1.81	0.073
	SH	0.238	0.037	0.385	0.090	2.34	0.021*
Macrophyte taxonomic richness	Intercept	0.041	–0.106	0.193	0.077	0.562	0.575
	%Agriculture	–0.171	–0.273	0.015	0.074	–1.73	0.086
	%Pasture	–0.355	–0.502	–0.182	0.082	–4.14	< 0.001***
	%Urbanization	–0.289	–0.419	–0.049	0.095	–2.45	0.016*
	WQD	–0.169	–0.253	–0.042	0.054	–2.72	0.008**
	Intercept	–0.239	0.585	1.09	0.423	0.599	< 0.001***

Fish FD	Rainforest	0.658	0.385	0.861	0.125	4.97	< 0.001***
	Grassland	–0.155	–0.361	0.116	0.118	–1.17	0.245
	%Agriculture	–0.204	–0.352	–0.057	0.077	–2.64	0.009**
	%Pasture	–0.114	–0.245	0.102	0.090	–0.789	0.432
	%Urbanization	–0.364	–0.513	–0.099	0.108	–2.82	0.006**
	WQD	–0.167	–0.239	–0.022	0.056	–2.31	0.023*
	Stream depth	0.208	0.024	0.323	0.078	2.22	0.028*
	Fish richness	0.300	0.002	0.402	0.095	2.66	0.009**
Arthropods FD	Intercept	0.083	–0.494	0.083	0.148	–1.383	0.169
	%Urbanization	–0.275	–0.460	–0.067	0.101	–2.610	0.010*
	SH	0.232	0.023	0.426	0.103	2.165	0.032*
	Arthropod richness	0.329	0.099	0.448	0.090	3.041	0.003**
Macrophyte FD	Intercept	–0.313	–1.398	0.771	0.547	–0.572	0.568
	Rainforest	–0.183	–1.018	–0.221	0.198	–3.071	0.035*
	Grassland	0.224	–0.347	0.461	0.200	0.389	0.717
	%Agriculture	–0.183	–0.267	–0.032	0.0604	–2.507	0.014*
	%Urbanization	–0.179	–0.320	0.030	0.090	–1.568	0.012*
	SH	0.183	0.029	0.336	0.079	2.286	0.024*
	Plant richness	0.477	0.275	0.559	0.073	5.733	<0.001***

Table S7. Best models from the model averaging procedure in linear mixed effect models (Appendix S1: Table 5; LMM) for diversity trait categories (CWV of recruitment and life-history, resource and habitat use, and body size) of each assemblages. MAP = mean annual precipitation; MAT = mean annual temperature; SH = Sediment heterogeneity; and WQD = water quality deterioration. *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$. Note that interactive effects (predictors vs. biomes) were never selected, suggesting a consistency of relationships across biomes.

		Estimate	CI(lower)	CI(upper)	Std error	t-value	*P*-value
Fishes							
Recruitment and life-history	Intercept	0.106	–0.017	0.273	0.068	1.609	0.393
	%Agriculture	–0.136	–0.194	–0.040	0.040	–2.846	0.005**
	%Urbanization	–0.170	–0.221	–0.018	0.052	–2.412	0.017*
	WQD	–0.169	–0.208	–0.098	0.028	–5.409	< 0.001***
	Stream depth	0.102	0.014	0.169	0.039	2.365	0.020*
Resource and habitat use	Intercept	–0.085	–0.358	0.014	0.096	–1.784	0.077
	%Agriculture	–0.145	–0.216	–0.039	0.045	–2.806	0.006**
	%Afforestation	0.312	0.201	0.414	0.055	5.597	< 0.001***
	SH	0.160	0.022	0.278	0.066	2.275	0.025*
	WQD	–0.131	–0.181	–0.040	0.036	–3.053	0.003**
Body size	Intercept	–0.040	–0.375	0.099	0.124	–1.103	0.272
	%Agriculture	–0.153	–0.249	0.017	0.069	–1.664	0.059
	%Urbanization	–0.168	–0.325	0.015	0.088	–1.745	0.054
	Temperature	–0.148	–0.261	–0.013	0.064	–2.124	0.036*
	WQD	–0.131	–0.237	–0.044	0.050	–2.790	0.006**
	SH	0.165	–0.004	0.325	0.0863	1.859	0.066
	Fish richness	–0.175	–0.375	–0.014	0.094	–2.073	0.040*
Arthropods							
Recruitment and life-history	Intercept	–0.205	–0.338	–0.037	0.079	–2.353	0.037*
	%Agriculture	–0.120	–0.153	–0.010	0.036	–2.273	0.025*
	SH	0.175	0.052	0.254	0.052	2.967	0.004**
	WQD	–0.082	–0.119	–0.008	0.028	–2.156	0.033*
	Arthropod richness	0.118	0.012	0.175	0.042	2.260	0.026*
Resource and habitat use	Intercept	–0.072	–0.261	0.041	0.070	–1.420	0.158
	%Urbanization	–0.217	–0.342	–0.068	0.070	–2.908	0.004**
	%Afforestation	0.305	0.179	0.439	0.067	4.604	< 0.001***
	Precipitation	0.158	0.020	0.259	0.061	2.273	0.025*
	WQD	–0.140	–0.197	–0.033	0.042	–2.724	0.007**
Body size	Intercept	0.116	–0.025	0.178	0.050	1.595	0.113
	%Agriculture	–0.136	–0.183	–0.035	0.038	–2.860	0.005**
	Temperature	–0.093	–0.218	–0.048	0.043	–3.050	0.003**
	Precipitation	0.086	0.032	0.209	0.045	2.638	0.009**
	Arthropod richness	0.077	0.015	0.192	0.045	2.269	0.025*
Macrophytes							
Recruitment and life-history	Intercept	0.065	–0.157	0.094	0.134	0.230	0.848
	%Pasture	–0.245	–0.364	–0.091	0.070	–3.074	0.003**
	%Urbanization	–0.291	–0.391	–0.123	0.069	–3.979	< 0.001***
	Precipitation	0.139	0.038	0.276	0.061	2.456	0.016*
	Temperature	–0.094	–0.213	0.011	0.057	–1.732	0.086
	Macrophyte richness	0.185	0.030	0.300	0.069	2.283	0.024*
	Intercept	–0.118	–0.500	0.059	0.143	–1.483	0.177

Resource and habitat use	%Agriculture	–0.173	–0.238	–0.014	0.057	–2.266	0.025*
	%Pasture	–0.245	–0.344	–0.067	0.071	–2.886	0.005**
	SH	0.195	0.014	0.334	0.082	2.141	0.034*
	WQD	–0.141	–0.182	0.006	0.047	–1.751	0.083
Body size	Intercept	–0.328	–0.814	0.206	0.246	–1.167	0.305
	%Agriculture	–0.241	–0.344	–0.045	0.076	–2.583	0.011*
	SH	0.415	0.175	0.605	0.110	3.550	< 0.001***
	WQD	–0.137	–0.260	–0.019	0.061	–2.225	0.028*

Table S8. Best models from the model averaging procedure in linear mixed effect models for community-weighted mean traits (CWMs) of each trait category (recruitment and life-history, resource and habitat use, and body size) of each assemblage. MAP = mean annual precipitation; MAT = mean annual temperature; SH = Sediment heterogeneity; and WQD = water quality deterioration. *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$.

		Estimate	CI(lower)	CI(upper)	Std error	t-value	*P*-value
Fishes							
Recruitment and life-history	Intercept	0.014	–0.120	0.175	0.066	0.213	0.863
	Fish richness	–0.114	–0.222	–0.012	0.054	–2.112	0.037*
	%Agriculture	–0.095	–0.171	–0.014	0.040	–2.350	0.021*
	%Urbanization	–0.109	–0.207	–0.008	0.051	–2.131	0.035*
	WQD	–0.170	–0.231	–0.117	0.029	–5.889	<0.001***
	Stream depth	0.089	0.012	0.166	0.039	2.260	0.026*
Resource and habitat use	Intercept	–0.134	–0.354	0.074	0.110	–1.210	0.259
	%Agriculture	–0.110	–0.196	–0.017	0.045	–2.414	0.017*
	%Afforestation	0.273	0.163	0.378	0.055	4.942	<0.001***
	WQD	–0.107	–0.183	–0.038	0.036	–2.939	0.004**
	SH	0.117	–0.011	0.243	0.065	1.790	0.076
Body size	Intercept	–0.050	–0.277	0.075	0.062	–0.815	0.416
	%Agriculture	–0.161	–0.213	–0.011	0.052	–2.164	0.033*
	%Urbanization	–0.112	–0.298	–0.024	0.070	–2.293	0.024*
	MPA	0.135	0.033	0.237	0.052	2.586	0.011*
Arthropods							
Recruitment and life-history	Intercept	–0.172	–0.309	–0.042	0.069	–2.499	0.030*
	%Agriculture	–0.113	–0.166	–0.057	0.028	–4.017	<0.001***
	MAT	0.060	–0.000	0.123	0.031	1.908	0.059
	SH	0.136	0.049	0.222	0.044	3.070	0.003**
Resource and habitat use	Intercept	–0.069	–0.327	0.155	0.109	–0.636	0.583
	%Urbanization	–0.135	–0.267	–0.005	0.067	–2.007	0.047*
	%Afforestation	0.303	0.177	0.427	0.064	4.701	< 0.001***
	MAP	0.129	0.016	0.244	0.058	2.214	0.029*
	WQD	–0.097	–0.178	–0.020	0.040	–2.416	0.017*
Body size	Intercept	–0.036	–0.105	0.028	0.032	–1.119	0.265
	%Agriculture	–0.058	–0.110	–0.005	0.027	–2.139	0.035*
	WQD	–0.092	–0.135	–0.050	0.021	–4.235	< 0.001***
	Stream depth	–0.066	–0.125	–0.007	0.030	–2.204	0.030*
Macrophytes							
Recruitment and life-history	Intercept	0.018	–0.057	0.094	0.038	0.488	0.626
	%Urbanization	–0.097	–0.171	–0.024	0.038	–2.563	0.012*
	%Afforestation	0.129	0.059	0.199	0.036	3.578	< 0.001***
	MAP	0.082	0.018	0.146	0.033	2.484	0.014*
	WQD	–0.049	–0.093	–0.005	0.022	–2.162	0.033*
Resource and habitat use	Intercept	–0.214	–0.431	–0.002	0.111	–1.923	0.090
	%Pasture	–0.172	–0.283	–0.062	0.056	–3.027	0.003**
	%Afforestation	0.186	0.074	0.290	0.055	3.377	0.001**
	SH	0.167	0.037	0.292	0.065	2.549	0.012*
	WQD	–0.089	–0.162	–0.021	0.035	–2.492	0.014*
Body size	Intercept	–0.328	–0.814	0.206	0.246	–1.167	0.305
	Rainforest	–0.145	–0.440	0.156	0.157	–0.929	0.371

	Grassland	0.423	0.181	0.674	0.131	3.210	0.037*
	%Afforestation	–0.236	–0.430	–0.043	0.100	–2.360	0.020*
	MAP	–0.212	–0.391	–0.032	0.092	–2.297	0.023*
	MAT	0.197	0.038	0.357	0.082	2.404	0.018*

Table S9. Pearson's correlations between weighted variance traits (CWV), and weighted mean traits (CWM) of traits categories: recruitment and life-history, resource and habitat-use, and body-size of fishes, arthropod, and macrophytes.

CWM		CMV	t-value	P-value
Fishes				
recruitment and life-histo	~~	recruitment and life-history	16.37	<0.001***
resource and habitat-use	~~	resource and habitat-use	23.38	<0.001***
body sizes	~~	body size	3.74	<0.001***
Arthropods				
recruitment and life-histo	~~	recruitment and life-history	10.39	<0.001***
resource and habitat-use	~~	resource and habitat-use	15.56	<0.001***
body size	~~	body size	10.55	<0.001***
Macrophytes				
recruitment and life-histo	~~	recruitment and life-history	4.78	<0.001***
resource and habitat-use	~~	resource and habitat-use	2.54	<0.001***
body size	~~	body size	6.78	<0.001***

Table S10. Coefficient estimates from mixed-effects models that included interaction terms to test whether land-use types (agriculture, pasture, urbanization and afforestation) influence the relationship between biodiversity andstanding biomass in each studied biome (i.e., rainforest and grassland). Biodiversity metrics included taxonomic and multivariate functional diversity of fish and arthropod assemblages. Bolded confidence intervals denote those that did not overlap zero. Analysis were performed to each biome separately. biomass and richness were modelled on the log-scale, and the presented confidence intervals are not back-transformed. $R^2_{marginal}$ = variance explained only by fixed effects; $R^2_{conditional}$ = variance explained by fixed plus random effects.

			Standing biomass			
Biome	Biodiversity metric	Explanatory variable	Estimate	95% CI	75% CI	*P*-value
Rainforest	Taxa richness	Fish rich	0.562	**0.25, 0.86**	**0.38,0.74**	< 0.001***
	$R^2_{marginal}$ = 0.533	Fish rich*agricul	–0.059	–0.14,0.02	–0.11,–0.00	0.183
	$R^2_{conditional}$ = 0.584	Fish rich*pasture	–0.067	**–0.29,–0.00**	**–0.22,–0.06**	0.049*
		Fish rich*urban	–0.067	–0.18,0.04	–0.13,0.00	0.253
		Fish rich*afforest	–0.067	–0.24,0.10	–0.16,0.03	0.446
Grassland	Taxa richness	Fish rich	0.829	**0.12,1.53**	**0.41,1.23**	0.022*
	$R^2_{marginal}$ = 0.459	Fish rich*agricul	–0.128	**–0.22,–0.03**	**–0.18,–0.07**	0.010*
	$R^2_{conditional}$ = 0.480	Fish rich*pasture	–0.025	–0.11,0.06	–0.07,0.02	0.588
		Fish rich*urban	–0.075	–0.28,0.13	–0.19,0.04	0.472
		Fish rich*afforesta	0.076	–0.01,0.16	0.02,0.12	0.084
Rainforest	Functional diversity	Fish FD	2.031	**0.11,3.94**	**0.92,3.14**	0.038*
	$R^2_{marginal}$ = 0.532	Fish FD*agricul	–0.420	–1.02,0.17	–0.76,–0.07	0.166
	$R^2_{conditional}$ = 0.591	Fish FD*pasture	–1.729	**–2.94,–0.51**	**–2.43,–1.02**	0.006**
		Fish FD*urban	–0.726	**–1.45,–0.00**	**–1.14,–0.30**	0.049*
		Fish FD*afforest	–0.247	–1.45,0.96	–0.94,0.45	0.682
Grassland	Functional diversity	Fish FD	2.755	**0.25,5.25**	**1.30,4.20**	0.031*
	$R^2_{marginal}$ = 0.465	Fish FD*agricul	–1.131	**–1.96,–0.30**	**–1.61,–0.65**	0.008**
	$R^2_{conditional}$ = 0.482	Fish FD*pasture	–0.420	–1.18,0.34	–0.86,0.02	0.276
		Fish FD*urban	–1.536	**–3.01,-0.05**	**–2.39,–0.67**	0.042*
		Fish FD*afforesta	0.276	–0.42,0.97	–0.12,0.68	0.430
Rainforest	Taxa richness	Arthropod rich	0.406	**0.21,0.75**	**0.32,0.64**	< 0.001***
	$R^2_{marginal}$ = 0.513	Arthropod rich*agricul	–0.089	–0.07,0.02	–0.05,–0.00	0.304
	$R^2_{conditional}$ = 0.531	Arthropod rich*pasture	–0.120	–0.19,0.05	–0.14,–0.00	0.275
		Arthropod rich*urban	–0.099	**–0.14,–0.00**	**–0.11,–0.03**	0.029*
		Arthropod rich*afforest	–0.039	–0.08,0.16	–0.02,0.11	0.485
Grassland	Taxa richness	Arthropod rich	0.640	**0.40,0.87**	**0.11,0.37**	< 0.001***
	$R^2_{marginal}$ = 0.565	Arthropod rich*agricul	–0.037	**–0.06, –0.01**	**–0.15,–0.07**	0.003**
	$R^2_{conditional}$ = 0.565	Arthropod rich*pasture	–0.010	–0.03,0.01	–0.07,0.01	0.440
		Arthropod rich*urban	–0.052	**–0.09,–0.00**	**–0.19, –0.0**	0.021*
		Arthropod rich*afforest	0.007	0.03,0.01	0.04,0.12	0.507
Rainforest	Functional diversity	Arthropod FD	3.606	**2.07, 5.13**	**2.72,4.49**	< 0.001***
	$R^2_{marginal}$ = 0.556	Arthropod FD*agricul	–0.561	**–1.06,–0.05**	**–0.85,–0.26**	0.031*
	$R^2_{conditional}$ = 0.582	Arthropod FD*pasture	0.271	–1.08,1.62	–0.51,1.05	0.689
		Arthropod FD*urban	–1.216	**–2.05,–0.38**	**–1.70,– 0.7**	0.005**
		Arthropod FD*afforest	–1.181	**–2.31,–0.04**	**–1.83, 0.52**	0.042*
Grassland	Functional diversity	Arthropod FD	1.760	**0.96,2.56**	**1.29,2.22**	< 0.001***
	$R^2_{marginal}$ = 0.492	Arthropod FD*agricult	–0.240	**–0.62,0.14**	**–0.46,–0.01**	0.214
	$R^2_{conditional}$ = 0.492	Arthropod FD*pasture	–0.087	–0.44,0.26	–0.29,0.11	0.624
		Arthropod FD*urban	–0.795	**–1.51,–0.07**	**–1.21,–0.38**	0.030*
		Arthropod FD*afforest	–0.169	–0.46,0.12	–0.34,0.02	0.256

Table S11. Standardized and unstandardized direct paths of all ecosystem drivers, including: composite land-use, climate (temperature and precipitation, environmental characteristics (water quality deterioration and sediment heterogeneity), stream depth, and species richness of fish, arthropods and macrophytes on standing biomass of fishes and arthropods (Fig. 8). This table includes all significant and nonsignificant path considered by our multigroup analysis, considering pathways for both rainforest and grassland biomes. Path coefficients that have been constrained are indicated with a **'c'** at the end of the row (i.e., paths that did not differ between rainforest and grassland biomes). In contrast, the paths that varied between rainforest and grassland biomes indicated with **'v'** at the end of the row and are highlighted in yellow. *= $P < 0.05$, **= $P < 0.01$, and ***= $P < 0.001$. This table show only results from model with species richness. Double-headed arrows (↔) indicate the variables that covary. MAP = mean annual precipitation; MAT = mean annual temperature; SH = sediment heterogeneity; WQD = water quality deterioration (PC1). The results of this table are shown in Figure 8a and c of the main article. Green lines represent the effects of ecosystem drivers on fish and arthropod biomasses.

Predictors	Response	Standardized coefficients	Regression weights	P-value	Paths difference between biomes
Taxonomic richness					
Rainforest streams; Fig. 8a					
Human land-use →	WQD	0.515	0.231	0.001**	0.0586[C]
MAP →	WQD	–0.194	–0.334	0.011*	0.1233[C]
Human land-use →	SH	0.019	0.003	0.871	0.3291[C]
MAP →	SH	0.338	0.335	0.001**	0.3272[C]
Stream depth →	SH	0.015	0.013	0.853	0.0624[C]
Human land-use →	Stream depth	–0.267	–0.047	0.118	0.2253[C]
MAP →	Stream depth	0.317	0.386	0.001**	0.2873[C]
Human land-use →	Fish rich	–0.442	–0.136	0.001**	0.0985[C]
MAP →	Fish rich	0.146	0.182	0.020*	0.0584[C]
MAT →	Fish rich	0.072	0.074	0.227	0.0464[V]
WQD →	Fish rich	–0.130	–0.093	0.044*	0.3206[C]
SH →	Fish rich	–0.092	–0.116	0.179	0.2561[C]
Stream depth →	Fish rich	0.085	0.089	0.177	0.3036 C
Arthropods rich →	Fish rich	0.429	0.558	0.001**	**0.0004*****[V]
Macrophyte rich →	Fish rich	–0.041	–0.039	0.469	0.0764[C]
Human land-use →	Arthropods rich	0.115	0.020	0.467	**0.02814***[V]
MAP →	Arthropods rich	–0.151	–0.144	0.209	0.1642[C]

MAT	→	Arthropods rich	0.147	0.114	0.187	0.2039[C]
WQD	→	Arthropods rich	–0.258	–0.068	0.180	0.05901[C]
SH	→	Arthropods rich	–0.129	–0.164	0.161	0.1032[C]
Stream depth	→	Arthropods rich	0.181	0.142	0.041*	0.1762[C]
Macrophyte rich	→	Arthropods rich	0.468	0.337	0.001**	0.2776[C]
Human land-use	→	Macrophyte rich	–0.617	–0.180	0.001**	0.2267[C]
MAP	→	Macrophyte rich	0.116	0.153	0.336	0.1198[C]
MAT	→	Macrophyte rich	–0.093	–0.100	0.416	0.1048[C]
WQD	→	Macrophyte rich	–0.149	–0.109	0.025*	0.1614[C]
SH	→	Macrophyte rich	0.179	0.235	0.094	0.1031[C]
Stream depth	→	Macrophyte rich	0.014	0.016	0.894	0.1685[C]
Human land-use	→	Fish biomass	–0.371	–0.090	0.021*	0.2427[C]
MAP	→	Fish biomass	0.104	0.125	0.100	0.2058[C]
MAT	→	Fish biomass	–0.067	–0.067	0.456	**0.00923**[V]**
Fish rich	→	Fish biomass	0.361	0.352	0.001**	0.1721[C]
Arthropods rich	→	Fish biomass	0.069	0.087	0.347	0.0962[C]
Macrophyte rich	→	Fish biomass	0.200	0.183	0.037*	0.0707[C]
Human land-use	→	thropod Biom	–0.410	–0.085	0.002**	0.1809 [C]
MAP	→	thropod Biom	0.072	0.068	0.121	0.29800[C]
MAT	→	thropod Biom	–0.015	–0.011	0.782	0.1091[C]
Fish rich	→	thropod Biom	–0.149	–0.112	0.063	0.05204[C]
Arthropods rich	→	thropod Biom	0.319	0.470	0.001**	0.07992[C]
Macrophyte rich	→	thropod Biom	0.202	0.145	0.010*	0.13121[C]
Fish biomass	↔	thropod Biom	–0.117	–0.029	0.368	

Grassland streams: Fig 8C

Human land-use	→	WQD	0.282	0.231	0.001**	0.0586[C]
MAP	→	WQD	-0.224	-0.334	0.011*	0.1233[C]
Human land-use	→	SH	-0.007	-0.003	0.953	0.3291[C]

MAP	→	SH	0.519	0.335	0.001**	0.3272[C]
Stream depth	→	SH	-0.106	-0.072	0.402	0.0624[C]
Human land-use	→	Stream depth	0.002	0.001	0.985	0.4074[C]
MAP	→	Stream depth	0.416	0.386	0.001**	0.2873[C]
Human land-use	→	Fish rich	-0.305	-0.136	0.001**	0.0985[C]
MAP	→	Fish rich	0.236	0.182	0.020*	0.0584[C]
MAT	→	Fish rich	0.222	0.160	0.039*	0.0464[V]
WQD	→	Fish rich	-0.180	-0.093	0.044	0.3206[C]
SH	→	Fish rich	-0.050	-0.059	0.647	0.2561[C]
Stream depth	→	Fish rich	0.084	0.067	0.443	0.3036 C
Arthropods rich	→	Fish rich	-0.067	-0.059	0.541	**0.0004*****[V]
Macrophyte rich	→	Fish rich	0.236	0.264	0.106	0.0764[C]
Human land-use	→	Arthropods rich	0.010	0.006	0.799	**0.02814***[V]
MAP	→	Arthropods rich	0.030	0.026	0.843	0.1642[C]
MAT	→	Arthropods rich	0.001	0.001	0.992	0.2039[C]
WQD	→	Arthropods rich	0.048	0.028	0.712	0.05901[C]
SH	→	Arthropods rich	0.218	0.293	0.078	0.1032[C]
Stream depth	→	Arthropods rich	0.153	0.142	0.041*	0.1762[C]
Macrophyte rich	→	Arthropods rich	0.298	0.337	0.001**	0.2776[C]
Human land-use	→	Macrophyte rich	-0.387	-0.180	0.001**	0.2267[C]
MAP	→	Macrophyte rich	-0.095	-0.073	0.471	0.1198[C]
MAT	→	Macrophyte rich	0.128	0.094	0.244	0.1048[C]
WQD	→	Macrophyte rich	-0.216	-0.109	0.025*	0.1614[C]
SH	→	Macrophyte rich	-0.018	-0.022	0.868	0.1031[C]
Stream depth	→	Macrophyte rich	0.200	0.163	0.069	0.1685[C]
Human land-use	→	Fish biomass	0.136	-0.090	0.021*	0.2427[C]
MAP	→	Fish biomass	0.060	0.067	0.519	0.2058[C]
MAT	→	Fish biomass	0.245	0.261	0.008**	**0.00923****[V]
Fish rich	→	Fish biomass	0.240	0.352	0.001**	0.1721[C]

Arthropods rich →	Fish biomass	-0.014	-0.018	0.883	0.0962[C]
Macrophyte rich →	Fish biomass	0.128	0.183	0.037*	0.0707[C]
Human land-use →	thropod Biom	-0.278	-0.085	0.002**	0.1809 [C]
MAP →	thropod Biom	0.143	0.075	0.127	0.29800[C]
MAT →	thropod Biom	0.033	0.016	0.727	0.1091[C]
Fish rich →	thropod Biom	-0.162	-0.112	0.063	0.05204[C]
Arthropods rich →	thropod Biom	0.490	0.305	0.001**	0.07992[C]
Macrophyte rich →	thropod Biom	0.228	0.145	0.010*	0.13121[C]
Fish biomass ↔	thropod Biom	-0.118	-0.029	0.365	0.0586[C]

Table S12. Standardized and unstandardized direct paths of all ecosystem drivers, including: composite land-use, climate (temperature and precipitation, environmental characteristics (water quality deterioration and sediment heterogeneity), stream depth, and functional diversity of fish, arthropods and macrophytes on standing biomass of fish and arthropods (Fig. 9). This table includes all significant and nonsignificant path considered by our multigroup analysis, considering pathways for both rainforest and grassland biomes. Path coefficients that have been constrained are indicated with a **'c'** at the end of the row (i.e., paths that did not differ between rainforest and grassland biomes). In contrast, the paths that varied between rainforest and grassland biomes indicated with **'v'** at the end of the row and are highlighted in yellow. *= $P < 0.05$, **= $P < 0.01$, and ***= $P < 0.001$. This table show only results from model with functional diversity. Double-headed arrows (◄►) indicate the variables that covary. MAP = mean annual precipitation; MAT = mean annual temperature; SH = sediment heterogeneity; WQD = water quality deterioration (PC1). The results of this table are shown in Figure 9a and c of the main article. Green lines represent the effects of ecosystem drivers on fish and arthropod biomass.

Predictors	Response	Standardize coefficient	Regressi weights	*P*-valu	Paths differ between bi
Functional diversity					
Rainforest streams: F 9A					
Human land-u →	WQD	0.469	0.259	<0.001*;	0.2272[C]
MAP →	WQD	-0.189	-0.327	0.014*	0.2399[C]
Human land-u →	SH	0.006	0.002	0.964	0.9418[C]
MAP →	SH	0.336	0.334	<0.001*;	0.8891[C]
Stream depth →	SH	-0.106	-0.072	0.401	0.1186[C]
Human land-u →	Stream depth	-0.268	-0.083	0.081	0.4074[C]
MAP →	Stream depth	0.311	0.380	<0.001*;	0.6288[C]
Human land-u →	Fish FD	-0.555	-0.240	<0.001*;	0.2411[C]
MAP →	Fish FD	0.122	0.164	0.278	0.1566[C]
MAT →	Fish FD	-0.240	-0.262	0.015*	0.02274*
WQD →	Fish FD	-0.262	-0.198	<0.001*;	0.6937[C]
SH →	Fish FD	-0.144	-0.193	0.141	0.04742*[
Stream depth →	Fish FD	0.241	0.264	0.001*;	0.405[C]
Arthropods Fl →	Fish FD	0.236	0.227	0.015*	0.9987[C]
Macrophyte F →	Fish FD	-0.214	-0.246	0.176	0.6607[C]
Human land-u →	Arthropods FI	-0.213	-0.086	0.027*	0.1109

MAP →	Arthropods FI	-0.198	-0.276	0.110	0.01482*
MAT →	Arthropods FI	0.085	0.097	0.422	0.3224[C]
WQD →	Arthropods FI	0.082	0.065	0.495	0.9049[C]
SH →	Arthropods FI	0.155	0.217	0.152	0.4845[C]
Stream depth →	Arthropods FI	-0.117	-0.136	0.291	0.5228[C]
Macrophyte F →	Arthropods FI	0.724	0.871	<0.001***	0.0000***
Human land-u →	Macrophyte FI	-0.490	-0.164	<0.001***	0.5203[C]
MAP →	Macrophyte FI	0.198	0.230	0.063	0.06523[C]
MAT →	Macrophyte FI	-0.020	-0.019	0.840	0.9368[C]
WQD →	Macrophyte FI	-0.119	-0.079	0.106	0.8037[C]
SH →	Macrophyte FI	0.149	0.174	0.044*	0.4447[C]
Stream depth →	Macrophyte FI	0.144	0.139	0.047*	0.9259[C]
Human land-u →	Fish biomass	-0.358	-0.129	0.007**	0.1373[C]
MAP →	Fish biomass	0.107	0.119	0.156	0.8338[C]
MAT →	Fish biomass	0.082	0.080	0.390	0.08141[C]
Fish FD →	Fish biomass	0.316	0.286	<0.001***	0.7327[C]
Arthropods FI →	Fish biomass	-0.070	-0.060	0.526	0.8671[C]
Macrophyte F →	Fish biomass	0.295	0.307	0.039*	0.1007[C]
Human land-u →	Arthropod Biom	-0.435	-0.101	0.003**	0.8257[C]
MAP →	Arthropod Biom	0.054	0.050	0.355	0.9528[C]
MAT →	Arthropod Biom	0.016	0.012	0.792	0.9681[C]
Fish FD →	Arthropod Biom	0.100	0.069	0.213	0.4883[C]
Arthropods FI →	Arthropod Biom	0.225	0.151	0.020*	0.9237[C]
Macrophyte F →	Arthropod Biom	0.153	0.125	0.049*	0.1248[C]
Fish biomass ↔	Arthropod Biom	0.025	0.011	0.852	
Grassland streams: Fig					
Human land-u →	WQD	0.314	0.259	<0.001***	0.2272[C]
MAP →	WQD	-0.222	-0.327	0.014*	0.2399[C]

Human land-u →	SH	-0.007	-0.003	0.954	0.9418[C]
MAP →	SH	0.518	0.334	<0.001***	0.8891[C]
Stream depth →	SH	-0.025	-0.016	0.846	0.1186[C]
Human land-u →	Stream depth	-0.023	-0.013	0.846	0.3533[C]
MAP →	Stream depth	0.408	0.380	<0.001***	0.6288[C]
Human land-u →	Fish FD	-0.425	-0.240	<0.001***	0.2411[C]
MAP →	Fish FD	-0.110	-0.108	0.384	0.1566[C]
MAT →	Fish FD	0.079	0.074	0.436	0.02274*
WQD →	Fish FD	-0.294	-0.198	<0.001***	0.6937[C]
SH →	Fish FD	0.146	0.223	0.223	0.04742*
Stream depth →	Fish FD	0.250	0.264	0.001***	0.405[C]
Arthropods FI →	Fish FD	0.142	0.227	0.015*	0.9987[C]
Macrophyte F →	Fish FD	-0.122	-0.148	0.242	0.6607[C]
Human land-u →	Arthropods FI	-0.242	-0.086	0.027*	0.1109
MAP →	Arthropods FI	0.326	0.200	0.030*	0.01482*
MAT →	Arthropods FI	-0.079	-0.046	0.528	0.3224[C]
WQD →	Arthropods FI	0.187	0.078	0.137	0.9049[C]
SH →	Arthropods FI	0.083	0.080	0.515	0.4845[C]
Stream depth →	Arthropods FI	-0.057	-0.037	0.652	0.5228[C]
Macrophyte F →	Arthropods FI	0.097	0.073	0.454	0.0000***
Human land-u →	Macrophyte FI	-0.341	-0.164	<0.001***	0.5203[C]
MAP →	Macrophyte FI	-0.105	-0.085	0.483	0.06523[C]
MAT →	Macrophyte FI	-0.010	-0.008	0.935	0.9368[C]
WQD →	Macrophyte FI	-0.142	-0.079	0.106	0.8037[C]
SH →	Macrophyte FI	0.139	0.174	0.042*	0.4447[C]
Stream depth →	Macrophyte FI	0.161	0.139	0.047*	0.9259[C]
Human land-u →	Fish biomass	-0.207	-0.129	0.007***	0.1373[C]
MAP →	Fish biomass	0.085	0.083	0.365	0.8338[C]
MAT →	Fish biomass	0.308	0.326	0.002***	**0.01265***
Fish FD →	Fish biomass	0.254	0.286	<0.001***	0.7327[C]

Arthropods FI →	Fish biomass	-0.015	-0.026	0.883	0.8671[C]
Macrophyte F →	Fish biomass	-0.018	-0.025	0.851	0.1007[C]
Human land-u →	Arthropod Biom	-0.307	-0.101	0.003**	0.8257[C]
MAP →	Arthropod Biom	0.095	0.050	0.355	0.9528[C]
MAT →	Arthropod Biom	0.025	0.012	0.792	0.9681[C]
Fish FD →	Arthropod Biom	0.129	0.069	0.213	0.4883[C]
Arthropods FI →	Arthropod Biom	0.177	0.151	0.020*	0.9237[C]
Macrophyte F →	Arthropod Biom	0.195	0.125	0.049*	0.1248[C]
Fish biomass ↔	Arthropod Biom	-0.105	-0.034	0.420	

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5 LONG-TERM CHANGES IN THE MULTI-TROPHIC DIVERSITY ALTER THE FUNCTIONING OF RIVER FOOD WEBS

ABSTRACT

1. Fish diversity is declining worldwide, which is predicted to alter the functioning of riverine food webs. Despite the importance of fishes for energy and nutrient turnover in riverine ecosystems, how changes in fish diversity affect the functioning of riverine food webs remain largely unknown.
2. Using a 17-year dataset, we show how changes in fish diversity affect the energy flux through fish food webs. We also analyze how multiple global drivers (human pressure, precipitation, and nutrient stoichiometry) affect fish diversity, and the consequences of this for energy flux.
3. We found declines in diversity of multiple fish trophic guilds; especially, top-predator species richness was reduced by 72% over time. The number of trophic links in the food webs decreased as well as the energy flux retained in each of the trophic compartment. Moreover, the proportion of energy flux in the higher trophic compartments decreased over time and the energy flux was concentrated in the lower trophic compartments. Human pressure has contributed substantially to the observed decline in diversity and energy flux.
4. These results reveal alarming decoupling in the energy flux through fish food webs, with potential negative consequences to riverine food web functions, such as detritivory, omnivory, and carnivory. Our findings further reinforce the urgent need to manage human pressure, as it was the major driver of the loss of diversity and decoupling of energy flux through natural food webs.

Keywords: energy flux, biodiversity, human pressure, ecosystem function, river ecosystem, fish

5.1 Introduction

A variety of disturbances resulting from global changes are causing species loss across many trophic groups (Gossner et al., 2016), with potential effects on the capacity of the ecosystem to maintain functions and provide services (Soliveres et al., 2016; Moi et al., 2022). Many ecosystem functions (e.g., primary production, decomposition and nutrient cycling) are

products of biological processes that are controlled by trophic interaction networks (Thompson et al. 2012). The functional consequences of a decline in biodiversity to ecosystem functioning can only be understood through the study of multiple trophic levels and their trophic interactions (Eisenhauer et al., 2019). Despite that, most studies are still limited to single trophic levels or simplified food webs (Hector et al., 1999; Hooper et al., 2005).

Quantifying energy dynamics in food webs has emerged as a powerful measure that describe ecosystem functioning across trophic levels, allowing for the analysis of biodiversity-ecosystem functioning (BEF) relationships from a multi-trophic perspective (Thompson et al., 2012; Barnes et al., 2018). This approach considers that all the organisms are interlinked by fluxes of matter and energy (Buzhdygan et al., 2020). Integrating energy flux through trophic levels provides quantitative measures of ecosystem functioning (Gauzens et al., 2018). For example, by quantifying the total energy flux (i.e., sum of all energy flux through all of trophic compartments), it is possible to describes the functioning of the entire multi-trophic network (Buzhdygan et al., 2020). The intake energy flux in trophic compartments describes different ecosystem functions that are driven by each trophic compartment. For instance, the energy flux through all decomposers is related to decomposition (Wall et al., 2008), and the flux of energy through all predators and omnivores is related to top-down control and food web stability, respectively (McCann & Hastings, 1997; Wang & Brose, 2018).

Although biodiversity and energy flux are strongly associated (Barnes et al., 2014; Wang et al. 2019), there is a general lack of empirical assessments using energy dynamics in food webs that consider biodiversity as a driver of ecosystem functioning. Such close association suggests that changes in biodiversity could alter the energy flux through ecosystems (Barnes et al., 2014). Moreover, we are unaware of how multiple drivers (e.g., human pressure and climatic variability) can affect biodiversity and energy flux in natural ecosystems. Experimental assessments have revealed that increased human pressure and extreme climatic events such as droughts can reduce biodiversity across many trophic groups, triggering shifts in the food web structure (Brose et al., 2019), and consequently altering energy flux through it (Ledger et al., 2013).

We compiled a unique long-term dataset (17 years) of standardized monitoring surveys of fish communities in a large Neotropical River (the Uruguay River; Figure S1). Our dataset comprised more than 20,000 individuals of 117 fish species surveyed from 2005 to 2021. We combined information on the local community (i.e., number of individuals, diet, and trophic

position), community metabolism, network topology, and resource-specific assimilation efficiencies (Gauzens et al., 2018) to quantified energy flux (joules per year; Figure 1). We calculated the energy flux necessary to support the energetic demands of each trophic compartment, which represents single functions carried out by each trophic compartment (e.g., omnivory, detritivory and carnivory). We combine the energy flux of all trophic compartments as a measure of multi-trophic ecosystem functioning (Barnes et al., 2014).

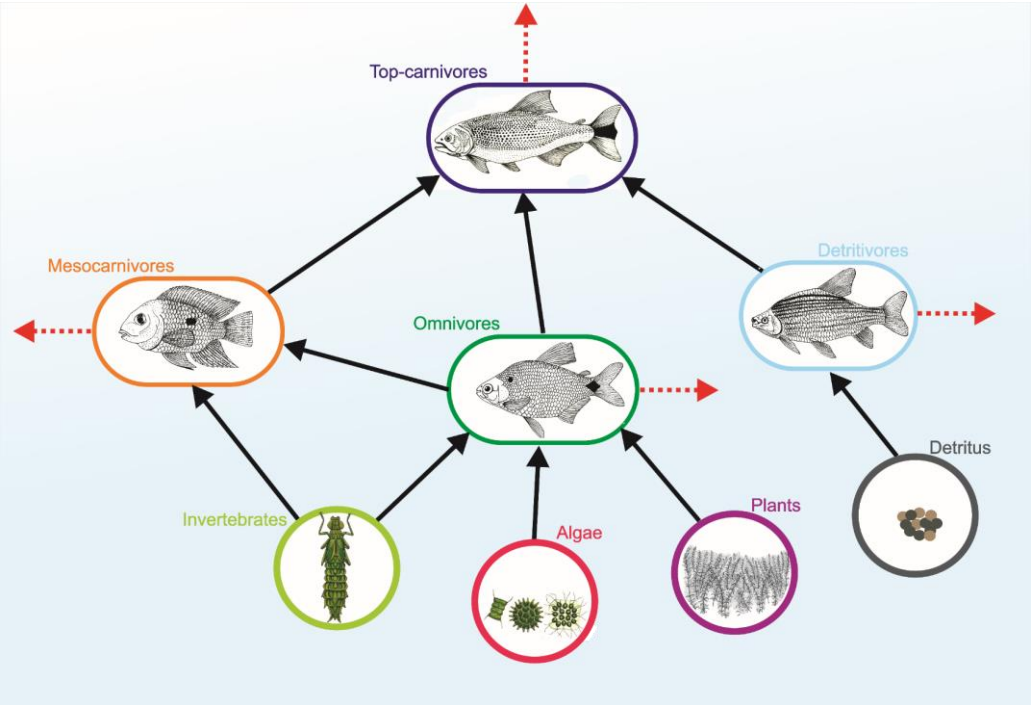

Figure 1. Conceptual diagram of the river trophic network model. The red dashed arrows represent flux leaving the system (i.e., fish respiration), and the black arrows represent flux transferring from one trophic compartment to another through the food web. Plants, invertebrates, algae, and detritus were considered basal resources for the fish community, so they have no input flux and respiration being represented here. Trophic interaction through the food web was defined by the use of gut contents. Illustration credit: Margenny Barrios (Departamento de Ecología y Gestión Ambiental CURE, Universidad de la República, Uruguay).

Here, we evaluated long-term trends in species richness, abundance, biomass and energy flux in the fish trophic guilds in a neotropical riverine system. Considering the previous evidence of changes in fish trophic guild diversity in Neotropical rivers, which affected food web topology (Moi & Teixeira de Mello, 2022; Borzone Mas et al., 2022), we would expect significant temporal changes in fish diversity, which would potentially affect the topology of the fish food webs over time. We next investigated the relationship between diversity and

energy flux over time. We hypothesize that changes in species diversity drive changes in the diversity-energy flux relationship. Lastly, we applied structural equation models (SEMs) to address the direct and indirect effects (via diversity) of human pressure, precipitation, nutrient stoichiometry, and river properties on energy flux.

5.2 Material and methods

5.2.1 Study area

This study was conducted in the Uruguay River, a large South America river, encompassing an extension of 1,838 km from headwaters tom mouth and a basin area of more than 350 km^2 (Figure S1). In the Uruguay River, flow patterns do not follow a seasonal predictable flood pulse (Krepper et al., 2003). Instead, flood pulse is determined by precipitation in the upper two thirds of its catchment, which increases during El Niño Southern Oscillation (ENSO) events, producing irregular flood peaks (Krepper et al., 2003). This region is characterized by a subtropical climate with a mean annual temperature of 17.4ºC and mean precipitation of approximately 1200 mm $year^{-1}$. The Uruguay River has a high fish diversity (López-Rodríguez et al., 2019), and along with Paraná River originated the largest estuaries worldwide (La Plata River). Despite its great diversity, the Uruguay River bears certain degradation caused by human activities, such as the input of effluents from industries, untreated sewage from cities and diffuse contamination from agricultural activities (e.g., Soutullo et al., 2020) as well as river fragmentation by three dams in the upper section and one in the middle-low river section. Fish are among the organisms most threatened by human-induced pressure (Su et al., 2021). The fish community was sampled in the three river sites from April 2005 to November 2021. To provide a comprehensive temporal assessment of fish communities, the survey included four standardized annual samples in each site, totaling 179 samples over 17 years. The field survey followed a rigorous and standardized sampling protocol (see Appendix: *Assessing fish community*)

5.2.2 Diversity measure

All fishes were identified at the species level, and then quantified the abundance (number of individuals per unit effort - CPUE) for each period. We calculated estimated rarefied richness as the Chao index with abundance-based data using the R package ‘iNEXT’ (Hsieh & Chao, 2016), which is based on rarefaction and extrapolation of Hill numbers and provides an unbiased estimate of asymptotic species richness.

5.2.3 Fish fresh body mass and assimilation efficiency

We measured individual body length (cm) using pachymeter digital. We also estimated the body mass of the fishes by weighing it on a semi-analytical balance (0.01 g precision). We then calculated community biomass (mg mass CPUE) for each of the 179 communities by summing together all individual body masses. The assimilation efficiency, *e* (the proportion of energy assimilated into fish biomass from total consumed energy), was assigned for each trophic interaction based on resources consumed (Lang et al., 2017). We defined assimilation efficiency as: 0.158 for fish consuming detritus, 0.545 for fish consuming plant and algae, and 0.906 for fish consuming other live fishes or invertebrates (Lang et al., 2017). These values are based on difference among trophic levels in their ability to extract energy from ingested material (Jochum et al., 2021).

5.2.4 Construction of the food webs

We analyzed the stomach contents of all fish species (see Appendix: *stomach contents*) and jointly with literature (e.g., Lopez- Rodriguez et al., 2019), we lumped fish communities into four feeding guilds: top-carnivores, mesocarnivores, omnivores, and detritivores (Table S1). Top-carnivores are individuals that feed only on other fish species. Mesocarnivores were assigned to individuals that feed on invertebrates, although there is one mesocarnivorous species (*Rhamdia quelen*) that can also consume other small fishes. Omnivores were designed for individuals that feed on at least two different resources: invertebrate–plant/algae, invertebrate–detritus or plant/algae–detritus. Detritivores are individuals that feed on detritus. We created an adjacency matrix of feeding links among all fish species (i.e., *network topology*) for each site in each sampling period, thereby creating 179 meta-webs for the fish community. A general meta-web with all fish species and their trophic interactions are provided (Figure S2 and Table S1).

5.2.5 Calculating fish metabolic rates

Metabolic rate represents rate at which energy and matter are taken up, transformed and allocated (Brown et al., 2004). For heterotrophs (e.g., fish), the metabolic rate equals to respiration rate, and is dependent on fresh body mass and temperature. We calculated the metabolic rates for individual fish using body masses and temperature. There were variations in body mass distributions among trophic compartments (Figure S6). Temperature was

measured below the water surface (using thermometer) during each sample period. Metabolic rate was calculated using following equation:

$$\ln I = ln\ i_o + a \times \ln M - \frac{E}{kT},$$

Where I is the metabolic rate, a is the allometric exponent, M is the body mass, E is the activation energy (1), k is the Boltzmann`s constant, T is the temperature, and i_o is a normalization factor (Ehnes et al., 2011).

5.2.6 Calculating food web energy fluxes

For energy flux calculations, we assumed a steady state, which the energy intake (via resource consumption) to a feeding guild exactly balances the energy losses of that feeding guild due to physiological processes and predation. Energy fluxes (joules per year^{-1}) among all feeding guilds were calculated, where consumer-resource links were assigned using the food web energetic approach (Barnes et al., 2018; Gauzens et al., 2018). F_{ik}, the flux of energy from resource i to consumer k, was calculated as:

$$\sum_i e_{ik}F_{ik} = X_k + \sum_k W_{ik}F_k$$

Where e_{ij} is the efficiency that consumer k converts energy coming from resource i into energy used for its metabolism and biomass production. This equation represents the balance between energetic gains of consumer k via consumption of resources (the left side of the equation: $\sum_i e_{ik}F_{ik}$), and the energetic losses due to its metabolism X_k (represented by sum of individual metabolic rates from fish in nodes k) and predation by higher trophic levels (Gauzens et al., 2018). The energy flux to each consumer was then defined as $F_{ik} = W_{ik}F_k$, where F_k is the sum of all ingoing fluxes to species k and W_{ik} define the proportion of F_k that is obtained from species i, which was obtained by scaling consumer preferences W_{ik} to the biomasses of different resources as:

$$w_{ik} = \frac{w_{ik}B_i}{\Sigma_k w_{kj}B_k}$$

where B_i is the biomass of resource i.

We started by calculating the energy flux to the top-carnivorous, which energy loss to predation should be assumed to be zero. There were cannibalistic links in some top-carnivorous fish (e.g., *Hoplias argentinensis*), but preference for cannibalism was set to 0.1 in the adjacency matrix to strongly down-weight the amount of energy a predator consumed from its own biomass pool. Total top-carnivorous flux was calculated as the sum of all outgoing energy flux from mesopredator, omnivorous and detritivorous fishes. Total mesocarnivorous flux was calculated as the sum of all outgoing energy flux from invertebrate and small fish prey. Total omnivorous flux represents the sum of all outgoing energy flux from plant, detritus, and invertebrates. Total detritivorous flux was assumed to come from only detritus. We also calculated the total energy flux (whole-community flux) by summing up fluxes along all trophic guilds. Producers (plants and algae), detritus, and invertebrates were assumed to be basal resources for the fish species; thus, it has no intake flux. We calculated the relative contribution (%) of the different fish trophic guilds to total energy flux in fish food web. Energy flux was calculated using the "fluxweb" package (Gauzens et al., 2018).

5.2.7 Statistical analyses

Using generalized mixed-effects models (GLMM`s; Bates et al., 2015), we investigated how the (a) species richness, (b) abundance, and (c) biomass, and (d) energy flux, and (e) relative energy flux (%) of whole-community and individual fish trophic guilds (top-carnivores, mesocarnivores, omnivores, and detritivores) changed over the years (17 years) in the three Uruguay river sites. We included site, year and their interaction as a fixed effect in all models to test for temporal and spatial consistency in responses. We used a negative binomial response distribution, which best represents the variation in these responses. To control for seasonality effects and to account for repeated measures, we nested the two samplings within each season as a random factor. We did not find temporal autocorrelation in our data using the function CAR1 in the car package (Fox & Weisberg, 2019). Additive and interactive models were analysed using type II sum of squares (SS). Probabilities were calculated using likelihood ratio tests (LRT, $\chi 2$).

We explored potential changes in the relationship between diversity (species richness) and energy flux using linear mixed-effect models (Pinheiro et al., 2018). We tested for the effect of on energy flux for overall data (whole-community energy flux) and repeated again for data from individual trophic guilds. To determine whether the diversity-energy flux relationship change over time, we added interaction terms for year × diversity to the mixed-effects models

and measured the estimated slope for these interactions. We analyzed the slope of diversity-energy flux relationship in each year period. We modelled the relationship between energy flux and diversity on a log–log scale because this specification has empirical supports in fish communities (Benkwitt et al., 2020). In the log–log models, the interpretation of β_1 is similar to the power coefficient; consequently, it enables a test of the shape of the relationship between diversity and ecosystem functioning (e.g., energy flux). Whether $\beta < 1$ this means a concave-down/saturating relationship between diversity and energy flux, whereas $\beta > 1$ mean a concave-up/non-saturating relationship (Mora et al., 2014). In other words, $\beta < 1$ indicates that the relationship between diversity and energy flux is weak, whereas $\beta > 1$ indicates that such relationship is strong. We test the pattern of diversity-energy flux relationships for the whole-community and for each trophic guild.

We used structural equation models (SEM), to address the direct and indirect pathways by which human pressure, precipitation, nutrient stoichiometry, and environmental variables affects the diversity and energy flux through each trophic compartment. We quantified human pressure using the human footprint index (*hereafter* HFP, Venter et al. 2016). To quantify nutrient stoichiometry, we calculate the N:P ratio, and to quantify environmental variables, we used water discharge and turbidity values. An explanation of the calculations for each of the drivers is provided in the Appendix (Section: *assessing global drivers*). The SEM was fitted based on a meta-model (Figure S3). We tested multicollinearity between predictors by calculating the variance inflation factor (VIF). VIF > 3 indicates possible collinearity, which was not observed in our model. We constructed SEMs for trophic guilds separately, and five SEMs were fitted: (i) entire community (ii) top-carnivores, (iii) mesocarnivores, (iv) omnivores, and (v) detritivores. The SEM was fitted using a linear mixed-effect model in the piecewiseSEM package (Lefcheck, 2016). We present the standardized coefficient for each path and estimated the indirect effects by coefficient multiplication. The significance of all path was obtained using maximum likelihood and SEM fit was examined using Shipley's test of d-separation through Fisher's C statistic ($p > 0.05$ indicates adequate model). All analyses were conducted in R 3.4.4 (RStudio Team, 2020).

5.3 Results

We found a decline in the species richness of top carnivores over time (Figure 2a; Table S2; Figure S4), which was overall estimated to be 72% along the period of 17 years (Figure 2d). In contrast, the species richness of whole-community, mesocarnivores, omnivores and

detritivores did not change over time (Figure 2a). The abundance of all trophic guilds decreased over time (Figure 2b). From 2005 to 2021, the abundance of top-carnivores, mesocarnivores, omnivores and detritivores reduced 96%, 85%, 80% and 82%, respectively (Figure 2e). Similarly, the biomass of all trophic guilds declined over time (Figure 2c); the biomass of top-carnivores, mesocarnivores, omnivores and detritivores reduced by 96%, 96%, 89% and 88%, respectively (Figure 2f).

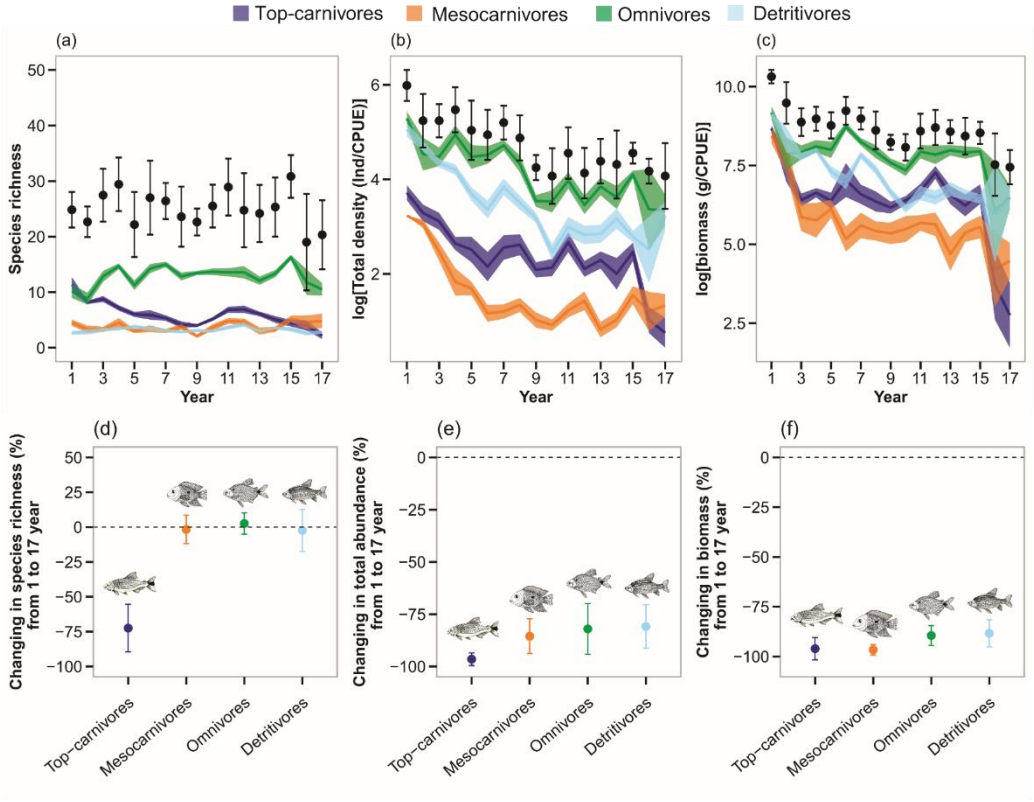

Figure 2. Long-term trends in species richness, total abundances, and biomass of fish trophic guilds**.** The mean (± s.e., n = 179) of species richness **(a)**, abundance **(b)**, and biomass **(c)** of whole-community (black points) and of each trophic guild (colored lines) along 17 years. Full model results are provided in Table S2. **d-f,** Proportion of change in the species richness, abundance, and biomass of fish trophic guilds from 1 to 17 year (error bars show 95% confidence intervals).

Comparing the food web structure between the first (2005) and last (2021) year, we found fewer trophic links in the latter (Figure 3), confirming the reduction in complexity of riverine food webs over time. Simultaneously, there was a general decrease in biomass (nodes sizes in Figure 3) and energy-processing rates through food webs (arrow widths in Figure 3).

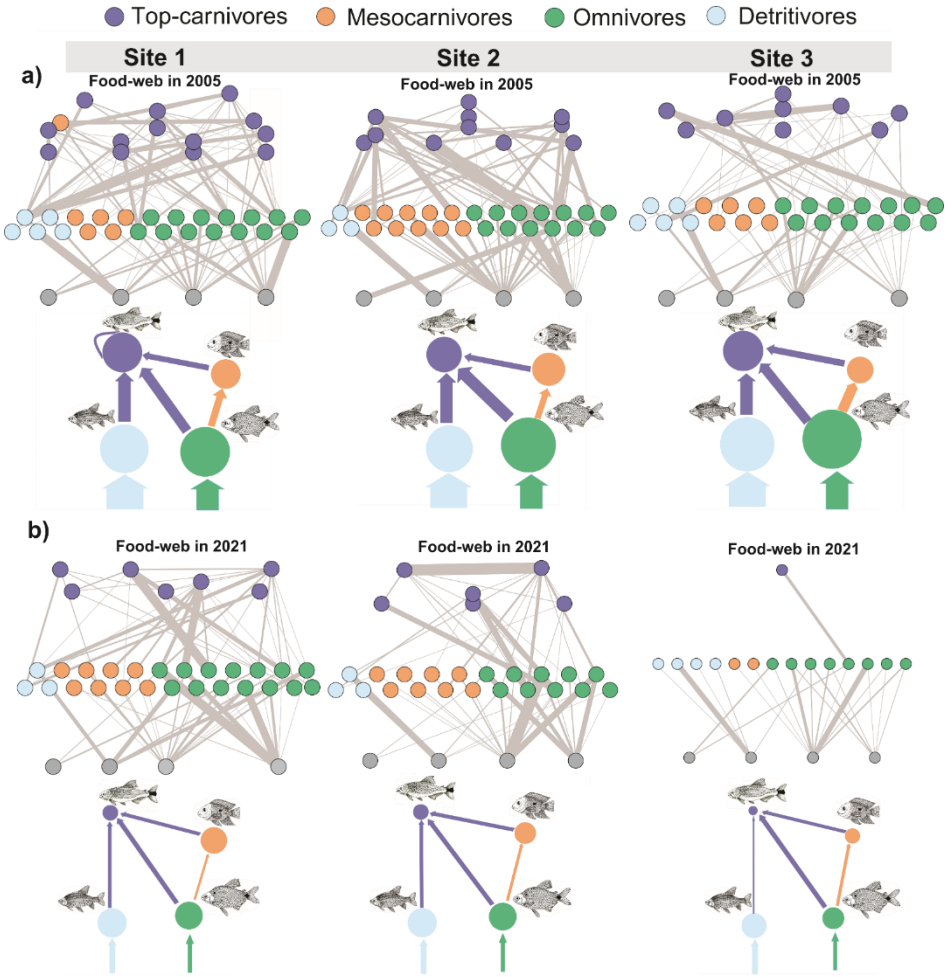

Figure 3. Change in the fish food web structure and community energy networks between first (a; 2005) and the last year (b; 2021) of the study. Food webs were constructed at species level interaction. Width of links scales with the log of fluxes. Nodes' labels correspond to the fish species ordering in the trophic level: green = omnivorous species, light blue = detritivorous species, orange = mesocarnivorous species, and purple = top-carnivorous species. Energy pathways displaying the relative annual energy flux (colored arrow width weighted by calculated energy flux (J/year)) and biomass (colored node diameter weighted by total biomass) among the trophic guilds: top-carnivore (purple), mesocarnivore (orange), detritivores (light blue) and omnivore (green). Each panel represents an energy network for one of the three river sites in 2005 (a) and 2021 (b).

Indeed, the energy flux to whole-community declined over time (Figure 4a; Figure S5). The energy flux to top-carnivores, mesocarnivores, omnivores and detritivores reduced by 95%, 87%, 19% and 60% over time, respectively (Figure 4b). In addition to the general decreases in energy fluxes, there were systematic changes in the proportion of energy flux among trophic guilds (Table S2); specifically, the proportion of energy flux to top-carnivores and mesocarnivores decreased by about 96% over time (Figure 4c).

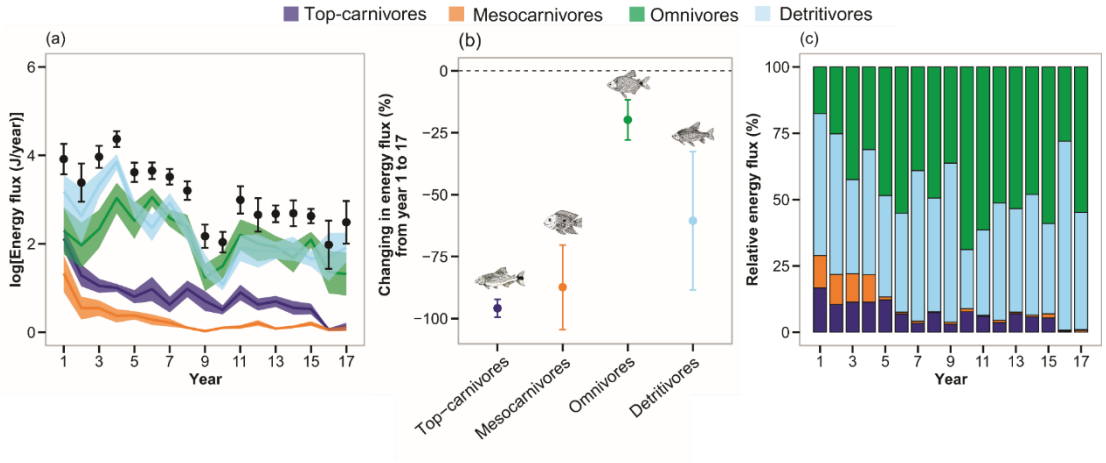

Figure 4. Long-term trends in energy fluxes through river food-webs. **a**, The mean (± s.e.) of energy flux of the entire community (black points) and of each trophic guild (colored lines) along 17 years. **b**, shows proportion of changes in the energy flux of each single trophic guild from year 1 to 17 year. Error bars show 95% confidence intervals. **c**, shows the relative contributions of fish trophic guilds to the whole food web energy flux (n = 179).

We found positives diversity-energy flux relationships, which was consistent across river sites (Figure 5a-e and Table S3). However, such relationships weakened along the years (Table S4). The relationship between whole-community diversity and energy flux shifted from strongly positive (non-saturating; β = 1.74 (1.99-1.08)) in the early years (year 1 to 9) to weakly positive (saturating; β = 0.60 (0.79-0.49)) in the remaining years (Figure 5f). The relationship between top-carnivore diversity and energy flux shifted from strongly positive (β = 1.12 (1.14-1.08)) in the initial years (year 1 to 3) to weakly positive or non-significant (β = 0.43 (0.73-0.09)) in the remaining years (Figure 5g). The relationship between mesocarnivore diversity and energy flux shifted from weakly positive (β = 0.41 (0.53-0.23)) in the initial five years to non-significant (β = 0.05 (0.13 to –0.01)) in the remaining years (Figure 5h). The relationship between omnivore diversity and energy flux remained strongly positive (β = 1.64 (2.01-1.09)) over the years (Figure 5i). The relationship between detritivore diversity and energy flux shifted from weakly positive or non-significant (β = 0.54 (1.21-0.002)) to negative (β = – 0.51 (–0.07 to –1.16) over the years (Figure 5j).

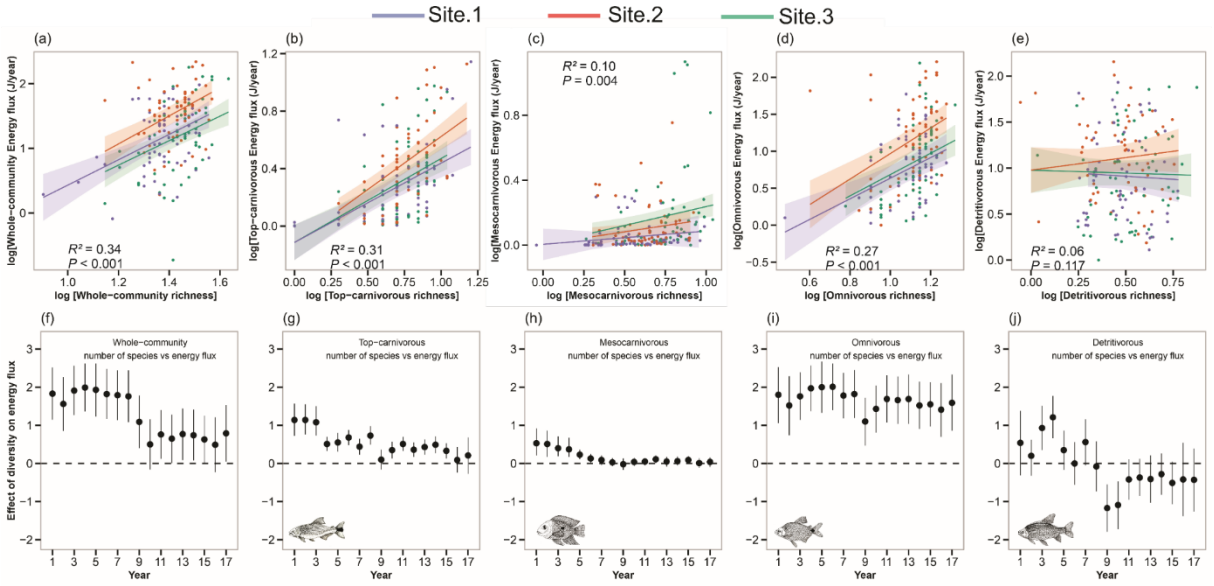

Figure 5. Long-term relationships between diversity and energy flux in the Uruguay River. **(a-e)** The linear association between energy flux and number of species of (a) the whole-community richness (all trophic guilds), and of (b-e) the four fish trophic guilds, for the three river sites; n = 179. Lines are predicted (fitted) values from LMMs after accounting for years and sample site differences, and areas represents 95% confidence intervals. **(f-j)** Estimated coefficients for the overall effect of diversity (number of species) on energy flux long 17 years. Coefficients are showed to each fish trophic guild and whole-community diversity. Points represent estimate values with their 95% confidence intervals. Coefficients represent the log-log association between diversity and energy flux.

Structural equation modeling revealed that human pressure, precipitation, N:P ratio, and environmental variables jointly influenced both diversity and energy fluxes (Figure 6, Table S5). Regarding climate, there were direct positive effects of precipitation on species richness of top-carnivore and detritivore (Figure 6b,k). Precipitation increased the energy flux to top-carnivore, both directly and indirectly mediated by an increase in species richness (Figure 6a,c). We found a direct negative effect of HFP on species richness of top-carnivore (Figure 6c). Conversely, there was a direct positive effect of HFP on species richness of omnivore (Figure 6f). The HFP had strong direct negative effects on the energy flux of all trophic guilds (Figure 6 and Figure S6). There was an indirect negative effect of HFP on the energy flux of the top-carnivore mediated by an increase in species richness (Figure 5c). The N:P ratio had a direct negative effect on species richness of top-carnivore and a direct positive effect on species richness of mesocarnivore (Figure 6b,e). The N:P ratio had a positive effect on the energy flux to the omnivores (Figure 6i). As for river properties, the water turbidity had a direct negative

effect on species richness and energy flux to top-carnivore and a direct positive effect on species richness and energy flux to omnivore (Figure 6b,h).

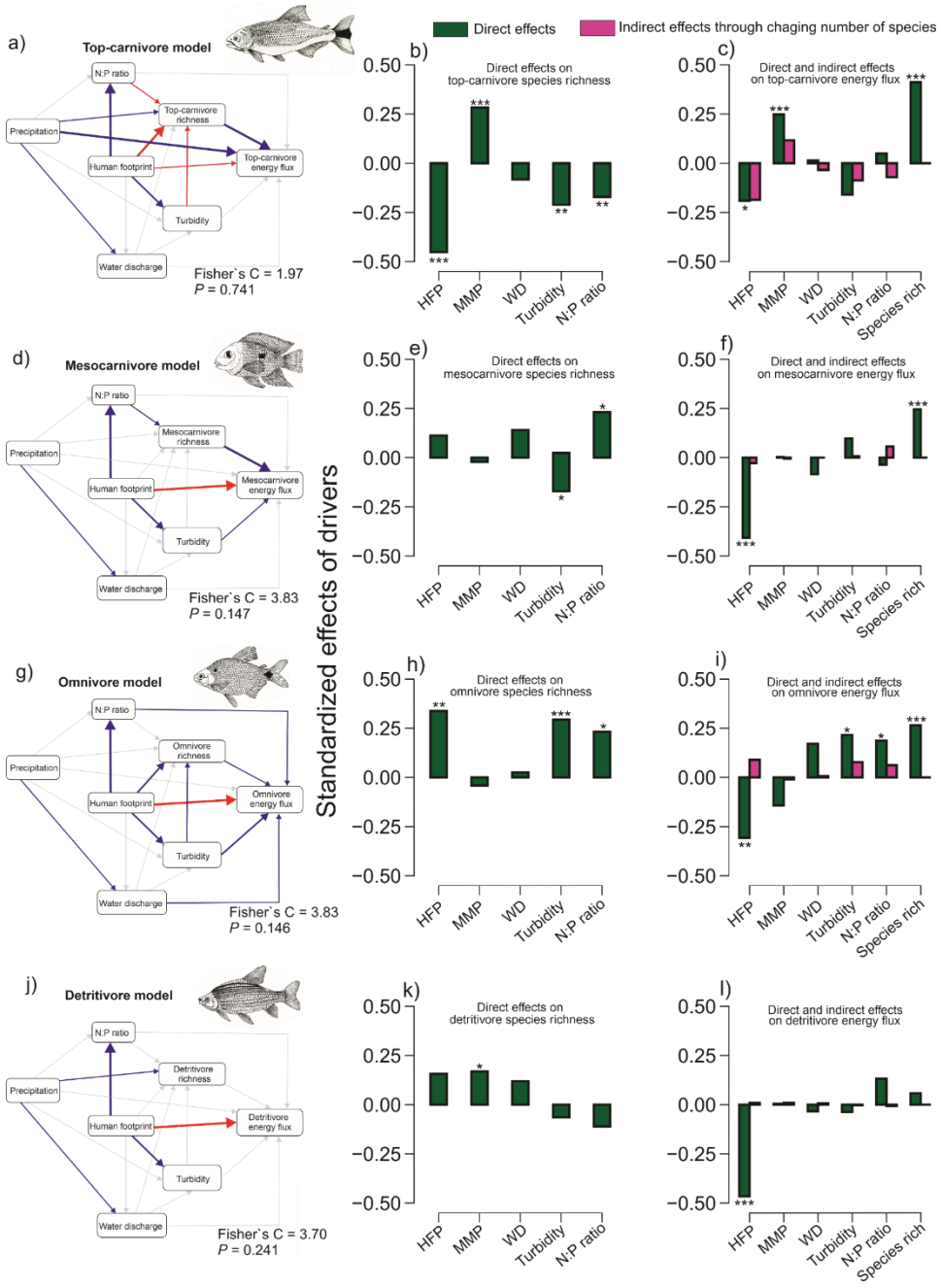

Figure 6. Direct and indirect effects of HFP, climate and water properties on the diversity and energy flux of fish trophic guilds. Direct and indirect pathways by which HFP, climate and water properties affect diversity and energy flux of fish trophic guilds. Specifically, the structural equation modelling was used to disentangle the direct and indirect diversity-mediated effects of HFP, climate, and water properties on energy flux of four fish trophic guilds, including (a-c) top-carnivores, (d-f) mesocarnivores, (g-i) omnivores, and (j-l) detritivores. Solid blue and red arrows represent significant positive and negative pathways, respectively ($P \leq 0.05$), while solid grey arrows non-significant pathways ($P \geq 0.05$). The thickness of the significant pathways (arrows) represents the magnitude of the standardized regression coefficient. Bar graphs illustrates the standardized effect size from SEMs (a, d, g, j) for both

species' richness and energy flux of (b-c) top-carnivores, (e-f) mesocarnivores, (h-i) omnivores, and (k-l) detritivores. Asterisk adjacent to bar represents the significance of the effects: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. HFP = human footprint, MMP = mean monthly precipitation and WD = water discharge. Indirect effects of drivers on energy flux are calculated by multiplying the path coefficient for the effect of drivers on species richness with the path coefficient for the effect of species richness on energy flux (see Table S4 for the individual path coefficients).

5.4 Discussion

Quantifying energy dynamics in food webs has proven powerful in describing the functioning of multi-trophic ecosystems (Thompson et al., 2012; Barnes et al., 2018). Combining a standardized long-term dataset and energetic food web approach, we explore the relationship between diversity (species richness) and energy flux, as well as their underlying mechanisms and human pressure driving them. First, while confirming the decline in the abundance and biomass of trophic guilds (Olden et al., 2008; Moi & Teixeira de Mello, 2022; Comte et al., 2021), our analyses reveal a considerable loss in top-carnivore species, suggesting a potential trophic downgrading (Estes et al., 2011). Second, we found pervasive decline in energy flux at the whole-network level over time, and along with it there was a breakdown in the distribution of flux among trophic guilds. Third, the ability of diversity to promote energy flux has weakened over time. Human pressure negatively influenced energy flux through direct and diversity-mediated, indirect pathways, and precipitation positively influenced energy flux. These results reveal long-term reductions in the energy flux in the riverine food webs, with negative consequences on ecosystem functions including detritivory, omnivory, and carnivory.

Our results contrast with findings from other studies (e.g., Moi & Teixeira de Mello, 2022; Colares et al., 2022), which found declining species richness of detritivorous and omnivorous fishes as consequence of human pressure. Importantly, top-carnivores have suffered the greatest losses in species richness over 17 years, with an estimated reduction of at least 72% (Figure 2d), along with their reduction in abundance and biomass. This extirpation of top-carnivore species suggests a process of trophic downgrading of fish communities (Estes et al. 2011). Albeit in lower intensities, the abundance and biomass of mesocarnivores, omnivores and detritivores also decreased over time, meaning that the ecosystem investigated is consistently becoming defaunated.

Reductions in abundance and biomass impair species' ability to assimilate resources, decreasing energy fluxes between trophic compartments (Barnes et al., 2019; Wan et al., 2022). Along with decreasing abundance and biomass, our analysis revealed declines in the whole-

food web energy flux, as well as in the individual trophic compartments. This result indicates a temporal decouple in energy transfer between trophic compartments (Barnes et al., 2018). Surprisingly, there were systematic imbalances in the distribution of energy flux between trophic compartments, driven by reductions in energy flux to the top of food webs (Figure 3c). Over time, detritivore and omnivore compartments accounted for 95% of all energy fluxes, suggesting a retention of energy flux in lower trophic compartments, as observed elsewhere (Barnes et al., 2014; Polazzo et al., 2022). The reduction in energy flux to the top of the food web combined with the reduction in top-carnivore species further reinforces the trophic downgrading of this ecosystem (Estes et al., 2011), with pronounced consequences on the functioning of riverine ecosystems. While our results evidenced declines in the energy flux in all trophic compartments, it was clear that such declines were stronger at the top than at the bottom of fish food webs. Lower trophic levels typically exploit a wide range of resources, including invertebrates, plants, detritus, and algae, with carbon from allochthonous (terrestrial) origin subsidizing most of its biomass (González-Bergonzoni et al., 2019). It is likely that lower trophic levels can maintain more stable energy fluxes over time as they exploit a wide variety of food (Rooney et al., 2006), and rely largely (directly or indirectly) on terrestrial organic matter inputs (González Bergonzoni at al., 2019), which highly abundant in riverine ecosystems (Marcarelli et al 2011). In contrast, higher trophic levels depend on the biomass generated within the fish community itself, and reductions in the biomass of the lower trophic compartments cascade up to top-carnivores.

Along with the decrease in energy flux, we showed dramatic shifts in diversity-energy flux relationships over time. Such relationships followed different trajectories within individual trophic compartments; in particular, our analysis revealed three significant changes in the relationships between diversity and energy flux: (i) changes from strongly positive to weakly positive or non-significant – whole-community and top-carnivore species; (ii) changes from weakly positive to non-significant – mesocarnivore species; (iii) changes from weakly positive to negative – detritivore species. To omnivore species the diversity-energy flux relationship remained strongly positive over time. These results reveal how complex the relationships between diversity and energy flux are in real-world ecosystems, where food webs are large and highly complex (Thompson et al., 2012). It also illustrates how diversity-energy flux relationships for the top-carnivores, mesocarnivores and detritivores weaken over time, while such relationship remains remarkably stable for omnivores. As aforementioned, omnivorous species exploit a wide variety of resources (López-Rodríguez et al., 2019); consequently, it is

likely that they can sustain high resource uptake over time, and maintain a stable energy flow. Conversely, top-carnivorous and mesocarnivorous fishes rely on the biomass generated within the fish community itself, thus the diversity-energy flux associations are highly variable for these trophic compartments.

Importantly, detritivorous fish are not usually limited by low resource availability, as detritus are highly abundant in large rivers (González Bergonzoni at al., 2019). Therefore, it should expect a stable energy flux for the detritivore species. However, this was not the case in our study, where the energy flux for detritivores was highly variable and decreasing over time, and the diversity-energy flux relationships changed substantially as well. This is because species diversity present not only depends on feeding strategies but in many other life history aspects (e.g., preferential habitat, migratory habits and reproduction successful; Borzone mas et al., 2022). Moreover, the role of all species is not symmetric, existing particular species whose may contribute disproportionally more to food web and energy flux (Layman et al., 2005). For instance, there was detritivore species that were high abundant (e.g., *Prochilodus lineatus*) and had disproportionately high energy flux. Therefore, changes in the diversity-energy flux relationship may depend on the identity of the species being lost with time and their particular role in the energy flux.

Our structural equation modelling revealed the underlying mechanisms driving decreasing in diversity and energy flux. As such, HFP caused a direct decrease in the energy flux to all trophic compartments in the fish food webs. These results add to those from experimental studies (e.g., Barnes et al. 2014; Polazzo et al. 2022), suggesting that, in real-world ecosystems, increased human activities may impair the energy flux at the whole-network level. The Uruguay River covers regions of intensive agricultural crops and pasture lands grazed by cattle, cities, and industries (Soutullo et al., 2020). It is likely that multiple human pressures jointly affect biodiversity and reduce energy flux through food webs. Decreased diversity disrupts and shortens food webs (Sanders et al., 2018). Our results revealed marked decreases in the number of trophic links over time, confirming the expected shrinking and simplification of the food web. Along with trophic simplification, there was a drastic reduction in the overall energy flux. A likely explanation for this is that human pressure decreases availability of resources that fuel trophic compartments, decreasing food webs complexities (Rooney et al., 2006). For instance, human activities have promoted the invasion of the golden mussel (*Limnoperna fortunei*) in this river since 2001. This mollusk rapidly became a predominant food resource for about one third of the fish species in the assemblage and subsidized >10% of

total fish community biomass by 2018 (González Bergonzoni et al., 2020). This have caused negative changes in the trophic niche of many fish species that consume this mollusk (González Bergonzoni et al., 2023). Alterations in food resources such as this one might be provoking a decreasing efficiency of energy transfer between trophic levels, causing energy flux to reduces at the whole-network level.

Human pressure has potentially stronger impacts on energy flux at the top of fish food webs (Strong & Frank, 2010). We highlight two major pathways by which human pressure generated the stronger impacts on the top of the food web in our system: (i) directly and indirectly reducing energy flux to top-carnivores (endogenous pathway) by affecting diversity and (iii) indirectly reducing energy flux to top-carnivore by affecting river properties and nutrient stoichiometry (exogenous pathway). Endogenous pathways were evidenced by the strong negative effects of HFP on energy flux to top-carnivores, both directly and indirectly mediated by the reduction in diversity. Top-carnivores are more sensitive to human pressure intensification (Estes et al., 2003) and enormous losses of apex predators occur in human-dominated environments (Myers & Worm, 2003). The close association between top-carnivore diversity and energy flux implies that diversity loss might impact the resource uptake by top-carnivores, reducing intake energy flux (Barnes et al., 2014). In general, human-dominated sites support more simplified food webs (Strong & Frank, 2010), in which efficiency of energy transfer between trophic levels is low (Barnes et al., 2014). This has an especially strong impact on the top carnivore species that depend on energy that is transferred through food webs.

Exogenous pathways were evidenced by positive effects of HPF on turbidity and N:P stoichiometry, which indirectly decreased energy flux to top-carnivores. Increased turbidity, which translates to decreased water transparency, is a result of increased concentration of suspended particles (Davies-Colley & Nagels, 2008). As we show, this negatively affected top-carnivore species that are visually oriented predators (Ortega et al., 2020). It is likely that turbidity decreased the prey encounter rates and resource uptake by top-carnivores, which was translated into lower energy flux to this trophic level. By contrast, increased turbidity had positive effects on diversity and energy flux of omnivorous fishes, suggesting a favoring of lower trophic guilds in turbid water (Turesson & Brönmark, 2007). Changes in N:P stoichiometry was another important driver in the reduction of top-carnivore diversity and energy flux. Although we did not observe temporal decreases in P concentrations, there is subtle increase in nitrogen levels over time (Figure S8). This has resulted in a stoichiometric imbalance towards increasing N saturation (Elser et al., 2007) with impacts on availability of

nutrients to primary producers, such a phytoplankton (Pineda et al., 2020). Changes in the composition of primary producers can create elemental imbalances between consumers and their resources, with negative consequences for energy flux. It has been documented that changes in primary producers can cascade-up to top-carnivore diversity (Moi et al., 2021) and as we show, negatively affect the intake energy flux to top-carnivore. This has two profound implications: (i) it makes the structure and functioning of food webs more vulnerable to external stressors and (ii) impact the dynamics of aquatic ecosystems since top-carnivores determine food webs architecture through top-down control (Ripple et al., 2014).

There were positive effects of precipitation on energy flux of detritivore and top-carnivore species. In our system, during periods of high precipitation, the biomass production of detritivores increases, as a result of the greater support of allochthonous matter (González-Bergonzoni et al., 2019). The increased energy flux with precipitation reflects the role of flood pulses in boosting energy transfer, which occurs when floodplains get accessible for fish for purposes of feeding, reproduction and as refuge to larvae and juveniles (Junk et al., 1989). A long-term fishing dataset showed that certain detritivore and top-carnivore migratory fishes increase in abundance after years with large flood pulses, probably due to increased recruitment of juveniles when the floodplains act as nurseries (Scarabotti et al., 2021). Our results seem concurrent with this, and also the high detritivore's biomass when recruitment in floodplains is successful, implies greater food availability for the top-carnivores. Moreover, the recruitment of many top-carnivores (e.g., Salminus brasiliensis) is directly benefited in years of flood, we suspect that energy flux from detritivore to top-carnivorous species increased during periods of elevated precipitation. Indeed, we found higher energy transference from detritivores to top-carnivorous fishes with increasing precipitation (Figure S9 and Table S7), supporting that the high biomass of detritivores fuels the top-carnivores during rainy years. These results suggest an effect of climate variability on the energy flux in fish food webs, and imply that precipitation affects both the bottom and the top of fish food webs. Considering the climate change predictions of a higher frequency and intensity of extreme precipitation events (IPCC 2022), the variability in energy flux as a key ecosystem function might increase more in the future

5.5 Conclusion

Freshwater ecosystems are particularly vulnerable to climate change and human pressure (Ledger et al., 2013; Reid et al., 2018). By employing a multi-trophic approach, we demonstrate an alarming decline in energy flux at the whole-network level in a natural riverine

ecosystem. Such decline occurred across multiple trophic compartments, including omnivorous, detritivorous and predators. Notwithstanding, we also show shifts in energy fluxes towards lower trophic levels – this means that energy flux is retained in the lower trophic compartments, thus not transferred to the higher trophic compartments. These findings indicate that fish food webs underwent considerable restructuring in trophic height and the distribution of energy fluxes over time. The composition of fish communities is shifting toward the dominance of generalist species. Our results suggest multi-trophic homogenization of fish communities (Moi & Teixeira de Mello, 2022), with potential impacts on ecosystem services, since the stocks of large carnivorous fish provide a rich resource of protein and financial support for the human well-being (Pelicice et al., 2022). Even when this might, in part, correspond to long-term dynamics in the composition of communities associated with large scale climatic variability. We also reveal strong influence of human-induced pressure in the observed decline of diversity and energy flux in fish food webs. Human pressure influences the top-carnivore compartment, decreasing their energy flux both directly and indirectly as human pressures reduce the diversity needed to maintain energy flux. We emphasize that conserving the stability and functioning of food webs in natural ecosystems will be a major challenge as human pressures continue to increase worldwide (Reid et al., 2018).

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APPENDIX D – Details of the study area and results

Assessing fish community

The fish community was sampled in the three sites twice a year, during autumn and spring over 17 years (2005-2021). We experimentally used standard Nordic multimesh gillnet that presents 30 m-long nets with 12 sizes of mesh from knot to knot (5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 33, 43, and 55 mm, respectively). Sampled were made always in the exact same locations. We used during each sampling at each site, two survey nets attached together, making a total of four 60 m-long sets of nets. We position two 60 m-long sets parallel to each other in the littorals zone at a maximum the distance of 100 m from the coast with 300 m between them with a deep of 1-2 m. The other two 60 m-long sets were set parallel to each other in the middle zone at a distance of 500 m from the coast with 300 m between them and deep of 2-5m. To a most comprehensive sampling, campaign nets were deployed for ca. 12 h during twilight and night periods. Sampling was repeated in an interval of a week, to account for small temporal scale variations in each season. In summary, a total of 16 Nordic multimesh gillnets were set in each site for a total period of 24 h during each sampling season. The collected fish were identified at the species level, counted, measured, and weighed (to more details see González-Bergonzoni et al. 2019). The fish sampling was approved by the national authorities (DINARA) and followed the ethical considerations recommended by the honorary commission for animal experimentation (CHEA) in Uruguay.

Assessing the stomach contents of fish

Gut content analysis (GCA) was performed in the laboratory. The occurring food items were classified broadly into eight item types as follows: Detritus, plankton (zooplankton and phytoplankton), periphyton (diatoms and filamentous algae), invertebrates (aquatic and terrestrial insects, molluscs, and macrocrustaceans), fish remains (entire fish, scales, fins and fish remains) aquatic macrophytes, and terrestrial vegetal (fruits, seeds and other vegetal tissues). The absolute volume of each food item was measured using standardized Hyslop's indirect volumetric (Hyslop 1980) method. With this information, the relative contribution of each food item type to the diet of individuals was calculated.

For the trophic classification of species, data from each individual belonging to a species from the different river sections was pooled. This procedure was applied in order to obtain a broader view of diet plasticity and to minimize the potential effect of the short time scale and the strong habitat specificity typically considered by GCA. This procedure was followed to use variability in space along the whole river as a proxy of the potential variability across time and different habitat scenarios for a given species. For the classification purpose, the term "omnivores" was used to define species feeding at contrasting trophic levels, such as primary producers and consumers of any kind. This is a pragmatic use of the definition that allows a rather conservative but unequivocal visualization of this feeding strategy, but acknowledging that omnivores are strictly those feeding on more than one trophic level.

Assessing global drivers

We quantified four drivers of biodiversity and energy flux: (i) human pressure, (ii) precipitation, (iii) nutrient stoichiometry, and (iv) environmental variables.

Human pressure

We quantified human pressure using the human footprint index (*hereafter* HFP, Venter et al. 2016). The HFP incorporates eight single human pressures: (1) built environments, (2) crop land, (3) pasture land, (4) human density, (5) nighttime lights, (6) railways, (7) roads and (8) navigable waterways into a cumulative index of human pressure (Venter et al. 2016). This index has allowed to consistently test how cumulative human pressures impacts diversity and ecosystem functioning (Crossley et al., 2020; Moi et al., 2022). The average HFP was extracted for each year from 2005 to 2019 using the global map (https://wcshumanfootprint.org/map/). We calculated the average HFP for the Uruguay River catchment. The HFP ranges between 0 and 50 (the cumulative sum of all eight individual human pressures), and the higher the HFP, the higher the human pressure.

We used the global Human Footprint (HFP) map (Venter et al., 2016) as a surrogate of the cumulative human pressure on the Uruguay river. This map is constructed using eight common human pressures worldwide: (i) the extent of built environments, (ii) crop land, (iii) pasture land, (iv) human population density, (v) night-time lights, (vi) railways, (vii) roads, and (vii) navigable waterways. Each human pressure was weighted according to their intensity. For example, (i) constructed environments areas, which are closely associated to urban centers was assigned a score of 10 (i.e., a score of 10 is assigned if there are built environments, otherwise

a score of 0 is assigned). (ii) Crop land is characterized by agricultural activities with high inputs of pesticides and fertilizers. The crop land pressures received a score between 0 and 7, where 7 indicates intensive agriculture and 0 indicates the absence of crop lands. (iii) Pasture land is one of the major human activities worldwide and is characterized by cattle and sheep farming. The pressure of pastures on Uruguay river was assigned a score of 4, which was scaled from 0 to 4 using the %pasture for each 1 km^2 pixel. (iv) Human population is an important underlying driver of the global change of natural ecosystems. Human density was mapped using gridded population downscaled to match the 1 km^2 resolution. All areas with a population above 1,000 people/km^2 were assigned a pressure score of 10. For less populated areas, the pressure score is logarithmically scaled using the following estimation: Pressure score = 3.333 x log (population density + 1). (v) Night-time lights include electric infrastructure related to more rural areas that are not part of built environments. To calculate the pressure of night-time lights, the areas were divided into 10 quantiles of increased night-time light intensity associated with scores between 1 and 10, while areas with no lights were assigned a zero score. (vi) Railways are essential human infrastructures that influence natural ecosystems. The direct pressure of railways was assigned a score of 8 for a distance of 0.5 km on either side of the railway. (vii) Likewise, roads modify the landscape where they are built. The direct and indirect pressure of roads on wetlands was assigned a score of 8 for 0.5 km (direct impact), while nearby areas up to 15 km received a score value that decayed exponentially on either side of the road (indirect impact). (viii) Navigable waterways act as conduits for people to access nature, resulting in impacts on wetlands. The pressure of navigable waterways was assigned a score of 4, which decayed exponentially out to 15 km away from the water banks. For full details of HFP estimation see (Venter et al. 2016). The average HFP of the 1 km^2 pixels (cell-size resolution) overlapping each river site was extracted to derive the cumulative pressure, and this average HFP ranged between 0 and 50 (cumulative sum of all individual human pressures). The average HFP was through global map of HFP (*available in:* https://wcshumanfootprint.org/). In particular, the HFP is available for each year from 2005 to 2019. The eight human pressures are not mutually exclusive, and may co-occur in the same site.

Precipitation

For each month, we calculated the mean monthly precipitation (MMP). The MMP data was obtained from the WorldClim 2.0 database (http://www.worldclim.org) at a 1-km^2 spatial resolution.

Nutrient stoichiometry

To quantify nutrient stoichiometry, we took water samples during the fish sampling to measure nutrient concentrations (total phosphorus and total nitrogen; $\mu g\ l^{-1}$). Total nitrogen (N) was analysed through the persulfate method (Bergamin et al., 1978) and determined in a spectrophotometer in the presence of cadmium, using a flow-injection system (Giné et al., 1980). Total phosphorus (P) was measured according to Golterman et al., (1978). We determined the N:P ratio to address the prediction that imbalanced N and P loads affect energy dynamic through food webs (Glibert et al., 2012).

Environmental variables

The water discharge was measured using online data available from the water volumes discharged by Salto Grande dam (located approximately 200 km upstream from the sampling sites), while turbidity (expressed in NTU) was measured using turbidimeter LaMotte, Chestertown, MD, USA.

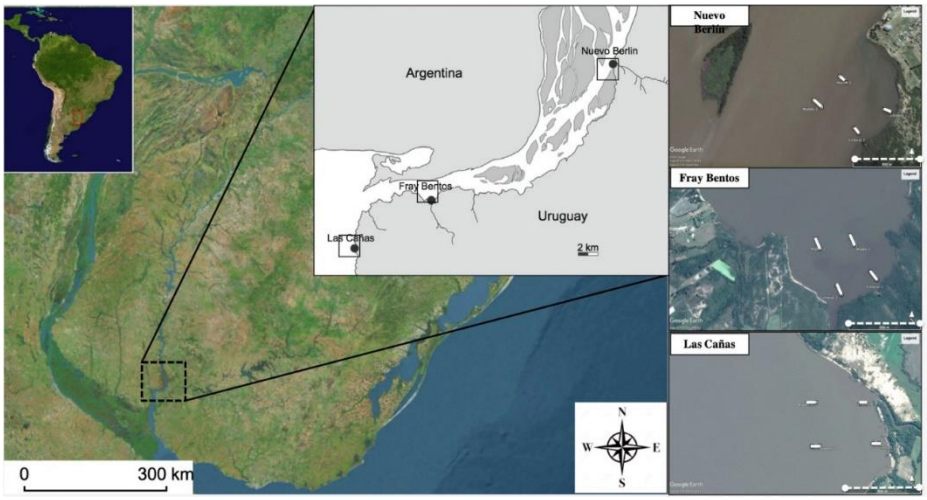


Figure S1. Sampling location and gillnet net deployment sites. Lower Uruguay river in South, America, indicating the sites Las Cañas, Fray Bentos and Nuevo berlin in lower Uruguay river where standardized fish sampling was taken over from 2005 to 2021, deploying 4 sets od Nordic Gillnets in the exact same locations shown in the right panel.

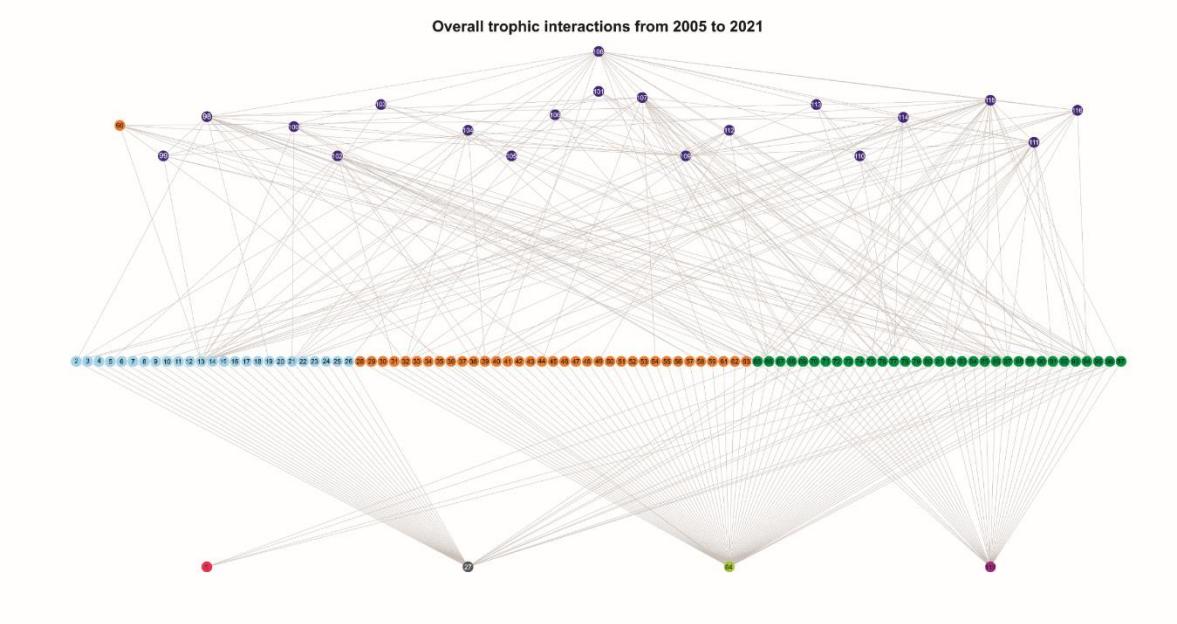
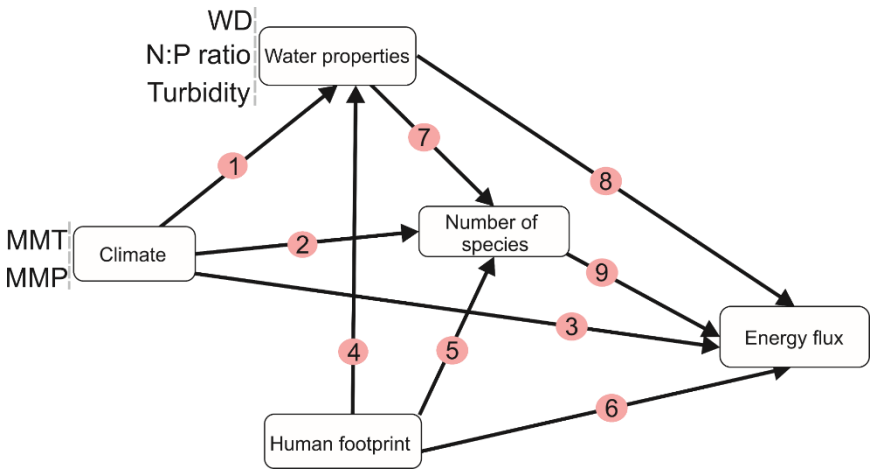


Figure S2. River level meta-food web constructed for all possible interactions among fish species in the Uruguay river. Color denotes species from specific trophic levels (red = algae; gray = detritus; light green = invertebrates; pink = aquatic plants; blue = detritivorous fishes, orange = mesocarnivorous fish; dark green = omnivorous fish; and purple = top-carnivorous fish. Links denote trophic interactions among fish feeding guilds in the food webs along which energy fluxes are calculated. Importantly, this is an overall meta food web representing all possible trophic interactions among fish species (a compilation of gut content analyses), which comprises 179 ‘sub ’meta food webs from each of three river sites surveyed from 2005 to 2021. Nodes’ labels correspond to the species ordering in the Table S1.



#	Links	*Rationale*	Ref.
1	**Climate → Water properties**	It is well known that climate affects the water characteristic of river ecosystems. For example, precipitation is expected to control water discharge and with increasing precipitation there is an increase in water discharge. Similarly, climate can influence nutrient stoichiometry in aquatic ecosystems	Wu et al., 2012; Zhao et al., 2017; Peacock et al., 2022; Moi et al., 2022
2	**Climate → Number of species**	Climate is also a key driver of number of species across multiple taxonomic groups. For example, precipitation has been reported to be positively related to the species richness of fish, macrophytes, zooplankton, and protists. Likewise, temperature is often positively related to the species richness of multiple aquatic organismal groups.	Rasconi et al., 2015; Guo et al., 2015; Moi et al., 2022; Tao et al., 2022
3	**Climate → Energy flux**	It has been increasingly recognized that climate can control energy flux through food webs, for instance, warming increases overall energy flux by increasing the metabolic demand of the species, while also decreasing the energy flux of specific trophic levels. Droughts it is expected to decrease trophic links and energy flux in the food webs.	Ledger et al, 2013; Schwarz et al. 2017
4	**HFP → Water properties**	Human pressures may control the water properties of aquatic ecosystems. For example, human activities such as agriculture, cities, and dams are expected to be negatively related to water discharge, turbidity, and nutrient stoichiometric.	Mander et al., 2000; Harrison et al., 2014; Winemiller et al., 2016; Zhao et al., 2017;

5	**HFP → Number of species**	Human pressures are considered to be major drivers of fish diversity. Human activities are expected to decrease species richness across multiple trophic groups	Utz et al., 2010; Filgueira et al., 2016; Moi and Teixeira de Mello, 2022; Moi et al., 2022
6	**HFP → Energy flux**	It has been proposed that human pressure can drive energy flux through food webs. Evidence suggests that energy flux decreases with increasing human pressure.	Barnes et al., 2014; Polazzo et al., 2022
7	**Water properties → Number of species**	Water properties are known to affect the number of species of multiple organismal groups in aquatic ecosystems. For example, water level fluctuation in natural aquatic systems is expected to have a strong positive effect on the species richness of fish species.	Agostinho et al., 2007
8	**Water properties → Energy flux**	Water properties has been showing to affect the biomass production of aquatic organisms, consequently, we can expect an effect of water properties on energy flux through aquatic organisms.	Fraley et al., 2020; Moi and Teixeira de Mello, 2022; Moi et al. 2022.
9	**Number of species → Energy flux**	Diversity has been a strong driver of energy flux in the food webs. There is evidence of a positive relationship between species richness and energy flux of trophic levels in food webs.	Barnes et al., 2014

Figure S3. Predictive figure which includes how the global drivers, namely human pressure (human footprint [HFP]), climate (precipitation and temperature), water properties (N:P ratio, water discharge, and turbidity), affect direct and biodiversity-mediated energy flux of fish trophic guilds in a neotropical river system. All variables were measured at the river level and represented the real conditions of the river environments. Different categories of predictors were grouped into the same box for graphical simplicity. However, they were considered individually in the model. In the table, we provide conceptual support for all the predictive links based on results from previous studies. Therefore, all causal relationships in our figure occur in nature and are not spurious. HFP = human footprint, MMT= mean monthly temperature, MMP = mean monthly precipitation and WD = water discharge**.**

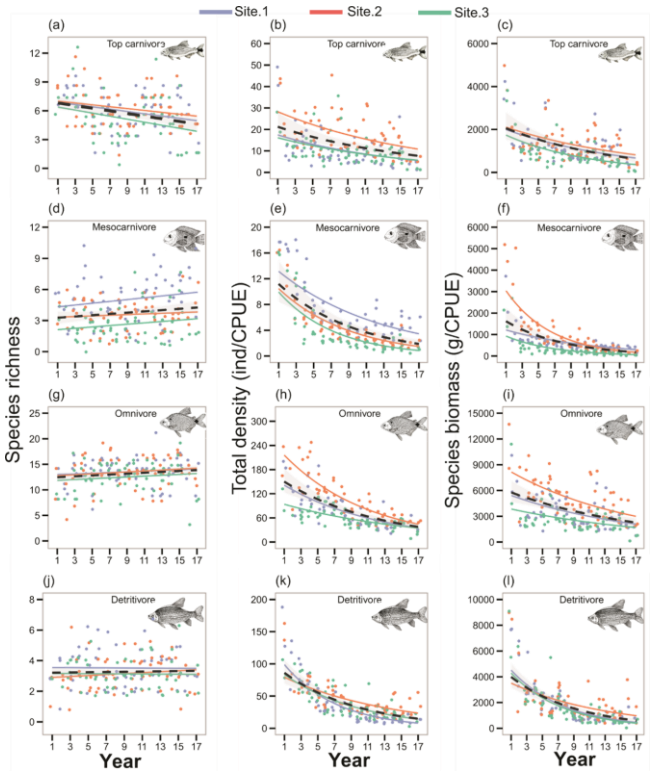


Figure S4. Long-term trends in species richness, total abundances, and biomass of fish trophic guilds. Generalized linear mixed models of temporal trends in species richness, total density, and standing biomass. Dashed black- and solid-colored lines are predicted (fitted) values from GLMMs for overall and local trends (for each site: blue line = site.1; orange line = site.2; green line = site.3), respectively. Shaded areas show the 95% confidence interval for the overall trend. Full model results are provided in Table 1. CPUE = catch per unit effort. Illustration credit: Margenny Barrios (Departamento de Ecología y Gestión Ambiental CURE, Universidad de la República, Uruguay).

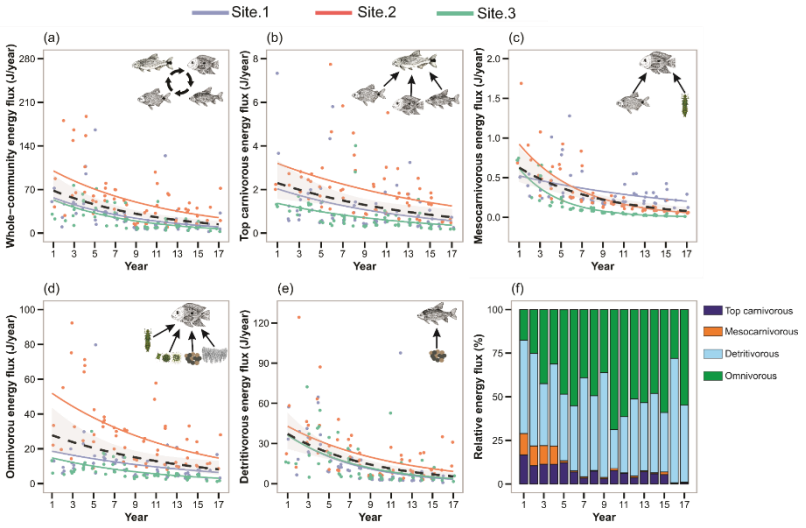


Figure S5. Long-term trends in energy fluxes through river food webs. Generalized linear mixed effects models of temporal trends in energy fluxes for (a) entire communities, (b) top carnivores, (c) mesocarnivores, (d) omnivores, and (e) detritivores. And (f) represents the relative contributions of fish trophic guilds over time. Dashed black- and solid-colored lines are predicted (fitted) values from GLMMs for overall and local trends (Uruguay River sites: blue line = site.1; orange line = site.2; green line = site.3). Shaded areas show the 95% confidence interval for the overall trend. Full model results are provided in Table 1. We calculated the intake flow for each trophic compartment of the river system. Total top-carnivorous flux was calculated as the sum of all outgoing energy flux from mesopredator, omnivorous and detritivorous fishes. Total mesocarnivorous flux was calculated as the sum of all outgoing energy flux from invertebrate and small fish prey. Total omnivorous flux represents the sum of all outgoing energy flux from plant, detritus, algae and invertebrates. Total detritivorous flux was calculated as the sum of outgoing energy flux from detritus.

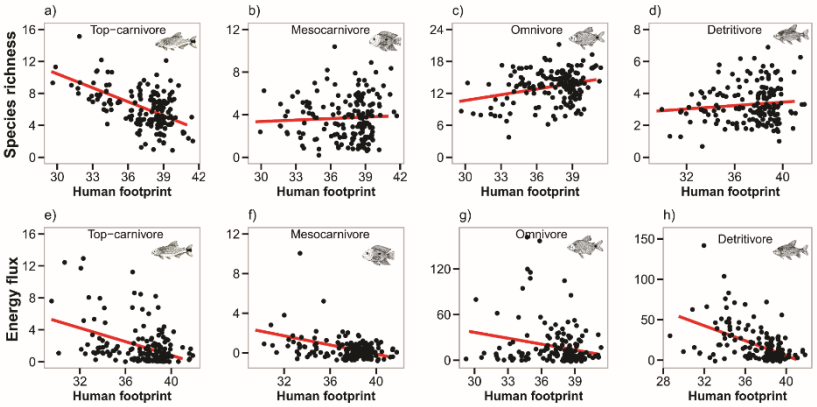


Figure S6. Relationship of human footprint with the number of species and energy flux in the fish trophic guilds. The linear association between the human footprint and the diversity and energy flux of the four selected trophic guilds of fish communities in a Neotropical River system; n = 179. Statistical analysis was performed using structural equation modeling (Figure 5, main manuscript). Solid lines are predicted (fitted) values from SEM. Full model results are provided in Table S4. Illustration credit: Margenny Barrios (Departamento de Ecologia e Gestão Ambiental CURE, Universidade da República).

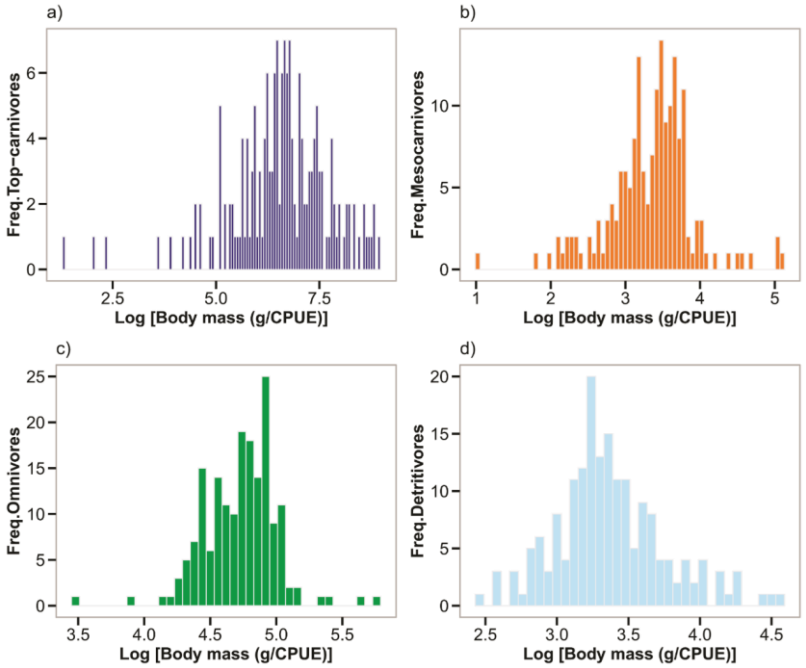


Figure S7. Body mass distributions for each of the four functional trophic guilds of the fish community. These trophic guilds included: top-carnivores (2685 individuals), mesocarnivores (979 individuals), omnivores (15099 individuals), and detritivores (7552 individuals).

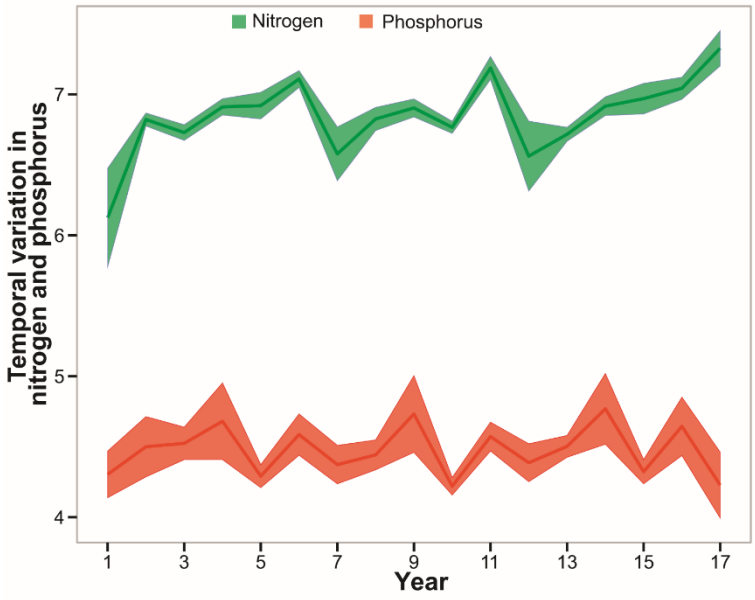


Figure S8. Long-term trends in nutrient variables. The mean (± s.e., n = 179) of nitrogen and phosphorus for each of 17-years.

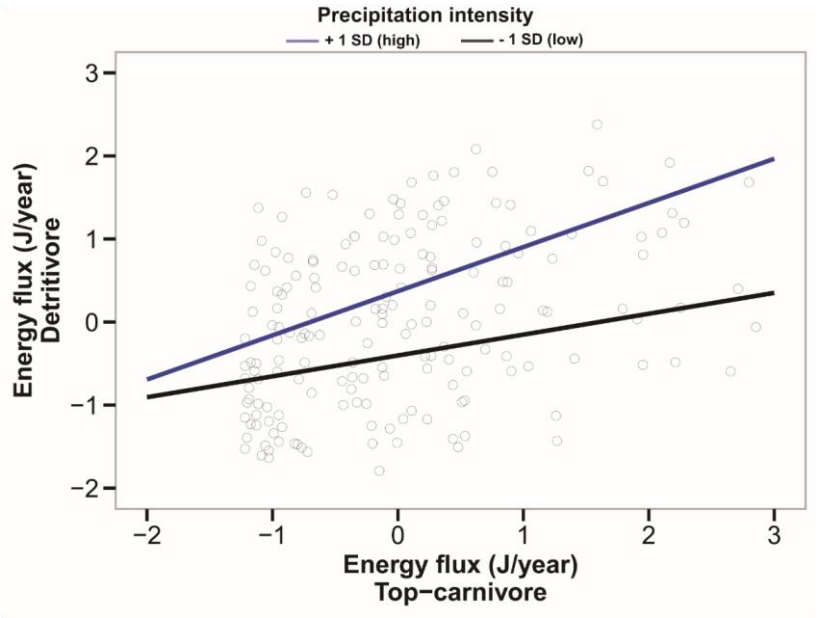


Figure S9. Effect of precipitation on the transfer of energy flux from detritivore to top-carnivore species. Linear transfer of energy flux from detritivore to top-carnivore species across precipitation gradient, including low (solid black line) and high precipitation (solid blue line). The lines are predicted (fitted) values from LMMs in which the effect of top-carnivore on detritivore is mediated at two levels of precipitation: (1) low: the standard deviation below the mean = −1 and the (2) high: the standard deviation above the mean = +1. Energy fluxes and precipitation were scaled to interpret parameter estimates at a comparable scale. Note that the transfer of energy flux from detritivore to top-carnivore increases significantly during periods of high precipitation compared with low precipitation periods (Table S6).

Table S1. The fish species and their food classification. The number in front of the species name corresponds to the species node in the meta food web (Figure S1).

Specie	Node position in meta Food-web	Trophic position
Cyphocharax voga	2	Detritivore
Loricariichthys.sp	3	Detritivore
Rhineloricaria.sp	4	Detritivore
Eigenmannia trinileneata	5	Detritivore
Hypostomus laplatae	6	Detritivore
Otocinclus.sp	7	Detritivore
Rineloricaria parva	8	Detritivore
Crenicichla vittata	9	Detritivore
Hypostomus aspilogaster	10	Detritivore
Potamorhina squamoralevis	11	Detritivore
Rhinelepis aspera	12	Detritivore
Prochilodus lineatus	13	Detritivore
Rineloricaria longicauda	14	Detritivore
Hypostomus.sp	15	Detritivore
Pseudohemiodon.sp	16	Detritivore
Hisonotus.sp	17	Detritivore
Hypostomus microstomus	18	Detritivore
Cyphocharax saladensis	19	Detritivore
Loricariinae.sp	20	Detritivore
Cyphocharax platanus	21	Detritivore
Hypostomus luteomaculatus	22	Detritivore
Hypostomus commersoni	23	Detritivore
Rhinodoras dorbignyi	24	Detritivore
Loricaria.sp	25	Detritivore
Pimelodella australis	26	Detritivore
Crenicichla.sp	28	Mesocarnivore
Crenicichlaminuano	29	Mesocarnivore
Pimelodus absconditus	30	Mesocarnivore
Crenicichla missioneira	31	Mesocarnivore
Cyphocharax spilotus	32	Mesocarnivore
Brochiloricaria chauliodon	33	Mesocarnivore
Gymnogeophagus australis	34	Mesocarnivore
Cheirodon interruptus	35	Mesocarnivore
Hyphessobrycon meridionalis	36	Mesocarnivore
Roeboides microlepis	37	Mesocarnivore
Odontesthes perugiae	38	Mesocarnivore
Eigenmannia virescens	39	Mesocarnivore
Bryconamericus iheringii	40	Mesocarnivore
Bunocephalus coracoides	41	Mesocarnivore
Corydoras paleatus	42	Mesocarnivore
Crenichla scotti	43	Mesocarnivore
Auchenipterus osteomystax	44	Mesocarnivore
Charax stenopterus	45	Mesocarnivore
Triportheus nematurus	46	Mesocarnivore
Pseudobunocephalus iheringii	47	Mesocarnivore
Leporinus lacustris	48	Mesocarnivore
Gymnotus omarorum	49	Mesocarnivore
Odontesthes argentinensis	50	Mesocarnivore
Characidium rachovii	51	Mesocarnivore
Microglanis	52	Mesocarnivore
Pseudopimelodus	53	Mesocarnivore
Synbranchus marmoratus	54	Mesocarnivore
Leporellus pictus	55	Mesocarnivore
Brachyhypopomus.sp	56	Mesocarnivore

Rineloricaria	57	Mesocarnivore
Paraloricaria vetula	58	Mesocarnivore
Pellona flavipinnis	59	Mesocarnivore
Rhamdia quelen	60	Mesocarnivore
Steindachnerina brevipinna	61	Mesocarnivore
Pterodoras granulosus	62	Mesocarnivore
Crenicichla lepidota	63	Mesocarnivore
Apareiodon affinis	65	Omnivore
Pimelodella gracilis	66	Omnivore
Auchenipterus nuchalis	67	Omnivore
Loricariichthys anus	68	Omnivore
Iheringichthys labrosus	69	Omnivore
Schizodon nasutus	70	Omnivore
Trachelyopterus lucenai	71	Omnivore
Odontesthes humensis	72	Omnivore
Loricariichthys edentatus	73	Omnivore
Ricola macrops	74	Omnivore
Bryconamericus stramineus	75	Omnivore
Astyanax lacustris	76	Omnivore
Odontostilbe pequira	77	Omnivore
Platanichthys platana	78	Omnivore
Characidium zebra	79	Omnivore
Leporinus striatus	80	Omnivore
Pimelodus maculatus	81	Omnivore
Trachelyopterus albicrux	82	Omnivore
Ramnogaster melanostoma	83	Omnivore
Hoplosternum littorale	84	Omnivore
Schizodon platae	85	Omnivore
Characidium tenue	86	Omnivore
Astyanax.sp	87	Omnivore
Astyanax erythropterus	88	Omnivore
Mylossoma duriventre	89	Omnivore
Callichthys callichthys	90	Omnivore
Homodiaetus anisitsi	91	Omnivore
Brycon orbignyanus	92	Omnivore
Megaleporinus obtusidens	93	Omnivore
Pachyurus bonariensis	94	Omnivore
Parapimelodus valenciennes	95	Omnivore
Pimelodus.sp	96	Omnivore
Loricariichthys melanocheilus	97	Omnivore
Acestrorhynchus pantaneiro	98	Top-carnivore
Oligosarcus oligolepis	99	Top-carnivore
Pygocentrus nattereri	100	Top-carnivore
Ageneiosus militaris	101	Top-carnivore
Galeocharax humeralis	102	Top-carnivore
Sorubim lima	103	Top-carnivore
Pseudoplatystoma corruscans	104	Top-carnivore
Potamotrygon brachyura	105	Top-carnivore
Serrasalmus marginatus	106	Top-carnivore
Pseudopimelodus mangurus	107	Top-carnivore
Ageneiosus inermis	108	Top-carnivore
Hoplias argentinensis	109	Top-carnivore
Lycengraulis grossidens	110	Top-carnivore
Catathyridium.sp	111	Top-carnivore
Cynopotamus argenteus	112	Top-carnivore
Luciopimelodus pati	113	Top-carnivore
Oligosarcus jenynsii	114	Top-carnivore
Rhaphiodon vulpinus	115	Top-carnivore
Salminus brasiliensis	116	Top-carnivore

Serrasalmus maculatus	117	Top-carnivore
Serrasalmus marginatus	118	Top-carnivore

Table S2. Results of generalized mixed-effects models for the effect of time × sites on biodiversity (species richness, abundance, and biomass), and energy flux across a river system. Probabilities were calculated using likelihood ratio tests (LRT, $\chi 2$). CPUE = catch per unit effort.

Transformation	Time			Sites			Time × sites		
	χ^2	**df**	***P*-value**	**χ^2**	**df**	***P*-value**	**χ^2**	**Df**	***P*-value**
Species richness									
Top-carnivore	12.91	1	**<0.001**	8.76	2	**0.012**	0.68	2	0.708
Mesocarnivore	3.58	1	0.058	54.81	2	**<0.001**	0.63	2	0.728
Omnivore	2.17	1	0.140	4.71	2	0.094	0.05	2	0.973
Detritivore	0.03	1	0.842	4.82	2	0.08	1.09	2	0.571
Abundance (ind/CPUE)									
Top-carnivore	32.05	1	**<0.001**	5.99	2	0.149	2.00	2	0.202
Mesocarnivore	9.25	1	**0.002**	4.01	2	0.134	0.53	2	0.765
Omnivore	4.85	1	**0.027**	1.76	2	0.414	0.16	2	0.919
Detritivore	18.45	1	**<0.001**	2.10	2	0.348	1.58	2	0.453
Biomass (g/CPUE)									
Top-carnivore	5.79	1	**0.016**	2.37	2	0.305	0.26	2	0.871
Mesocarnivore	14.58	1	**<0.001**	8.26	2	**0.016**	1.21	2	0.545
Omnivore	4.71	1	**0.029**	5.23	2	0.072	0.03	2	0.981
Detritivore	0.62	1	0.427	0.03	2	0.981	0.05	2	0.973
Energy flux (J/year)									
Whole food web	19.57	1	**<0.001**	14.15	2	**<0.001**	0.41	2	0.811
Top carnivore	6.09	1	**0.013**	10.82	2	**0.004**	0.12	2	0.937
Mesocarnivore	1331.2	1	**<0.001**	1.71	2	0.423	0.31	2	0.801
Omnivore	4.42	1	**0.035**	11.73	2	**0.002**	0.10	2	0.950
Detritivore	27.80	1	**<0.001**	5.83	2	0.053	0.93	2	0.626
Relative energy flux (proportion)									
Top carnivore	4.276	1	**0.038**	0.19	2	0.906	1.63	2	0.440
Mesocarnivore	25.41	1	**<0.001**	7.20	2	**0.027**	3.33	2	0.415
Omnivore	2.76	1	0.096	0.75	2	0.685	0.26	2	0.876

Detritivore	0.17	1	0.672	0.70	2	0.701	0.40	2	0.817

Table S3. Results of linear mixed-effects models for the effect of biodiversity × sites on energy flux. Biodiversity is represented by number of species. Model was performed for entire fish community: (i) whole community biodiversity ~ whole-community energy flux, and for each single trophic guild: (ii) top-carnivore biodiversity ~ top-carnivore energy flux, (iii) mesocarnivore biodiversity ~ mesocarnivore energy flux, (iv) omnivore biodiversity ~ omnivore energy flux, and (v) detritivore biodiversity ~ detritivore energy flux.

Energy flux	Biodiversity			Sites			Biodiversity × sites			
	$F_{(1,170)}$	**df**	***P*-value**	$F_{(2,170)}$	**df**	***P*-value**	$F_{(2,170)}$	**df**	***P*-value**	R^2
(i) Whole-community	21.92	1	**<0.001**	2.93	2	0.055	2.33	2	0.100	0.349
(ii) Top-carnivore	13.84	1	**<0.001**	1.85	2	0.160	4.56	2	**0.011**	0.311
(iii) Mesocarnivore	8.15	1	**0.004**	3.63	2	**0.028**	3.82	2	**0.023**	0.107
(iv) Omnivore	21.35	1	**<0.001**	2.80	2	0.063	2.14	2	0.119	0.276
(v) Detritivore	2.70	1	0.102	4.14	2	**0.017**	2.25	2	0.107	0.067

Table S4. Results of linear mixed-effects models for the effect of biodiversity on energy flux over each year. Biodiversity is represented by number of species.

			Energy flux	
Trophic guild	Explanatory variables	Estimate	95% CI	*P*-value
Whole community	Biodiversity: 2005	1.83	**1.15, 2.52**	<0.001
	Biodiversity: 2006	1.56	**0.85, 2.26**	<0.001
	Biodiversity: 2007	1.91	**1.27, 2.56**	<0.001
	Biodiversity: 2008	1.99	**1.36, 2.62**	<0.001
	Biodiversity: 2009	1.93	**1.23, 2.62**	<0.001
	Biodiversity: 2010	1.82	**1.17, 2.48**	<0.001
	Biodiversity: 2011	1.79	**1.13, 2.44**	<0.001
	Biodiversity: 2012	1.76	**1.08, 2.44**	<0.001
	Biodiversity: 2013	1.09	**0.40, 1.78**	0.002
	Biodiversity: 2014	0.50	**-0.16, 1.16**	<0.001
	Biodiversity: 2015	0.76	**0.12, 1.40**	<0.001
	Biodiversity: 2016	0.65	**0.01, 1.28**	<0.001
	Biodiversity: 2017	0.77	**0.09, 1.44**	<0.001
	Biodiversity: 2018	0.74	**0.08, 1.41**	<0.001
	Biodiversity: 2019	0.63	**0.00, 1.25**	<0.001
	Biodiversity: 2020	0.49	**-0.23, 1.21**	<0.001
	Biodiversity: 2021	0.79	**0.05, 1.53**	<0.001
Top-carnivore	Biodiversity: 2005	1.14	**0.72, 1.57**	<0.001
	Biodiversity: 2006	1.14	**0.76, 1.55**	0.001
	Biodiversity: 2007	1.08	**0.73, 1.50**	<0.001
	Biodiversity: 2008	0.51	**0.33, 0.70**	<0.001
	Biodiversity: 2009	0.55	**0.31, 0.80**	<0.001
	Biodiversity: 2010	0.68	**0.48, 0.88**	<0.001
	Biodiversity: 2011	0.44	**0.23, 0.65**	<0.001

	Biodiversity: 2012	0.73	**0.49, 0.98**	<0.001
	Biodiversity: 2013	0.10	-0.16, 0.36	0.463
	Biodiversity: 2014	0.35	**0.13, 0.57**	0.001
	Biodiversity: 2015	0.51	**0.33, 0.70**	<0.001
	Biodiversity: 2016	0.36	**0.17, 0.55**	<0.001
	Biodiversity: 2017	0.43	**0.23, 0.63**	<0.001
	Biodiversity: 2018	0.49	**0.27, 0.71**	<0.001
	Biodiversity: 2019	0.33	**0.13, 0.53**	0.001
	Biodiversity: 2020	0.09	-0.24, 0.43	0.573
	Biodiversity: 2021	0.21	-0.27, 0.68	0.394
Mesocarnivore	Biodiversity: 2005	0.53	**0.21, 0.92**	<0.001
	Biodiversity: 2006	0.51	**0.17, 0.86**	0.004
	Biodiversity: 2007	0.40	**0.19, 0.71**	<0.001
	Biodiversity: 2008	0.37	**0.17, 0.67**	<0.001
	Biodiversity: 2009	0.23	**0.11, 0.36**	<0.001
	Biodiversity: 2010	0.13	**0.02, 0.25**	0.023
	Biodiversity: 2011	0.09	-0.04, 0.21	0.168
	Biodiversity: 2012	0.03	-0.07, 0.14	0.531
	Biodiversity: 2013	-0.02	-0.17, 0.13	0.804
	Biodiversity: 2014	0.04	-0.07, 0.15	0.456
	Biodiversity: 2015	0.05	-0.05, 0.14	0.348
	Biodiversity: 2016	0.11	**0.01, 0.21**	0.028
	Biodiversity: 2017	0.05	-0.08, 0.17	0.469
	Biodiversity: 2018	0.06	-0.05, 0.18	0.295
	Biodiversity: 2019	0.09	0.00, 0.19	0.055
	Biodiversity: 2020	0.01	-0.12, 0.14	0.876
	Biodiversity: 2021	0.04	-0.09, 0.16	0.556

Omnivore	Biodiversity: 2005	1.80	**1.06, 2.53**	<0.001
	Biodiversity: 2006	1.52	**0.74, 2.29**	<0.001
	Biodiversity: 2007	1.76	**1.14, 2.39**	<0.001
	Biodiversity: 2008	1.97	**1.37, 2.57**	<0.001
	Biodiversity: 2009	2.00	**1.33, 2.67**	<0.001
	Biodiversity: 2010	2.01	**1.40, 2.62**	<0.001
	Biodiversity: 2011	1.78	**1.18, 2.37**	<0.001
	Biodiversity: 2012	1.82	**1.19, 2.45**	<0.001
	Biodiversity: 2013	1.10	**0.47, 1.72**	<0.001
	Biodiversity: 2014	1.43	**0.81, 2.04**	<0.001
	Biodiversity: 2015	1.69	**1.07, 2.31**	<0.001
	Biodiversity: 2016	1.66	**1.04, 2.28**	<0.001
	Biodiversity: 2017	1.69	**1.04, 2.33**	<0.001
	Biodiversity: 2018	1.52	**0.90, 2.14**	<0.001
	Biodiversity: 2019	1.55	**0.97, 2.13**	<0.001
	Biodiversity: 2020	1.41	**0.70, 2.11**	<0.001
	Biodiversity: 2021	1.59	**0.86, 2.33**	<0.001
Detritivore	Biodiversity: 2005	0.54	-0.31, 1.38	0.212
	Biodiversity: 2006	0.20	-0.32, 0.62	0.591
	Biodiversity: 2007	0.93	**0.35, 1.51**	0.001
	Biodiversity: 2008	1.21	**0.66, 1.77**	<0.001
	Biodiversity: 2009	0.35	-0.17, 0.86	0.190
	Biodiversity: 2010	0.00	-0.56, 0.56	0.991
	Biodiversity: 2011	0.56	-0.05, 1.16	0.072
	Biodiversity: 2012	-0.08	-0.73, 0.58	0.819
	Biodiversity: 2013	-1.17	**-1.79, -0.55**	<0.001
	Biodiversity: 2014	-1.09	**-1.72, -0.47**	<0.001

	Biodiversity: 2015	-0.42	-0.95, 0.11	0.121
	Biodiversity: 2016	-0.37	-0.85, 0.12	0.135
	Biodiversity: 2017	-0.41	-1.03, 0.21	0.198
	Biodiversity: 2018	-0.28	-0.82, 0.26	0.299
	Biodiversity: 2019	-0.51	-1.07, 0.05	0.074
	Biodiversity: 2020	-0.42	-1.38, 0.54	0.389
	Biodiversity: 2021	-0.43	-1.26, 0.39	0.301

Table S5. Standardized direct paths of ecosystem drivers, including climate (temperature and precipitation), space (distance from equator), local aquatic properties (N:P ratio, water discharge, and turbidity), besides human pressure (human footprint index) on the diversity (number of species) and energy flux of fish trophic guilds; (a) top-carnivores, (b) mesocarnivores, (c) omnivores and (d) detritivore (see Figure 5, main article). This table includes all significant and nonsignificant path considered by our model. *= $P < 0.05$, **= $P < 0.01$, and ***= $P < 0.001$. (n = 179). MMP = mean monthly precipitation; WD = water discharge.

Predictors		Response	Standardized coefficients	Std.Error	*P*-value
Fig. 5 a-c: Top-carnivore					
MMP	→	Energy flux	0.248	0.067	<0.001***
Human footprint	→	Energy flux	–0.190	0.129	0.015*
Water discharge	→	Energy flux	0.014	0.000	0.863
N:P ratio	→	Energy flux	0.049	0.029	0.500
Turbidity	→	Energy flux	–0.160	0.018	0.421
Species richness	→	Energy flux	0.412	0.079	<0.001***
MMP	→	Species richness	0.283	0.063	0.048*
Human footprint	→	Species richness	-0.451	0.108	0.003**
Water discharge	→	Species richness	-0.0826	0.000	0.184
N:P ratio	→	Species richness	–0.1716	0.028	0.009**
Turbidity	→	Species richness	–0.2108	0.017	0.008**
Fig. 5 d-f: Mesocarnivore					
MMP	→	Energy flux	0.004	0.038	0.945
Human footprint	→	Energy flux	–0.407	0.075	<0.001***
Water discharge	→	Energy flux	–0.084	0.000	0.257
N:P ratio	→	Energy flux	–0.025	0.018	0.609
Turbidity	→	Energy flux	–0.035	0.011	0.165
Species richness	→	Energy flux	0.246	0.055	<0.001***
MMP	→	Species richness	–0.021	0.055	0.856
Human footprint	→	Species richness	0.112	0.094	0.411

Water discharge	→	Species richness	0.140	0.000	0.502
N:P ratio	→	Species richness	0.231	0.025	0.040*
Turbidity	→	Species richness	–0.187	0.015	0.024*
Fig. 5 g-i: Omnivore					
MMP	→	Energy flux	–0.142	0.778	0.058
Human footprint	→	Energy flux	–0.307	1.174	0.003**
Water discharge	→	Energy flux	0.170	0.000	0.142
N:P ratio	→	Energy flux	0.186	0.355	0.030*
Turbidity	→	Energy flux	0.216	0.228	0.038*
Species richness	→	Energy flux	0.264	0.725	<0.001***
MMP	→	Species richness	–0.042	0.086	0.586
Human footprint	→	Species richness	0.337	0.146	0.005**
Water discharge	→	Species richness	0.025	0.000	0.778
N:P ratio	→	Species richness	0.232	0.039	0.043*
Turbidity	→	Species richness	0.293	0.023	<0.001***
Fig. 5 j-l: Detritivore					
MMP	→	Energy flux	0.004	0.620	0.953
Human footprint	→	Energy flux	–0.466	0.965	<0.001***
Water discharge	→	Energy flux	–0.033	0.000	0.688
N:P ratio	→	Energy flux	0.132	0.278	0.098
Turbidity	→	Energy flux	–0.037	0.169	0.636
Species richness	→	Energy flux	0.057	1.293	0.396
MMP	→	Species richness	0.168	0.034	0.033*
Human footprint	→	Species richness	0.156	0.042	0.076
Water discharge	→	Species richness	0.119	0.000	0.133
N:P ratio	→	Species richness	–0.110	0.015	0.175

Turbidity	→	Species richness	–0.064	0.009	0.435
Interactions among drivers					
MMP	→	Turbidity	0.015	0.280	0.829
Human footprint	→	Turbidity	0.352	0.527	0.004**
Water discharge	→	Turbidity	–0.036	0.000	0.676
MMP	→	N:P ratio	0.099	0.167	0.159
Human footprint	→	N:P ratio	0.478	0.350	<0.001***
MMP	→	Water discharge	0.158	1.942	0.014*
Human footprint	→	Water discharge	–0.011	3.713	0.920

Table S6. Proportion of variance explained for each endogenous variables in SEM. This table showed a R^2, marginal (variance explained by fixed factors) and conditional (variance explained by fixed factors plus random factors) of all endogenous variables (i.e., turbidity, water discharge, N:P ratio, species richness and energy flux) in the Figure. 5. MAP = mean annual of precipitation.

Endogenous variables	**$R^2_{marginal}$**	**$R^2_{conditional}$**
Species richness model – Fig 6,d,g,j		
Turbidity	0.13	0.41
Water discharge	0.02	0.59
N:P ratio	0.20	0.49
Top-carnivore species richnes	0.39	0.51
Top-carnivore energy flux	0.35	0.57
Mesocarnivore species richnes	0.16	0.44
Mesocarnivore species flux	0.17	0.67
Omnivore species richness	0.15	0.32
Omnivore species flux	0.14	0.34
Detritivore species richness	0.19	0.29
Detritivore species flux	0.21	0.31

Table S7. Coefficient estimates and confidence intervals from mixed-effects models that include interaction terms to test whether transference of energy from detritivore species to top-carnivore species change from low to high precipitation periods (Figure S8) in a river system (n = 179). *P* < 0.001***.

Predictor	Estimate	CI (lower)	CI (Upper)	Std.Error	t-value	p-value
Energy flux from detritivore to top-carnivore species – Figure S9						
Top-carnivore	0.361	0.217	0.505	0.072	4.9	<0.001***
Top-carnivore*Precipitation	0.412	0.271	0.582	0.073	6.12	<0.001***

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CONCLUDING REMARKS

Freshwater systems provide countless goods and services that fuels human well-being, such as drinking water, food and transportation. In history, human civilizations have always developed near freshwater systems, impacting the quality of these environments. These impacts intensify as the human population increases, – now with 8 billion people inhabiting the Earth, they have reached the highest levels ever recorded. We conducted four independent studies to assess how human-induced pressures affect biodiversity, ecosystem functioning, and the biodiversity-ecosystem functioning relationship across different freshwater systems. These studies were conducted in lakes, rivers and streams and ranged from short (1 year) to long (17 years) time scales. We measure different human-induced pressures, including regime shifts (clear, turbid and shaded), human land-uses (urbanization, agriculture, pasture and afforestation), and *human footprint*. To test the consistency of human impacts across all studies, we accounted for space (latitude), climate (temperature and precipitation), and local environmental variables (water level, pH, conductivity).

The results revealed that freshwater ecosystems are losing biodiversity across multiple organismal groups of varying trophic levels. We found decreases in the biodiversity (species richness, abundance, and functional diversity) of fish, macrophytes, microcrustaceans, rotifera, phytoplankton, and protozoa. Importantly, higher trophic levels (top-carnivores) are the ones that suffered the greatest biodiversity loss. Such biodiversity decline was a general pattern in freshwater systems, occurring in lakes, rivers, and streams. Moreover, the decline in biodiversity became more pronounced over time. This illustrates that more species losses were observed over long periods than over short periods.

It has been proposed that the decline in biodiversity could affect the ability of freshwater ecosystems to maintain their functioning. As such, our results reveal a strong positive relationship between diversity and the functioning of freshwater systems. This illustrates that the greater the biodiversity, the greater the performance of lakes, rivers, and streams to sustain multiple ecosystem functions. Increased biodiversity has elevated the performance of various ecosystem functions, and also increased the uniformity of

energy flux through fish food webs. However, increasing human-induced pressures reduce the biodiversity of freshwater systems. Human-induced pressures including regime shifts, human land-uses and *human footprint* had strong negative effects on species richness, abundance and functional diversity of all organismal groups studied (fish, macrophytes, microcrustaceans, rotifera, phytoplankton and protozoa). Such negative effects of human-induced pressures were remarkably consistent across freshwater systems, and also became stronger over time.

We also found that the positive relationship between biodiversity and ecosystem functioning was broken-down, which occur both directly and indirectly as human pressures reduce the biodiversity needed to maintain numerous ecosystem functions. The break-down of the positive biodiversity-ecosystem functioning relationship, in turn, caused the collapse of important ecosystem processes. In particular, our findings revealed declines in primary productivity, biomass production, nutrient cycling, and energy flow through food webs in freshwater systems. Collectively, these four studies highlight that conserving the biodiversity and functioning of freshwater systems will be a major challenge as human pressures continue to increase in these systems worldwide. More broadly, reducing human pressures must be addressed urgently and encompass all freshwater systems that are under human threat. Finally, biodiversity conservation is necessary if we are to safeguard the functioning of freshwater systems to maintain the services these systems provide for the human well-being.

UMA NOTA DE INFORMAÇÃO COMPLEMENTAR – Lista de trabalhos publicados, aceitos, e submetidos.

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