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Experimental effects of multiple agricultural stressors on diversity and size structure of subtropical stream macroinvertebrates

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ABSTRACT

Agricultural activities cause changes in land use and affect the ecological integrity of streams and rivers, with some of the main impacts being related to increased nutrient and fine sediment inputs. Agricultural water diversion and prolonged droughts due to climate change can also cause alterations in these ecosystems, such as modifications in flow dynamics. However, the interactions between stressors related to agricultural production and climate change are still poorly understood. Therefore, the singular and combined effects of three agricultural and climate change stressors were tested: i. nitrate enrichment (ambient/80 %/140 %/220 %), ii. fine sediment deposition (ambient and 80 % increase), and iii.- flow reduction (ambient and 66 % reduction) on the benthic macroinvertebrate community of a subtropical stream. For this purpose, we used a streamside mesocosm setup consisting of 64 experimental mesocosms. These were continuously supplied with stream water, allowing for comparable physicochemical and climatic characteristics to the adjacent stream. We investigated the individual and combined effects of the stressors on invertebrate community richness, abundance, Shannon diversity, equitability (species dominance), community composition and community size structure (size spectra). Our findings revealed that the macroinvertebrate community was affected by all three stressors, with each one influencing different structural aspects of the community. Nitrate addition had a positive effect on community evenness up to moderate enrichment levels. Sedimentation primarily decreased richness and the abundance of larger individuals, notably impacting chironomid communities that are closely tied to sediment characteristics. Flow reduction altered diversity and equitability, benefiting species such as ostracods that thrive in slow-flow environments. When combined, sedimentation and reduced flow decreased the occurrence of several rarer taxa. These findings suggest that agricultural intensification and climate change may negatively impact macroinvertebrate communities in subtropical streams through single and combined stressor mechanisms.

Introduction

Streams in tropical and subtropical regions are among the most biodiverse aquatic ecosystems in the world, yet they are also among the most understudied and threatened due to anthropogenic activities such as agricultural production and climate change (Dudgeon et al., 2006;

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Lambin et al., 2003; Taniwaki et al., 2017; Valente-Neto et al., 2024). Intensification of agricultural practices substantially contributes to the deterioration of the functions and biological structure of stream ecosystems (Martinelli et al., 2010; Schiesari and Corrêa, 2015; Schiesari and Grillitsch, 2011). Streams located in agricultural regions are often impacted by sediment accumulation and high nutrient concentrations, stressors that affect stream ecosystems worldwide (Dudgeon, 2010; Foley et al., 2005; Vorosmarty et al., 2000). As the intensity of agricultural activities increases, the impact of multiple stressors on freshwater communities is correspondingly amplified (Beermann et al., 2018; Elbrecht et al., 2016). Nutrient enrichment of streams, primarily from surface runoff and contaminated groundwater inputs, accelerates the eutrophication process and accumulation of heavy metals in sediment beds, leading to negative impacts on stream communities and shifts in community structure (Camargo et al., 2005; Schwantes et al., 2021; Wagenhoff et al., 2012). Such changes include the bioaccumulation of heavy metals, the exclusion of sensitive species or functional processes and disruption of freshwater food webs (Arnold et al., 2021; Espinoza-Toledo et al., 2021; Lima et al., 2022). Moreover, agricultural activities increase the input of fine sediment into streams, for example through deforestation associated with monocultures and the use of heavy machinery (Martinelli et al., 2010), thus increasing streambed sedimentation and altering the habitat of the benthic community (França et al., 2023; Pedersen, 2009).

Degradation of running-water ecosystems can be effectively monitored using benthic macroinvertebrates, which are widely recognized as reliable bioindicators of aquatic ecosystem health due to their abundance, ease of collection, and wide distribution (de Mello et al., 2023; Rosenberg and Resh, 1993). These organisms have long life cycles that include larval, juvenile and adult stages, with the duration of each stage varying according to species and environmental conditions. Further, their relatively sedentary nature enables them to provide a localized representation of the environment (Rosenberg and Resh, 1993). The worldwide popularity of benthic macroinvertebrates as bioindicators is due to their high taxonomic diversity and their sensitivity to different stressors (Beermann et al., 2018; Blöcher et al., 2020; Juvigny-Khenafou et al., 2021; Piggott et al., 2015). Moreover, benthic macroinvertebrates span a wide range of body sizes, and larger taxa with smaller populations are usually expected to be more sensitive to environmental degradation. This can be described by a size-based assessment tool such as the size spectrum which depicts the negative relationship between abundance/biomass and density in a community, where a steeper slope indicates that less biomass of large organisms is maintained in the community (Collyer et al., 2023; Petchey and Belgrano, 2010). All these properties make benthic macroinvertebrates highly suitable bioindicators in running waters for detecting changes in environmental quality at different time scales.

Alongside agricultural stressors, the consequences of climate change may further impact aquatic environments (Gesualdo et al., 2019; Taniwaki et al., 2017). According to reports from the Intergovernmental Panel on Climate Change (IPCC), higher and more variable temperatures are forecasted in subtropical regions, as well as prolonged drought periods, causing significant changes in streamflow dynamics in subtropical streams (IPCC, 2019). Drought-related reductions in stream flow and/or current velocity can cause changes in the hydrological patterns of streams and water quality, as well as affecting the composition of the benthic macroinvertebrate community (Blöcher et al., 2020; Dewson et al., 2007). These changes can include increased nutrient loading during drought periods, reduced oxygen availability which excludes sensitive species, as well as increased sediment loads during the rainy season, causing habitat changes and the loss of macrofaunal diversity (Fan and Shibata, 2015).

Given that most present-day ecosystems are affected by multiple stressors, understanding how they act alone and interact with each other is crucial for mitigating their negative impacts on aquatic ecosystems (Ormerod et al., 2010). In Brazil, a number of studies have investigated the individual effects of agricultural stressors on lotic ecosystems (e.g Hepp and Santos, 2009; Mello et al., 2018; Tanaka et al., 2015; Taniwaki et al., 2017). However, studies on the interactions between multiple stressors in Brazilian streams are rare, due to the difficulties in separating the effects of individual stressors in the environment and due to the challenges in conducting multiple-stressor experiments in the field. This is an important knowledge gap, as there is a global consensus that simultaneously acting stressors can alter structure and functioning of these ecosystems more profoundly than individual stressors (Ormerod et al., 2010; Piggott et al., 2015; Taniwaki et al., 2017).

To address the challenge to understand the interactions of multiple stressors (Schäfer and Piggott, 2018), the present study investigated the individual and combined effects of three key agricultural and climate change stressors (nutrient enrichment, sedimentation, and flow reduction) on the benthic macroinvertebrate community in stream mesocosms fed by a subtropical stream. We tested five hypotheses: (H1) Flow reduction will have predominantly negative effects on the invertebrates, due to the interplay of decreased oxygen availability, limitation in dispersal capacity, and reduced feeding opportunities (Elbrecht et al., 2016; Matthaei et al., 2010); (H2) Nutrient enrichment effects will be positive at moderate but negative at high levels where reduced dissolved oxygen concentrations impact the invertebrates (Townsend et al., 2008; Wagenhoff et al., 2012); (H3) Added sediment and reduced flow will interact synergistically. This is due to exacerbated competition for limited resources under reduced flow (which also increases sedimentation), the possibility of suffocation and habitat loss due to sedimentation, changes in food availability, restrictions on migration and dispersal of aquatic organisms, and physiological stress caused by the combination of these stressors (Beermann et al., 2018; Blöcher et al., 2020); (H4) The positive effects of nutrient enrichment alone may become negative when combined with sediment addition, as the two combined stressors can cause habitat structure degradation and exacerbated algal growth, triggering adverse responses in the benthic community. (H5) The stressor effects described above should also result in changes in the size structure of the invertebrate communities. Because larger organisms require more energy flowing through the food chain, the three stressors should synergistically cause a decrease in the abundance of larger organisms due to higher metabolic costs associated with dealing with suffocation and physiological stress. This should result in steeper negative relationships between abundance and body size under these conditions (Petchey and Belgrano, 2010).

Methods

Study area

The study was conducted in the Experimental Station of Forest Sciences (Itatinga- ESALQ/USP- EECFI) of the University of São Paulo, situated in the Itatinga municipality, São Paulo State (latitude 23°10′ S, longitude 48°40′ W, average altitude 850 meters a.s.l., and average terrain slope 10 %). The station covers a total area of 2119 hectares, characterized by gently undulating topography and a predominance of latosol and podzolic soils. The vegetation cover is mainly composed of *Eucalyptus* and *Pinus*, used for both experimental and commercial purposes, with some areas of preserved native vegetation (Gonçalves et al., 2012). According to the Köppen classification (Alvares et al., 2013), the climate of the region is characterized as Cwa (humid subtropical climate), with dry winters and hot summers. Average annual temperature is 20 °C, and average annual precipitation is 1350 mm. The stream feeding our mesocosm setup was a 2nd-order stream draining *Eucalyptus* plantations mixed with native forest. The stream has 30+ m of riparian vegetation buffer along both banks, with background concentrations of nitrate being around 3 mg/L and an annual mean discharge of 67 L/s (Estação Experimental de Ciências Florestais – USP, unpublished data).

Experimental design

The study was conducted using the ExStream System, an outdoor flow-through mesocosm system used in several countries, resulting in more than 30 peer-reviewed journal articles to date. This system offers precise control of experimental variables, excellent statistical power, and a high degree of field realism (Elbrecht et al., 2016; Hunn et al., 2024; Piggott et al., 2015). The system used in this study, ExStream Brazil, was installed 100 m from the stream and consisted of a structure 4.1 m high and 20 m long, divided into two levels (Fig. 1). The upper level supported four polyethylene tanks with a capacity of 135 L each, while the lower level was composed of a wooden support measuring 1 meter high by 1.2 meters wide, designed to accommodate the 64 mesocosms. These had an external diameter of 25 cm, a height of 9 cm, and a capacity of 3.5 liters. Each mesocosm was equipped with a flow control valve to regulate water flow. A centrifugal pump (Schneider Monoestágio BC-92S 1B 3 CV with maximum capacity of 11.4 m^3/h) was installed on the stream to maintain the continuous flow of water and organisms through the system (Fig. 1a). The pump inlet was protected by a 4.5-mm metal mesh to prevent the pump from clogging with branches and leaves, with an additional enclosure with an overlay screen (mesh opening 3 cm). Both meshes were cleaned daily in the morning and afternoon using a brush and a shovel.

The stream water flowing through the pump was transported to a central collector via a 38-mm diameter pipe. This central collector distributed water to the four header tanks (each controlled by a manual ball valve) along 19-mm diameter polyethylene pipes, with each tank gravity-feeding water to 16 mesocosms through 12.7-mm internal diameter hoses. The pump was powered by the three-phase electrical grid, and although there were a few power outages during the experiment, these never exceeded 2 hours in duration.

The experiment began on January 12, 2022 and lasted for 6 weeks.

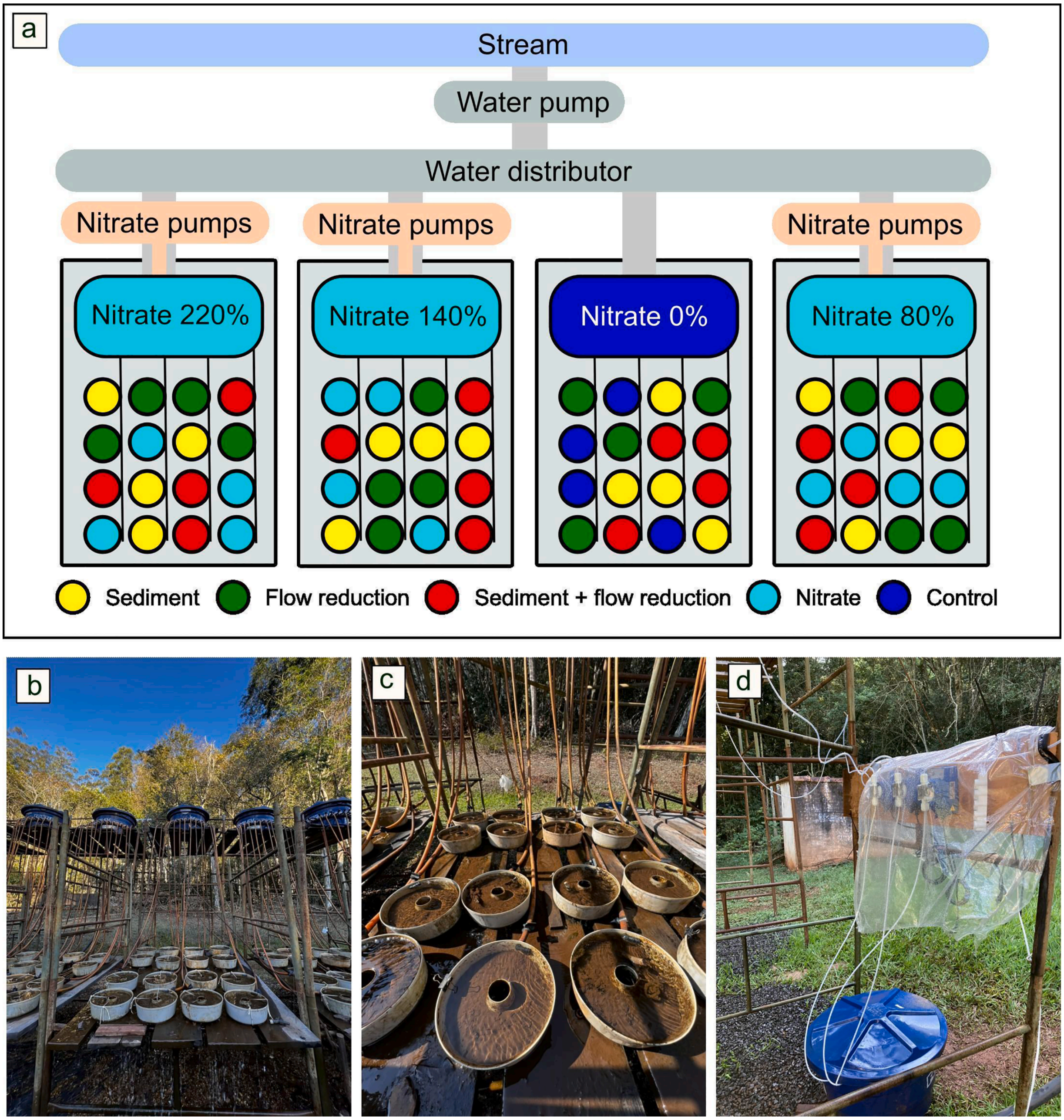

Fig. 1. Experimental setup of the ExStream Brazil system used in our study. (a) Schematic representation of the treatments of stressors used. (b) Photo of the system. (c) Photo of the mesocosms. (d) Photo of the nitrate pumps.

Minimum and maximum temperature during the study period in the station were 13.9 °C and 32.5 °C, respectively. The accumulated precipitation for January 2022 was 399.7 mm and 198.9 mm for February 2022. We applied four concentrations of nitrate (ambient stream concentration / 80 %, 140 %, and 220 % more), two levels of sediment (none added / 80 % cover of the bed surface of the mesocosm), and two flow velocity levels (no alteration / 66 % reduction) in 64 mesocosms, using a full-factorial design. Nutrient blocks were assigned randomly at the header-tank level, with all 16 mesocosms in each block receiving the same nutrient concentrations. Within each nutrient block, sediment and flow velocity treatment combinations were randomly assigned to individual mesocosms, resulting in four replicates of each treatment combination (Fig. 1a).

On the first day of the experiment, the mesocosms were filled with dry substrate (500 mL of gravel ranging from 2 to 20 mm, plus sixteen 20-mm surface stones), simulating the bed of small streams in the Itatinga region. The natural colonization period occurred over 3 weeks, from day -21 to day -1, during which the flow rate through the system remained constant at 1.5 L/min per mesocosm. On day -4, to supplement the natural colonization process with taxa rare in the drift and organisms bigger that 4mm (Elbrecht et al., 2016; Piggott et al., 2015), all mesocosms received a standard load of benthic invertebrates. These organisms were collected from the same stream through kick sampling using D nets with 500 μm mesh and sampling approximately 3,4m^2 for 5 minutes each, totaling around 40 m^2. Each mesocosm received the amount of organisms from around 1.5x the area of each mesocosm. The organisms were transported to the experimental site in polyethylene buckets containing stream water and were distributed evenly into the mesocosms using a 200-mL plastic container, mixing the bucket before each distribution.

Stressors were applied from day 0 to day 21, where treatments were added to the corresponding mesocosms. From day 2 to day 21, to conduct a simultaneous study on the effects of stressors on decomposition, 3 leaf litter bags (height 8 cm, width 7 cm) made of coarse mesh (6 mm) were added to all mesocosms, each containing 10 leaf discs (15 mm in diameter) from the species *Heliconia tarsais*, native to the Atlantic Forest in South America and commonly found and collected near streams in the experimental station.

For the nitrate enrichment, water-soluble KNO_3 (YaraTera™ Krista™ K – 13 % N, 43 % K_2O, 1 % Mg) was used. The KNO_3 was dissolved in a polyethylene container, at a ratio of 50 g of nitrate per 100 L of water (Fig. 1d). The resulting solution was introduced into the mesocosms through 3 pressure-compensating drippers (Seko model Kompact AML 200 with 0.52 ml per injection and 160 pulses per minute), connected to each corresponding upper tank, and individually adjusted to different drip rates to achieve the desired nitrate conditions. Nitrate concentrations were measured twice daily (morning and afternoon) according to the APHA 4500 NO3-B method, in a randomly selected mesocosm from each experimental block, using a UV spectrophotometer (Model AJX-6100P6 double-beam spectrophotometer) with wavelengths of 220 and 270 nm (APHA, 2005).

The nitrate enrichment treatment was based on findings by Taniwaki et al. (2017), which observed that nitrate concentrations in streams influenced by sugarcane are up to five times higher than in streams draining pasture regions and up to eight times higher when compared to forested headwaters. Therefore, the nitrate enrichment treatments were ambient concentration (no nitrate addition - median 3.8 mg/L), low increase (median 8.5 mg/L), medium increase (median 11.9 mg/L), and high increase (median 14.9 mg/L).

The added sediment consisted of fine sand (average grain size 0.2 mm), which was collected in the region and commercialized by a local business (São Caetano Materiais para Construção - Itatinga, SP, Brazil). The influence of fine sediment was evaluated at two levels: the natural stream condition (no added load) and 80 % sediment cover of each mesocosm. To achieve the latter, 400 g of fine sand was added to each mesocosm designated for sediment treatment. The choice of grain size was based on a study conducted by Blöcher et al. (Blöcher et al., 2020), which analyzed the effects of sediment grain size on the benthic community and identified greater negative impacts when particle size was $\leq$ 0.2 mm. The decision to increase sediment cover to 80 % of the mesocosms was based on a study that encompassed 86 streams draining various watersheds with different land uses in the state of São Paulo (Molina et al., 2017).

Flow treatments comprised two categories: ambient (1.5 L/min) and reduced (0.5 L/min), representing a 66 % discharge reduction. This reduction was based on a study conducted in the state of São Paulo that projected a 35-50 % decrease in stream flow under climate change scenarios (Gesualdo et al., 2019). The discharge in the system was measured twice daily (morning and afternoon) in all mesocosms using a digital flow meter (Model: SEA1245/ Brand: SEA, Sea Zhongjiang, China), and manually regulated through the flow control valve attached to each mesocosm.

On the last day of the experiment, we measured pH, electrical conductivity (μS/cm), temperature (°C), and dissolved oxygen (mg/L and %) in the stream and in four mesocosms from each block (representing one randomly chosen mesocosm of each treatment combination). Measurements were taken using a multiparameter meter from Hanna (model HI 9828, Hanna Instruments, Brazil).

Sampling of benthic macroinvertebrates

On day 21, after the system was turned off, all mesocosms and litter bags were elutriated through a 250 μm sieve. Subsequently, the samples were transferred to plastic containers and stored in 70 % ethanol. All invertebrate samples were sorted, counted, and identified in the laboratory. To identify the genera of Chironomidae (Diptera), permanent glass slides containing Hoyer's Liquid were prepared, and all midge larvae were analyzed under a binocular biological microscope with magnifications of 40x to 400x (Model: TNB-41B-PL/ Brand: Option). For the other organisms, a DI150B binocular stereoscopic microscope equipped with an auxiliary objective lens of 0.7x (Model: New light/ Brand: Digilab, Brazil) was used. All organisms were identified to genus, family or class (for Ostracoda). Identification was based on taxonomic keys (Hamada et al., 2014; Mugnai et al., 2010; Segura et al., 2011; Shepard et al., 2020; Trivinho-Strixino, 2023). We calculated the body mass of every individual from the experiment by measuring body dimensions and applying allometric size-mass equations (Collyer et al., 2023).

Four community-level indices were calculated: Richness - Total number of taxa in each sample, Abundance - Total number of individuals, Shannon- Wiener Index – Community diversity and Pielou's Equitability Index – Species dominance.

Statistical analyses

All analyses were conducted using the software R (version 3.5.3) (R Core Team, 2018). To compare the physical and chemical characteristics of water among different blocks and the stream source, we applied Kruskal-Wallis and Mann-Whitney tests. To determine stressor effects on macroinvertebrate community composition, we used a permutational multivariate analysis of variance (PERMANOVA) with the Bray-Curtis dissimilarity index as response matrix and all stressors and their interactions as explanatory variables. We complemented this analysis by exploring the effects on individual taxa using a SIMPER analysis when PERMANOVA effects were significant. Because these data were also used for the size spectra analyses, we only evaluated the communities outside of the litter bags. This was done to avoid mixing 'brown' and 'green' food-web channels, one based on algae and other based on litter biomass, which would confound interpretations of size spectra changes.

We applied generalized linear models to analyze the singular and combined (interactive) effects of nitrate, sediment, and flow on benthic macroinvertebrate richness, abundance, Shannon diversity and evenness, mixing the communities inside and outside of the litterbags. All predictor variables were classified as fixed categorical predictors, and the four specific benthic invertebrate variables as responses. We used Poisson-regression for abundance and species richness data, Quasi-Binomial regression for equitability (which is bounded between 0 and 1), and simple linear regression for Shannon diversity. Applying data transformations or using non-linear link-functions changes the interpretation of the interaction term (Spake et al., 2023), therefore we specified the identity-link for all regression models to evaluate interactions on the arithmetic scale. This corresponds to the simple addition null model for multiple-stressor effects (Schäfer and Piggott, 2018). Model validation and statistical evaluation was done with the car package (version 3.1-2) (Fox and Weisberg, 2019). Residual plots were used to check compliance with model assumptions. We used ANOVA type II sum of squares for statistical inference, applying either the F-test (quasibinomial and simple regression model) or the LRT-Test (poisson regression models). If nitrate had a significant effect on the response variable, we applied post-hoc tests with the emmeans package (version 1.10.3) (Lenth, 2024). All nitrate levels were compared pairwise, and *p*-values were corrected with Tukey's method.

We applied a biomass-size-spectrum approach named LBNBiom (Edwards et al., 2017) to the communities outside of the litter bags. This method is an a-taxonomic approach, meaning that taxonomic identities are ignored and individual body masses are grouped in size classes (bins) defined by different methods (White et al., 2007). In our case, we separated bins of equal sizes comprising a range of body masses and assigned each individual to the mean of their bin. We then plotted these bins against their summed biomasses on double-log axes. This method can identify the decrease or increase in the biomass of large organisms in proportion to smaller organisms and has already been used to test the effects of stressors on benthic communities (Collyer et al., 2023). In our study, the effects of stressors on size spectra were investigated by applying a mixed-effect model with mesocosms as random intercepts using R package Size Spectra (version 3.5.3) (Edwards et al., 2017). The summed biomasses were treated as response variables while binned size classes and treatments were used as fixed factors with interactions. A significant interaction between binned size classes with treatments would indicate that the slope of size spectra changes with the stressor treatments.

Results

Physical and chemical characteristics in the stream and in the mesocosms

Water physicochemistry in the mesocosms varied significantly across the nitrate treatments and differed marginally from the source stream (see Supplement 1 and 2 for comparison results and post-hoc tests). In general, the mesocosms were significantly acidic (stream pH = 6.02 ± 0.11, mesocosms = 6.01 ± 0.10), with higher concentrations of dissolved oxygen (stream = 6.6 ± 0.20 mg/L, mesocosms = 7.34 ± 0.27 mg/L), and slightly higher water temperatures (stream = 23.1 ± 0.1 °C, mesocosms = 23.5 ± 0.1 °C) (Supplement 1). Water conductivity increased as nitrate concentrations increased (stream = 53 ± 0 µS/cm, enriched mesocosms = 66 ± 6 µS/cm) (Supplement 1).

Taxonomic composition and stressor effects on individual taxa

A total of 7531 individuals belonging to 113 taxa were collected and identified. The five taxa with the highest abundances were Ostracoda, representing 33.3 % of all individuals, Oligochaeta (Clitellata, Haplotaxida) with 11 %, Tanytarsini (Chironomidae, Diptera) with 10.3 %, Tanypodinae (Chironomidae, Diptera) with 7 %, and *Pentaneura* spp. (Chironomidae, Diptera) with 5.3 % (Fig. 2). At the family level, there was a predominance of Chironomidae, which represented 39.2 % of all individuals. Within Chironomidae, Tanytarsini represented 26.2 %, Tanypodinae 17.8 %, *Pentaneura* 13.5 %, Chironominae 8.4 % and Orthocladiinae 6.7 %.

The PERMANOVA detected a significant influence of flow reduction ($F = 2.85$, $R^2 = 0.020$, $p = 0.03$) and the interaction between flow and sedimentation ($F = 2.98$, $R^2 = 0.021$, $p = 0.02$) on community

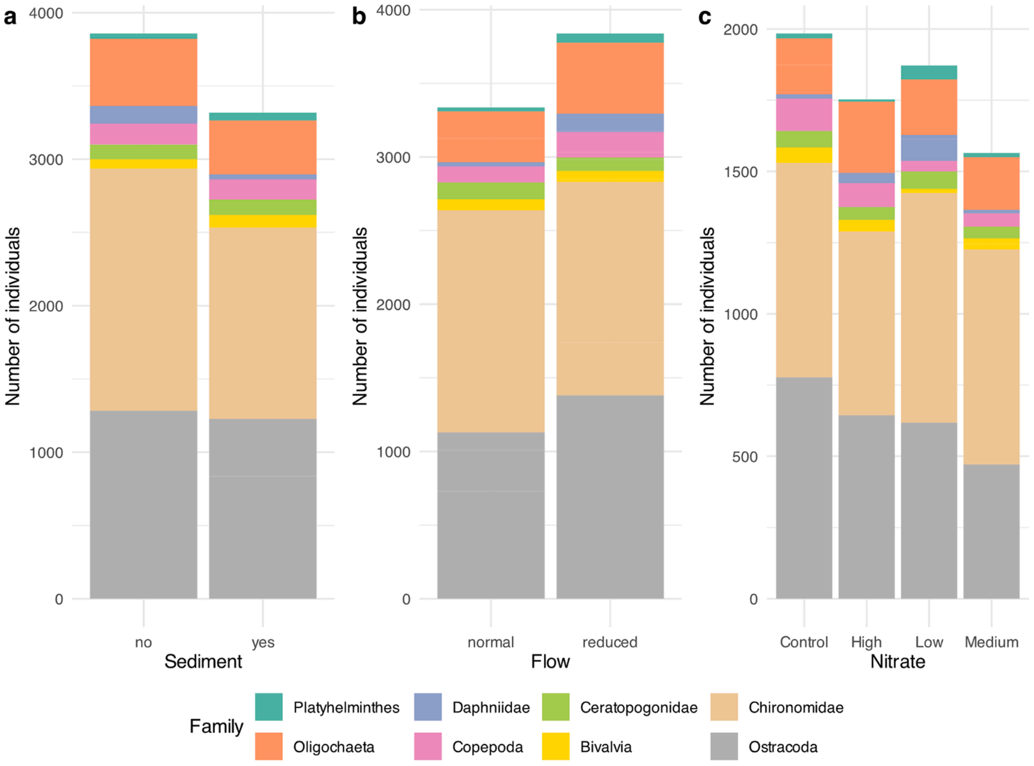

Fig. 2. Factor main effects of sediment addition, flow reduction and nitrate enrichment on the taxonomical groups of benthic macroinvertebrates.

composition, though with low explanatory power. The SIMPER analyses were applied according to the significant results of PERMANOVA, thus testing only which taxa were affected by flow reduction and the interaction between flow and sedimentation. The genera *Cryptochironomus* and *Labrundinia* were more common for normal flow, whereas the genus *Chironomus* was more abundant at reduced flow (Table 1). Despite these significant effects, the absolute differences were fairly small.

We found no differences in abundances across the two sediment levels in the SIMPER analysis for normal water flow. However, such differences were observed for 23 taxa for reduced water flow (Table 2). All these taxa were more common at reduced flow in treatments without added sediment, suggesting a synergistic significant interaction between reduced flow and fine sediment. Further, 19 rare taxa (singletons) were found only in treatments without increased sedimentation.

Stressor effects on community-level invertebrate metrics

We observed only significant stressor main effects on the four community-level response variables (Table 3). Flow reduction affected abundance and diversity, sedimentation changed species richness and abundance, and nitrate enrichment affected equitability. None of the stressor interactions were significant (Table 3).

Under reduced flow, macroinvertebrate diversity and equitability were both lower, indicating that the community shifted towards a less diverse one with more dominant species (Fig. 3). Sediment addition reduced both the number of taxa and the number of individuals (Fig. 3). Nitrate enrichment significantly increased community equitability only at medium enrichment conditions (Fig. 3, Supplement 3).

Biomass size spectra

The LME model identified the expected negative linear decrease in biomass with increasing individual (binned) body sizes. Generally, organisms of smaller sizes contributed more to the community biomass (Table 4). However, this relationship was mostly unrelated to our experimental manipulations. We only found a marginally significant effect of the interaction between size classes, nitrate concentration and sediment addition (Table 4). With increased sedimentation, there was a tendency for less biomass of large size classes in two nitrate concentrations, meaning a loss of large organisms with added sediment in these cases (Fig. 4). The estimated marginal means comparison indicated that these changes occurred with nitrate 80 % (t ratio = -1.75, P = 0.08) and 140 % (t ratio = -1.70, P = 0.09) (Fig. 4).

Discussion

This experiment evaluated the individual and combined effects of sedimentation, flow reduction and nitrate enrichment on benthic invertebrate communities in streamside mesocosms fed by a subtropical stream. Our primary aim was to investigate whether these combined stressors could lead to unexpected outcomes, considering that these stressors are widely spread through agricultural regions in Brazil. Our results revealed that flow reduction negatively impacted macroinvertebrate diversity and equitability (species dominance), while high nitrate concentrations adversely affected community equitability. The combination of sedimentation and flow reduction resulted in the loss of rare species and the combination of nitrate and sedimentation slightly reduced the abundance of larger taxa. These findings underscore the varied impacts of agricultural and climate-related stressors on macroinvertebrate communities, highlighting the need for refined assessments of taxonomical, size-based, and community structures. Below, we discuss the main findings related to the stated hypotheses of this study.

Table 1
Taxa that differed in their abundance in relation to flow velocity in the SIMPER analysis. Normal (1.5 L/m) and reduced (0.5 L/m).

Taxa	*p* value	Normal flow	Reduced flow
Cryptochironomus	0.017	44	34
Chironomus	0.044	9	13
Labrundinia	0.014	5	2

Table 2
Taxa that differed in their abundance in relation to sediment addition under reduced flow velocity (0.5 L/s) in the SIMPER analysis.

Taxa	*p* value	Without sediment	With sediment
Pentaneura	0.009	104	69
Polypedilum	0.007	12	1
Larsia	0.046	11	3
Coelotanypus	0.012	8	1
Parapentaneura	0.007	6	0
Corynoneura	0.007	2	0
Zygoptera	0.005	1	0
Endotribelos	0.009	2	0
Paracladius	0.017	2	0
Thienemanniella	0.017	2	0
Coleoptera	0.004	1	0
Tanypus	0.010	1	0
Clinotanypus	0.015	1	0
Paramerina	0.015	1	0
Tanytarsini	0.015	1	0
Demicryptochironomus	0.007	1	0
Metriocnemus	0.010	1	0
Pseudochironomus	0.017	1	0
Chironomini	0.018	1	0
Dicrotendipes	0.018	1	0
Orthocladinae	0.016	1	0
Procladius	0.001	2	0
Tanytarsus (Caladomyi)	0.001	6	0

Hypothesis i. predicted that a reduction in flow velocity would adversely affect benthic macroinvertebrates. Our results corroborate this hypothesis, with flow reduction negatively impacting community diversity and equitability, as observed in previous related studies (Blöcher et al., 2020; Elbrecht et al., 2016; Matthaei et al., 2010). While some of these studies employed more drastic flow reductions, our study applied moderate reductions based on forecasts for the São Paulo Metropolitan Region (Gesualdo et al., 2019), yet significant effects were still observed. Besides flow reduction negatively affecting diversity indexes, it increased the presence of ostracods and oligochaetes in the mesocosms, thereby negatively impacting diversity and dominance indexes. Ostracods, which generally avoid fast flows (Ruiz et al., 2013), were expected to benefit from flow reduction. Similarly, the presence of oligochaetes is often associated with higher organic matter availability, which is positively influenced by reduced flow due to accumulation processes (Cortelezzi et al., 2011). Consequently, reduced flows favour tolerant taxa and diminish biodiversity and equitability in macroinvertebrate communities. Additionally, it is important to note that chironomids, which rely on drift for colonization, may experience reduced drift and colonization due to flow reduction. Therefore, with projections of prolonged droughts in the São Paulo region due to climate change (Gesualdo et al., 2019), and stream water abstraction due to agricultural irrigation, our results indicate that stream macroinvertebrate communities are likely to lose diversity.

Our second hypothesis predicted that low concentrations of nitrate enrichment positively affect the invertebrate community, whereas high concentrations have negative effects. Our results support this hypothesis, showing increased community equitability at low and intermediate nitrate levels, but a reduction at high nitrate levels. These findings align with other experiments conducted in New Zealand, which demonstrated positive effects at low to moderate nutrient concentrations, turning negative at high concentrations (Matthaei et al., 2010; Townsend et al., 2008; Wagenhoff et al., 2012). Nutrient effects on benthic macroinvertebrates typically occur indirectly by influencing food supply in the short term and causing long-term impacts such as eutrophication, which can lead to oxygen depletion (“Multiple-stressor effects of sediment,

Table 3
Results of the generalized linear models evaluating the individual and combined effects of sedimentation, nitrate enrichment and flow reduction on the community-based variables evaluated in this study. n.s = non-significant.

Dependent	Model	Independent	SumSq	F_value	Chi square	Df	*p*_value
Species richness	Poisson	**Sediment**			7,86	1	**0.01**
		Flow			0,63	1	n.s
		Nitrate			1,63	3	n.s
		Sediment:Flow			3,61	1	n.s
		Sediment:Nitrate			6,76	3	n.s
		Flow:Nitrate			2,30	3	n.s
		Sediment:Flow:Nitrate			4,73	3	n.s
Shannon Diversity	Gaussian	Sediment	0,24	3,59		1	n.s
		Flow	0,47	7,04		1	**0.01**
		Nitrato	0,23	1,16		3	n.s
		Sediment:Flow	0,00	0,02		1	n.s
		Sediment:Nitrate	0,34	1,68		3	n.s
		Flow:Nitrate	0,24	1,17		3	n.s
		Sediment:Flow:Nitrate	0,09	0,43		3	n.s
Equitability (J)	Quasibinomial	Sediment	0,00	0,08		1	n.s
		Flow	0,24	6,14		1	**0.02**
		Nitrate	0,34	2,91		3	**0.04**
		Sediment:Flow	0,08	2,03		1	n.s
		Sediment:Nitrate	0,03	0,30		3	n.s
		Flow:Nitrate	0,08	0,67		3	n.s
		Sediment:Flow:Nitrate	0,07	0,58		3	n.s
Total abundance	Quasipoisson	**Sediment**	60,47	4,62		1	**0.04**
		Flow	38,15	2,91		1	n.s
		Nitrate	62,75	1,60		3	n.s
		Sediment:Flow	41,80	3,19		1	n.s
		Sediment:Nitrate	16,87	0,43		3	n.s
		Flow:Nitrate	38,20	0,97		3	n.s
		Sediment:Flow:Nitrate	26,08	0,66		3	n.s

phosphorus and nitrogen on stream macroinvertebrate communities,” 2018). Additionally, we observed a systematic reduction in ostracods with increased nutrient levels, which favoured equitability in the invertebrate community. Some species of Ostracods can respond highly sensitively to nutrient increases (Ruiz et al., 2013), which would explain our results. In scenarios of agricultural intensification, higher concentrations of nitrate in streams are expected, especially because of the use of fertilizers (R.H. Taniwaki et al., 2017). This increase in nitrate levels is likely to lead to reductions in the equitability and increase the dominance of resistant taxa in the agricultural streams.

Our third and fourth hypotheses predicted synergistic effects of sedimentation and reduced flow (H3), and sedimentation and nitrate enrichment (H4). Our results partially support these hypotheses, as interactive effects were observed among the studied stressors in the taxonomic composition of benthic invertebrates, but not in the four studied community-level metrics. The combination of sedimentation and flow reduction caused the loss of rare taxa and reduced the abundances of certain common taxa. However, the same stressor interaction did not cause any significant effects on the community-level metrics. These results align with previous ExStream studies conducted in other countries (Germany and New Zealand), which found that the combined effects of sedimentation and reduced flow impacted specific taxa, especially members of the pollution-sensitive EPT groups (mayflies, stoneflies and caddisflies) (Beermann et al., 2018; Blöcher et al., 2020). By contrast, the main effects of these stressors influenced the overall community structure (Beermann et al., 2018; Blöcher et al., 2020).

In our experiment, sedimentation and flow reduction impacted invertebrate community structure in different ways. Sedimentation primarily affected abundance and richness, reducing the number of individuals and taxa, particularly chironomid communities that are closely tied to sediment characteristics (Suren and Jowett, 2001). Flow reduction reduced diversity and equitability, favouring already abundant groups such as ostracods that thrive in low-flow conditions (Ruiz et al., 2013). Nitrate addition impacted community equitability positively up to moderate enrichment levels. Therefore, the findings of our study indicate that agricultural and climate-related stressors affect macroinvertebrate communities in distinct ways. Flow reduction due to climate change leads to the creation of uniform habitats, favouring a limited number of taxa associated with slow-flow conditions, while increased sedimentation generally has detrimental effects on the community as a whole. The combined impact of low flow and sedimentation exacerbates these negative effects. However, local actions, such as reducing sediment inputs to streams, can mitigate some of the harmful consequences of reduced flow caused by climate change. This highlights the need for refined assessments of both taxonomical and community structures.

Our fifth hypothesis predicted that the effects of the studied stressors would result in changes in the size structure of the invertebrate communities. As expected by metabolic scaling theory, the biomass of larger invertebrate size classes was systematically smaller than that of small organisms in all conditions (Brown et al., 2004). This means that energetic constraints on the abundance and biomass of large taxa are likely operating in our system (White et al., 2007). However, our hypothesis was only partially supported, considering that only a marginally significant effect of the interaction between size classes, nitrate and sedimentation were observed. With increased sedimentation, there was a tendency for less biomass of large size classes in two nitrate concentrations, indicating a loss of large organisms with sedimentation. In accordance with our previous explanation, the changes in size spectra are likely to reflect impacts of suffocation and loss of habitats leading to a loss of diversity. In our study, this was especially evident for larger taxa, which in many cases tend to be predators with smaller populations (e.g. damselflies were significantly affected by sedimentation in the SIMPER analysis). The consequences of having fewer large-sized invertebrates in a community are not only a loss of biodiversity, but also potential changes in the functioning of the ecosystem, as changes in specific trophic groups may impact patterns and rates of energy transfer.

Implications for conservation

Our study demonstrates that the evaluated agricultural stressors and flow reduction (as a predicted consequence of climate change) have the

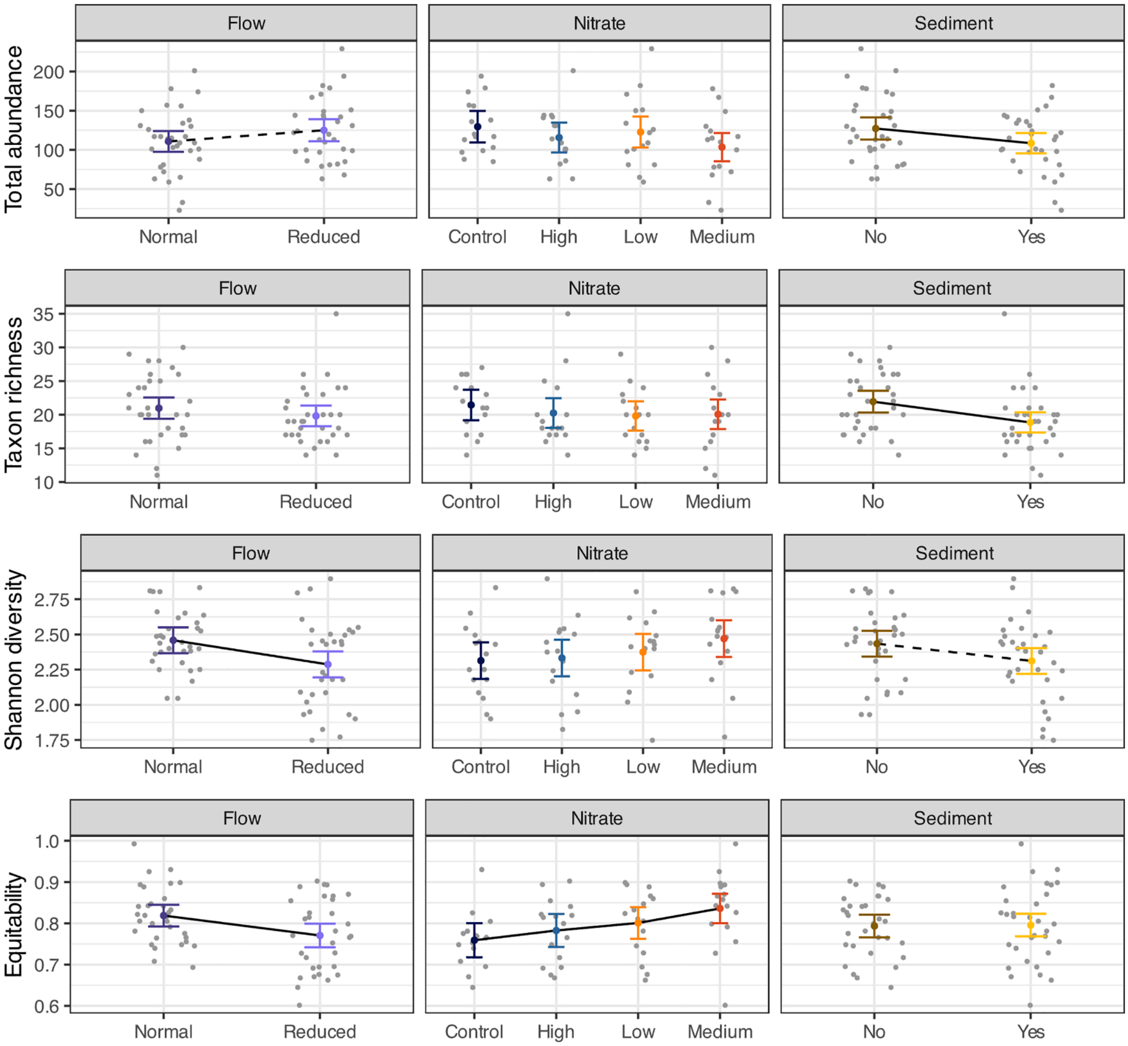

Fig. 3. Factor main effects of flow reduction, nitrate enrichment and sediment addition on community-level invertebrate metrics. Solid lines indicate significant effects ($p < 0.05$). Dashed lines indicate marginally significant effects ($0.05 \leq p < 0.10$).

Table 4

Linear mixed-effect (LME) associating normalized biomass from different size classes and the experimental treatments. Individual mesocosms were treated as random intercepts. Significance of the model parameters was assessed by ANOVA type II Wald Chi-Square tests. A significant association between size classes and treatments indicates a change in the biomass size spectrum slope.

Dependent	Chi square statistic	Degrees of freedom	*p* value
Size classes	16.37	1	<0.01
Nitrate	4.45	3	0.22
Sediment	2.37	1	0.12
Flow	1.73	1	0.19
Nitrate:Sediment	5.25	3	0.15
Nitrate:Flow	0.52	3	0.92
Sediment:Flow	0.15	1	0.70
Size classes:Nitrate	5.75	3	0.12
Size classes:Sediment	1.56	1	0.21
Size classes:Flow	0.51	1	0.48
Nitrate:Sediment:Flow	6.23	3	0.10
Size classes:Nitrate: Sediment	**7.51**	**3**	**0.06**
Size classes:Nitrate:Flow	1.8	3	0.61
Size classes:Sediment:Flow	0.31	1	0.58
Size classes:Nitrate:Sediment: Flow	0.26	3	0.97

potential to change the structure of macroinvertebrate communities. Macroinvertebrates play a crucial role in many streams, due to their importance for organic matter processing as the primary source of energy and their subsequent contribution to food webs (Rosenberg and Resh, 1993). Sedimentation, which is exacerbated during rainy periods in agricultural streams, affected community abundance and richness, reducing the numbers of individuals and taxa. Flow reduction, which is intensified during dry periods and by climate change, influenced community diversity and equitability, favouring species that are characteristic for slow-flow conditions. Nitrate concentrations, which are typically elevated in agricultural streams, especially during fertilization periods (usually during dry seasons to reduce N loss), negatively impacted community equitability at high levels. Considering all these effects in combination, we can expect lower macroinvertebrate abundance and richness during wet seasons and lower diversity during dry seasons in subtropical streams in Brazil. To avoid the intensification of these stressors, we speculate that the implementation of riparian vegetation buffers to minimize sedimentation during the wet season could reduce the negative impacts on the macroinvertebrate community, as seen in other studies (Espinoza-Toledo et al., 2021; Lorion and Kennedy, 2009). Moreover, thoughtful design of roads (by following contour lines and reducing the length of the roads) (Rodrigues et al., 2019) and the identification of hydrologically sensitive areas (Siefert and Santos, 2015) may also reduce sedimentation in streams. In addition, the

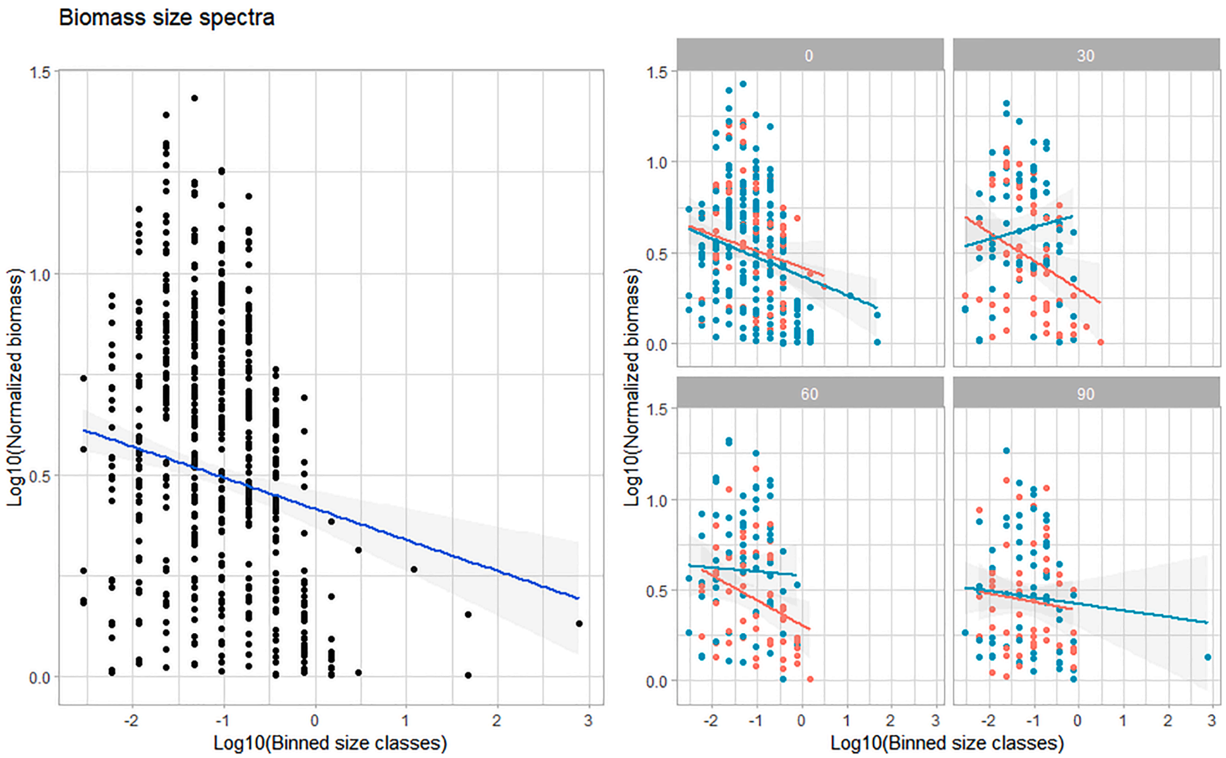

Fig. 4. Biomass size spectra from the experimental mesocosms using the LBNBiom method (see Methods), which estimates the linear association between organisms from different binned size classes and their normalized summed biomasses in double-log axes. Left: The negative association depicting less biomass from larger size classes. Right: Marginal differences (see Table 4 for details) between mesocosms with added sediment (red) and without added sediment (green) in the four different levels of nitrate concentration.

controlled use of fertilizers and the use of low-impact fertilization, such as biological nitrogen fixation, slow-release fertilizers and precision agriculture would likely reduce the negative impacts in the macroinvertebrate community during dry seasons (Austin et al., 2013; Filoso et al., 2015; Martinelli et al., 2010). Without such mitigation measures, there is the possibility of losing rare species and reduced abundances of stress-sensitive common species, given that the combination of sedimentation and flow reduction affected these community characteristics in our experiment.

Conclusions

Our study evaluated the individual and combined effects of three stressors—nutrient enrichment, flow reduction, and sedimentation—on the benthic macroinvertebrate community in mesocosms fed by a subtropical stream. Our results indicated that invertebrate taxonomic composition was influenced by the combination of sedimentation and reduced flow, affecting rare taxa in particular. Community structure was influenced by the singular effects of each stressor, with each impacting different structural characteristics. Sedimentation mainly reduced abundance and richness, particularly organisms that depend on sediment layers as their primary habitat. Flow reduction influenced diversity and equitability, favouring species that thrive in slow-flow conditions. Nitrate addition positively impacted community equitability up to moderate enrichment levels. Our findings suggest that agricultural intensification and climate change will affect macroinvertebrate communities in subtropical streams through distinct mechanisms, with single stressors affecting community structure and combined stressors disproportionally reducing rare taxa as well as changing community size structure and metabolic characteristics.

AI statement

During the preparation of this work the author(s) used ChatGPT in order to translate and improve the readability of the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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CRediT authorship contribution statement

Gabrielle S.S. Almeida: Writing – original draft, Methodology, Investigation, Formal analysis. **Victor S. Saito:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Milena Sartori:** Methodology, Investigation, Formal analysis. **Hugo H.L. Saulino:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Lyandra O. da Penha:** . **Paulo S.C.T. Miranda:** Methodology, Investigation, Formal analysis. **Mariana Morilla:** Methodology, Investigation, Formal analysis. **Maria Gabrielle Rodrigues-Maciel:** Methodology, Investigation. **Giovanna Collyer:** Methodology, Formal analysis. **Marcelo S. Moretti:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Fabiana Schneck:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Jeremy J. Piggott:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Iris Madge Pimentel:** Writing – review & editing, Software, Methodology, Investigation. **Christoph D. Matthaei:**

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ricardo Taniwaki reports financial support was provided by State of Sao Paulo Research Foundation. Gabrielle S. S. Almeida reports financial support was provided by Coordination of Higher Education Personnel Improvement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2025.100630.

Data availability

Data will be made available on request.

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