Universidade Federal do Rio Grande - FURG
Instituto de Ciências Biológicas
Pós-graduação em Biologia de Ambientes Aquáticos Continentais

Efeitos de múltiplos estressores sobre diatomáceas perifíticas em ambientes lóticos

Maria Gabrielle Rodrigues Maciel

Orientador: Profa. Dra. Fabiana Schneck
Coorientador: Prof. Dr. Ricardo H. Taniwaki

Rio Grande
2025

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ATA DE DEFESA DE TESE DE DOUTORADO EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS – Nº 005/2025

Às 08h30 (oito horas e 30 minutos) do dia 10 (dez) do mês de dezembro de 2025 (dois mil e vinte e cinco), no endereço eletrônico: https://meet.google.com/egs-dtqb-jjg, reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Tese de Doutorado da acadêmica Maria Gabrielle Rodrigues Maciel. A Tese intitulada **"Efeitos de múltiplos estressores sobre diatomáceas perifíticas em ambientes lóticos"** foi avaliada pela Banca Examinadora composta pela Profª. Dra. Fabiana Schneck (Orientadora, FURG), Prof. Dr. Ricardo Taniwaki (Co-orientador, UFABC), Prof. Dr. Leandro Bugoni (FURG), Profª. Dra. Gisele Carolina Marquardt (UFPR) e Profª. Dra. Bárbara Dunck Oliveira (UFRA). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **DOUTOR EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 12h (doze horas) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pela Coordenadora do Curso.

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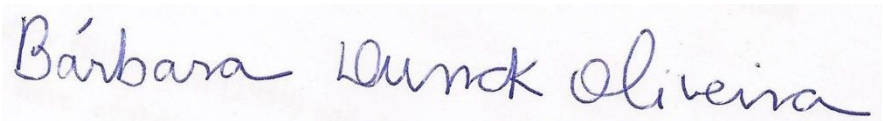

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DEDICATÓRIA

Dedico aos meus pais e às minhas irmãs, minha fonte de inspiração, de força e determinação.

EPÍGRAFE

Carrego mundos inteiros no microscópio.
Pequenas vidas que, silenciosamente, ensinam
que existir é adaptar-se.
(Maciel, M.G.R. 2025)

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RESUMO

Os ecossistemas aquáticos continentais abrigam elevada biodiversidade, mas estão entre os mais ameaçados globalmente, sobretudo em regiões tropicais. A intensificação conjunta do uso da terra e das mudanças climáticas exige uma compreensão de como múltiplos estressores ambientais influenciam a composição e a diversidade das comunidades aquáticas. Esta tese teve como principal objetivo investigar os efeitos de múltiplos estressores ambientais em comunidades de diatomáceas perifíticas de riachos subtropicais, integrando uma abordagem experimental, conduzida em mesocosmos na Mata Atlântica, e uma abordagem observacional, realizada em riachos do bioma Pampa. No Capítulo 1, um experimento em mesocosmos, avaliou os efeitos do enriquecimento com nitrato, deposição de sedimentos finos e redução do fluxo sobre a biomassa de algas bentônicas (clorofila-a) e atributos da comunidade de diatomáceas perifíticas. Os resultados indicaram que o enriquecimento por nitrato foi o principal fator responsável pelas mudanças na biomassa algal e diversidade de diatomáceas (redução da riqueza e equitabilidade; aumento da dominância). A composição de espécies respondeu tanto aos efeitos individuais quanto às interações, e a redução do fluxo diminuiu a heterogeneidade entre comunidades (diversidade beta). O Capítulo 2 avaliou a importância relativa de variáveis ambientais locais, de uso da terra e espaciais na variação da riqueza de espécies e na estrutura da comunidade de diatomáceas perifíticas, utilizando o conjunto total de espécies, espécies comuns, raras e muito raras. Os resultados indicaram que os mecanismos que estruturam a metacomunidade de diatomáceas dependem tanto do atributo avaliado (riqueza versus composição) quanto do grau de raridade das espécies. Gradientes ambientais governam a riqueza em todos os grupos, mas processos espaciais dominam as mudanças na substituição composicional, especialmente para táxons comuns e muito raros. O Capítulo 3 focou na taxonomia, descrevendo uma espécie do gênero *Encyonema*, evidenciando o potencial taxonômico ainda pouco explorado no bioma Pampa e contribuindo para o avanço do conhecimento florístico. As informações desta Tese demonstram que múltiplos estressores influenciam as comunidades de diatomáceas de forma interativa, que a estrutura das comunidades varia conforme o grau de raridade das espécies e que o bioma Pampa abriga diversidade subdocumentada. Ao integrar abordagens experimentais e observacionais, reforça-se a importância de controlar o enriquecimento nutricional, preservar a vegetação ripária e considerar diferentes escalas ecológicas na conservação dos ecossistemas aquáticos.

Palavras-chave: Algas bentônicas; ambiente de água doce; Bioma Pampa; mesocosmos; riachos tropicais; taxonomia; uso do solo.

ABSTRACT

Continental aquatic ecosystems harbor high biodiversity, but are among the most threatened globally, especially in tropical regions. The combined intensification of land use and climate change requires an understanding of how multiple environmental stressors influence the composition and diversity of aquatic communities. The main objective of this thesis was to investigate the effects of multiple environmental stressors on periphytic diatom communities in subtropical streams, integrating an experimental approach, conducted in mesocosms in the Atlantic Forest, and an observational approach, carried out in streams in the Pampa biome. In Chapter 1, a mesocosm experiment evaluated the effects of nitrate enrichment, fine sediment deposition and flow reduction on benthic algal biomass (chlorophyll-a) and periphytic diatom community attributes. The results indicated that nitrate enrichment was the main factor responsible for changes in algal biomass and diatom diversity (reduced richness and evenness; increased dominance). Species composition responded to both individual effects and interactions, and the reduction in flow decreased heterogeneity between communities (beta diversity). Chapter 2 assessed the relative importance of local environmental, land use and spatial variables in the variation of species richness and the structure of the periphytic diatom community, using the total set of species, common, rare and very rare species. The results indicated that the mechanisms that structure the diatom metacommunity depend on both the attribute evaluated (richness versus composition) and the degree of rarity of the species. Environmental gradients govern richness in all groups, but spatial processes dominate changes in compositional substitution, especially for common and very rare taxa. Chapter 3 focused on taxonomy, describing a species of the genus *Encyonema*, highlighting the taxonomic potential that has yet to be explored in the Pampa biome and contributing to the advancement of floristic knowledge. The information in this thesis demonstrates that multiple stressors influence diatom communities interactively, that community structure varies according to the degree of rarity of species and that the Pampa biome is home to underdocumented diversity. By integrating experimental and observational approaches, the importance of controlling nutrient enrichment, preserving riparian vegetation and considering different ecological scales in the conservation of aquatic ecosystems is reinforced.

Key-words: Benthic algae; freshwater environment; land use; mesocosms; Pampa Biome; tropical streams; taxonomy.

APRESENTAÇÃO

Esta Tese segue o modelo de formatação sugerido pelo Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais (PPGBAC) da Universidade Federal do Rio Grande - FURG. Na **Introdução Geral** abordo os principais temas conceituais trabalhados nessa Tese, bem como o **objetivo geral** e **objetivos específicos** que serão investigados nos capítulos. Os principais tópicos da introdução geral são: (I) Ecossistemas aquáticos continentais: funções ecológicas, serviços ecossistêmicos e biodiversidade; (II) Uso da terra e impactos sobre comunidades aquáticas; (III) Múltiplos estressores em ecossistemas aquáticos: conceitos, interações e desafios na previsão de respostas ecológicas; (IV) Espécies comuns e raras na estruturação de comunidades bentônicas: implicações ecológicas e taxonômicas; (V) Diatomáceas perifíticas como bioindicadores em ecossistemas aquáticos: respostas a múltiplos estressores e relevância taxonômica; (VI) Objetivos. Esta seção está formatada seguindo as normas da Associação Brasileira de Normas Técnicas (ABNT). **O primeiro capítulo** da tese intitulado "*Multiple stressors in tropical streams: nitrate, sediment and flow interactions mediate benthic biomass and diatom community responses in experimental streams*" segue as normas de formatação e está publicado no periódico *Inland Waters*. Neste capítulo, empregamos a estrutura ExStream (mesocosmos de riachos ao ar livre) para avaliar os efeitos de estressores típicos de áreas agrícolas, enriquecimento por nitrato, adição de sedimentos finos e redução de fluxo, sobre a biomassa de algas bentônicas (utilizando clorofila-a como *proxy*) e a estrutura da comunidade de diatomáceas. **O segundo capítulo**, "*The relative importance of local environmental, land use and spatial factors in explaining species richness and community composition varies for common and rare stream diatom species"* segue as normas de formatação e será submetido ao periódico *Freshwater Biology*. Neste capítulo, avaliamos como variáveis ambientais locais, de uso da terra e espaciais explicam a variação na riqueza de espécies e na estrutura de comunidades, com base em três conjuntos de dados: (i) todas as espécies que compõem a metacomunidade de diatomáceas, (ii) as espécies raras e (iii) as espécies comuns. **No terceiro capítulo**, intitulado "*A Encyonema species from from the Brazilian Pampa biome"* segue as normas do periódico *Diatom Research* ao qual foi submetido. Nele, descrevemos uma espécie de diatomácea do gênero *Encyonema* para a região do Bioma Pampa discutindo suas diferenças com espécies semelhantes e sua ecologia. Finalizamos com a seção de **Considerações Finais e Perspectivas obtidas** com o desenvolvimento da Tese; nesta última seção, as citações e referências bibliográficas seguem as normas da ABNT.

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INTRODUÇÃO GERAL

I. Ecossistemas aquáticos continentais: funções ecológicas, serviços ecossistêmicos e biodiversidade

Os ecossistemas aquáticos continentais, como rios, lagos, igarapés, planícies alagáveis e zonas úmidas, embora ocupem uma pequena fração da superfície terrestre (LEHNER; DÖLL, 2004; DUDGEON *et al.*, 2020), concentram uma proporção elevada da biodiversidade global (REID *et al.*, 2019; DUDGEON, 2020). Por exercerem funções ecológicas essenciais, esses ambientes sustentam a integridade dos sistemas naturais e fornecem benefícios diretos e indiretos à sociedade. Atuam como componentes estruturantes da paisagem, desempenhando um papel central na manutenção da conectividade ecológica, na regulação do ciclo hidrológico e na conservação da biodiversidade (DUDGEON *et al.*, 2006; VÖRÖSMARTY *et al.*, 2010; DUDGEON *et al.*, 2019; ALBERT *et al.*, 2021). Essas funções suportam uma ampla gama de serviços ecossistêmicos, que incluem: (i) provisão de água doce e recursos pesqueiros; (ii) regulação do clima, controle de cheias e purificação da água; (iii) serviços culturais, como atividades recreativas e valores espirituais; e (iv) suporte à ciclagem de nutrientes e à biodiversidade (MEA, 2005; VÖRÖSMARTY *et al.*, 2010).

Apesar de sua importância, esses ecossistemas permanecem entre os mais vulneráveis às pressões antrópicas em escala global (FEIO *et al.*, 2022) e continuam sub-representados em avaliações quantitativas de biodiversidade, especialmente nas regiões tropicais e subtropicais (por exemplo, SALA *et al.*, 2000; PEREIRA *et al.*, 2010; HE *et al.*, 2023). Esse quadro torna-se ainda mais preocupante ao se considerar a situação crítica dos ecossistemas aquáticos continentais, que apresentaram os maiores declínios globais entre todos os ambientes monitorados. Nas últimas cinco décadas (1970-2020), o tamanho médio das populações de vertebrados monitoradas globalmente diminuiu 73%, conforme o Living Planet Index (LPI), que se baseia em quase 35.000 séries temporais de 5.495 espécies de anfíbios, aves, peixes, mamíferos e répteis (WWF, 2024). Nos ecossistemas de água doce, essa redução foi ainda mais acentuada, atingindo 85%, contrastando com os ambientes terrestres (69%) e marinhos (56%) (WWF, 2024). O cenário é particularmente alarmante, considerando que esses sistemas abrigam uma biodiversidade altamente especializada e estão entre os menos monitorados. Além disso, essas estimativas não contemplam grupos menos estudados, como micro-organismos, por exemplo, algas, fungos e outros, cujo conhecimento da diversidade permanece negligenciado (REID *et al.*, 2019; WWF, 2024). A perda de diversidade

compromete o funcionamento dos ecossistemas, reduz sua resiliência (ALI *et al.*, 2023) e os benefícios ecossistêmicos associados (CARDINALE *et al.*, 2012). Ao mesmo tempo, as lacunas de conhecimento dificultam a formulação de estratégias eficazes de monitoramento e conservação em diferentes escalas (PEREIRA *et al.*, 2010; AZEVEDO-SANTOS *et al.*, 2018; HOPPENREIJS *et al.*, 2024), tornando urgente o fortalecimento dos esforços de pesquisa e proteção desses ecossistemas vitais (REID *et al.*, 2019; ALBERT *et al.*, 2021; BAROUILLET *et al.*, 2023; HOPPENREIJS *et al.*, 2024).

A redução e perda de diversidade em ecossistemas aquáticos reflete o acúmulo de múltiplas pressões antrópicas que afetam esses sistemas em diferentes escalas. As principais pressões sobre esses ecossistemas incluem a degradação e perda de habitat, relacionadas às alterações de uso da terra, à sobre-exploração dos recursos, à poluição, às alterações do regime hidrológico e à introdução de espécies exóticas (DUDGEON, 2019; REID *et al.*, 2019; WWF 2024; CAFARO *et al.*, 2022). Esses fatores são agravados pelas mudanças climáticas e pelas crescentes pressões socioeconômicas (DUDGEON, 2019; RIPPLE *et al.*, 2021) (Figura 1). Entre essas pressões, destaca-se o uso intensivo dos recursos hídricos, frequentemente extraídos, desviados ou contaminados, o que compromete sua disponibilidade e a integridade ecológica dos habitats (DUDGEON, 2000). Por estarem situados nas zonas de drenagem, rios e lagos acumulam sedimentos e poluentes, mas, diferentemente dos oceanos, possuem baixa capacidade de diluição, o que os torna especialmente sensíveis a desequilíbrios ecológicos (HYNES, 1975; NAIMAN; LATTERELL, 2005). Esse cenário reforça o conflito entre uso humano e conservação, especialmente em regiões densamente povoadas, onde a manutenção dos fluxos ecológicos é frequentemente negligenciada (POSTEL; RICHTER, 2003; THARME, 2003; DUDGEON; STRAYER, 2024).

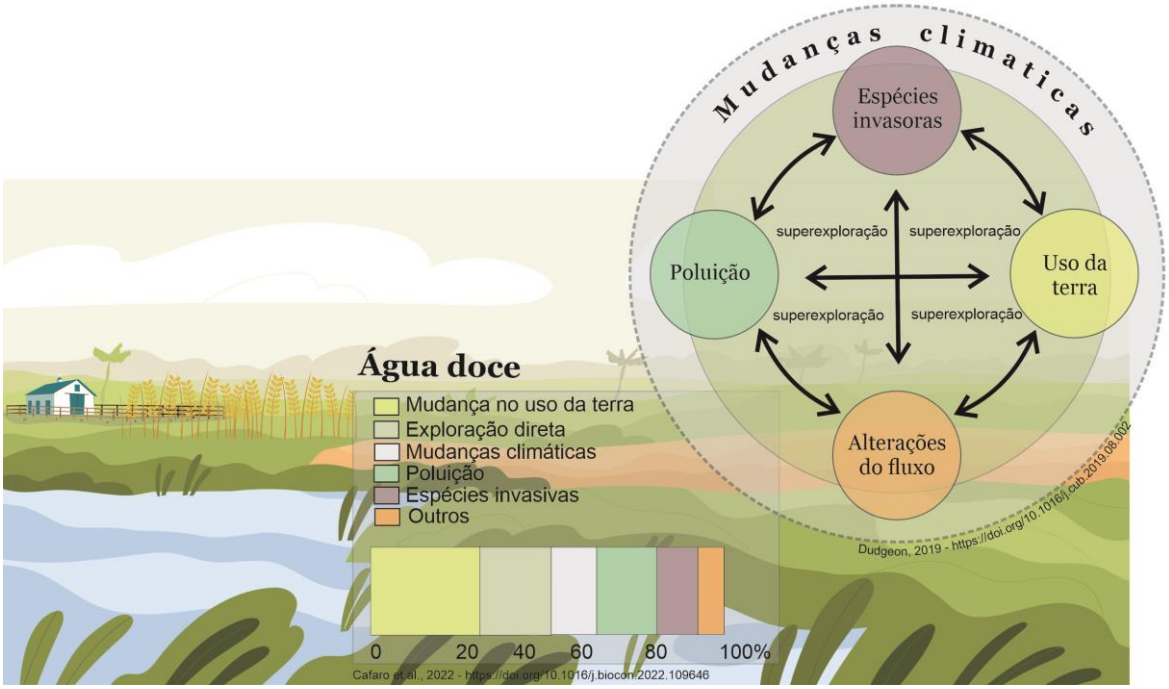

Figura 1 Fatores de impacto na biodiversidade de água doce e suas interações. Gráfico de barras (esquerda): porcentagem dos seis fatores diretos que impactam a biodiversidade de água doce; diagrama (direita): interações entre os quatro principais fatores que afetam ecossistemas aquáticos (poluição, uso da terra, alterações do fluxo e espécies invasoras). O fator superexploração intensifica todas as interações e o quadro completo é agravado pelas mudanças climáticas (Adaptado de DUDGEON, 2019; CAFARO *et al.*, 2022).

Considerando que a biodiversidade sustenta serviços críticos para a sociedade, torna-se fundamental compreender os fatores que ameaçam sua integridade, bem como os mecanismos ecológicos que mantêm seu funcionamento. Essa compreensão é necessária para que o valor ecológico e funcional desses sistemas seja efetivamente reconhecido e incorporado às políticas públicas, assegurando o uso sustentável da água e a conservação de longo prazo dos ecossistemas aquáticos continentais (DUDGEON, 2006; DUDGEON; STRAYER, 2024). Diante de um futuro climático incerto e de ameaças cada vez mais complexas, conservar a biodiversidade de água doce é um desafio fundamental. Nesse contexto, a conservação dos ecossistemas lóticos tropicais e subtropicais deve ser prioridade nas agendas de políticas públicas e estratégias de manejo ambiental, as quais devem ser fundamentadas em evidências científicas robustas que considerem a estrutura, a diversidade e o funcionamento das comunidades aquáticas diante de múltiplos estressores ambientais (IRVINE *et al.*, 2016; CARDOSO *et al.*, 2022). Entre esses estressores, destaca-se de forma particularmente crítica a intensificação das mudanças no uso e cobertura da terra, cujos efeitos sobre os habitats aquáticos e suas comunidades biológicas têm sido amplamente documentados (SALA *et al.*, 2000; AGOSTINHO *et al.*, 2005; CARDOSO *et al.*, 2020).

II. Alterações no uso da terra e seus impactos aos ecossistemas aquáticos

Em escala global, a conversão de áreas naturais em paisagens agrícolas, urbanas ou industriais tem promovido a fragmentação de habitats, a perda de conectividade hidrológica e o aumento das cargas de sedimentos e nutrientes nos corpos d'água (FOLEY *et al.*, 2005; FULLER *et al.*, 2015; IPBES, 2019). No Brasil, esses processos são particularmente evidentes na expansão da fronteira agrícola e no avanço desordenado da urbanização, frequentemente associados à supressão da vegetação nativa e ao uso intensivo de poluentes, como agrotóxicos e fertilizantes (DIAS *et al.*, 2021; PACHECO *et al.*, 2022).

A conversão da cobertura vegetal nativa para usos antrópicos tem gerado impactos significativos no equilíbrio hidrológico das bacias hidrográficas. A redução da infiltração da água no solo e o aumento do escoamento superficial intensificam o transporte de poluentes difusos para os sistemas lóticos, comprometendo a qualidade da água, os processos hidrológicos e os ciclos biogeoquímicos, especialmente em áreas submetidas a mudanças intensas no uso da terra (ALLAN *et al.*, 2004; MELLO *et al.*, 2018). Essas modificações também afetam a disponibilidade hídrica e a dinâmica dos fluxos, ampliando os efeitos negativos sobre o funcionamento e a integridade dos ecossistemas aquáticos (LIMA *et al.*, 2022).

A simplificação da paisagem e a supressão da vegetação comprometem a estrutura e o funcionamento das comunidades aquáticas, promovendo alterações na composição e na diversidade biológica que favorecem a homogeneização biótica e resultam na perda de espécies mais sensíveis (ALLAN *et al.*, 2004; ZORZAL-ALMEIDA *et al.*, 2017). A redução da cobertura vegetal, especialmente em áreas ripárias, aumenta a incidência de luz e o aporte de matéria orgânica nos ambientes aquáticos, afetando a composição taxonômica (ZORZAL-ALMEIDA *et al.*, 2017) e funcional das comunidades (MARQUES *et al.*, 2021), bem como sua estrutura trófica e os processos de produção primária e secundária, em função de alterações na dinâmica de nutrientes e do carbono (WRONA *et al.*, 2006).

Entre os grupos mais impactados por essas mudanças destacam-se as comunidades bentônicas, formadas por organismos que vivem associados ao fundo dos ecossistemas aquáticos, sobre superfícies sólidas ou em outros tipos de substratos (BRINKHURST; BOLTT 1974; STITES 1999). O contato direto com o sedimento torna esses organismos particularmente sensíveis a alterações na estrutura do substrato, ao aumento da carga orgânica e à redução da disponibilidade de oxigênio (BIGGS, 1998; BIGGS; KILROY, 2000; BOËCHAT *et al.*, 2021). Como consequência, essas pressões ambientais podem levar à perda

de táxons sensíveis e à simplificação da estrutura das comunidades, resultando em reduções expressivas na riqueza de espécies em comparação com ambientes florestados, conforme documentado em estudos com macroinvertebrados (EGLER *et al.*, 2012; FERREIRA *et al.*, 2014; GUIMARÃES-SOUTO *et al.*, 2019; BOËCHAT *et al.*, 2021). Padrões semelhantes têm sido observados também em comunidades de microalgas perifíticas, cuja riqueza (BIXBY et al. 2009), composição e biomassa respondem rapidamente às mudanças ambientais associadas ao uso da terra (BERE; TUNDISI, 2011). O perifíton representa uma forma específica de organização das algas bentônicas, caracterizada por comunidades aderidas a superfícies submersas e estruturadas em biofilmes complexos, compostos por algas, bactérias e matriz extracelular (WETZEL, 1983; STEVENSON *et al*., 1996). De forma mais ampla, as algas bentônicas correspondem ao conjunto de algas associadas ao fundo dos ambientes aquáticos, independentemente do tipo de substrato ou do modo de fixação, descrevendo sua posição ecológica no sistema (WETZEL, 2001). Assim, enquanto o termo "bentônico" se refere à localização dos organismos no ambiente, "perifítico" descreve o modo de vida aderido ao substrato.

Dentre as algas perifíticas, Bere e Tundisi (2011) demonstraram que as diatomáceas perifíticas são particularmente sensíveis às variações de uso e ocupação da terra. Gradientes de degradação ambiental resultam em quedas significativas na riqueza, diversidade e equitabilidade dessas comunidades. Os autores observaram que áreas urbanas apresentaram maior predominância de espécies oportunistas e tolerantes, enquanto áreas agrícolas e florestais sustentaram comunidades mais diversas e equilibradas. Tais mudanças são impulsionadas por múltiplos fatores, como o aumento da concentração de nutrientes (eutrofização), a deposição de sedimentos finos, a diminuição da transparência da água e a variação na disponibilidade de luz, condições que favorecem a dominância de espécies adaptadas a ambientes instáveis, empobrecendo a estrutura trófica (LOBO *et al.*, 2004; SALOMONI *et al.*, 2011; COSTA; SCHNECK, 2022, COSTA *et al.*, 2025). Em riachos com diferentes níveis de urbanização e impacto agropecuário, essas microalgas demonstram respostas ecológicas rápidas às perturbações, o que reforça seu potencial como ferramentas eficientes em programas de monitoramento ambiental (LOBO *et al.*, 2004; MÉNDEZ-ZAMBRANO *et al.*, 2024; COSTA *et al.*, 2025).

Complementando esse entendimento, estudos têm enfatizado que o uso de microalgas perifíticas particularmente as diatomáceas, permite detectar variações ambientais em múltiplas escalas espaciais e temporais (LIU *et al.*, 2013; BARTOZECK *et al.*, 2018; SOININEN *et al.*, 2019). As mudanças estruturais e funcionais observadas nas comunidades

epifíticas de diatomáceas, indicam forte influência de fatores relacionados ao uso da terra (SOININEN 2007). Contudo, esses efeitos raramente ocorrem isoladamente; na maioria das vezes, resultam da ação conjunta de múltiplos estressores, como alterações geomorfológicas, poluição difusa, modificações no regime hidrológico e aumento da temperatura (PIGGOTT *et al.*, 2015a). Essa complexidade impõe novos desafios à compreensão e previsão das respostas ecológicas, exigindo abordagens integradas que considerem os efeitos combinados, e, muitas vezes, não lineares, desses múltiplos estressores sobre os ecossistemas aquáticos (COTÉ *et al.*, 2016). Diante da crescente pressão sobre os ambientes aquáticos continentais, compreender como diferentes tipos de perturbações interagem e afetam a biodiversidade torna-se essencial. Essa abordagem ganha ainda mais relevância no contexto do conceito de múltiplos estressores, um dos grandes desafios contemporâneos da ecologia aquática, que envolve a interação sinérgica, aditiva ou antagônica entre diferentes pressões antrópicas (CRAIN *et al.*, 2008; PIGGOTT *et al.*, 2015a; COTÉ *et al.*, 2016).

III. Múltiplos estressores em ecossistemas aquáticos: conceitos, interações e desafios na previsão de respostas ecológicas

Em estudos ecológicos, "estressor" é frequentemente tratado como sinônimo de poluição ou pressão antrópica, assumindo-se que seus efeitos são sempre negativos (FOLT *et al.*, 1999). No entanto, uma mesma variável pode ser prejudicial para algumas espécies e benéfica para outras, dependendo das interações ecológicas. Além disso, a resposta a estressores pode seguir um gradiente de subsídio-estresse, isso significa que em baixas concentrações, esse fator pode estimular o crescimento, aumentar a produtividade ou favorecer algumas espécies. Em altas concentrações, o mesmo fator passa a prejudicar o organismo ou a comunidade, causando estresse (ODUM *et al.*, 1979; BIGGS, 2000). Esta relação já foi demostrada em alguns estudos, com comunidades de riachos expostas a diferentes concentrações de nutrientes dissolvidos (NIYOGI *et al.*, 2007; DUNCK *et al.*, 2015; ALMEIDA *et al.*, 2025; RODRIGUES-MACIEL *et al.*, 2025). Assim, adotamos aqui o termo "estressor" para designar qualquer variável que, em decorrência de atividades humanas, ultrapasse sua faixa natural de variação e modifique, positiva ou negativamente, táxons, a composição da comunidade ou o funcionamento ecossistêmico, em comparação a uma condição de referência (PIGGOT *et al.*, 2015d), sendo os estressores ecológicos condições ambientais que se afastam significativamente do "ótimo" e prejudicam o desempenho ou as funções das espécies (BARRETT *et al.*, 1976; ZHOU; WANG, 2023).

O termo “múltiplos estressores” refere-se à ocorrência concomitante de dois ou mais estressores, de origem antrópica ou natural, que interagem de maneira complexa sobre os ecossistemas (FOLT *et al.*, 1999; CRAIN *et al.*, 2008). Essa é uma condição frequente, pois os agentes de estresse ambiental não atuam de forma isolada; seus efeitos são muitas vezes interativos e imprevisíveis (PIGGOTT *et al.*, 2015a; 2015b). Entre os principais estressores antrópicos destacam-se as mudanças no uso da terra, a poluição difusa, a introdução de espécies exóticas, as alterações climáticas, que impactam simultaneamente comunidades biológicas e processos ecológicos (FOLKE *et al.*, 2004; CÔTÉ *et al.*, 2016). A natureza não linear dessas interações constitui um desafio significativo para a compreensão dos mecanismos que estruturam e regulam os ecossistemas, dificultando a previsão de respostas ecológicas e a formulação de estratégias de manejo eficazes (PIGGOTT *et al.*, 2015a; 2015b; 2015c).

As interações entre estressores podem assumir diferentes formas: aditivas, quando o efeito combinado equivale à soma dos efeitos isolados; sinérgicas, quando os efeitos se amplificam mutuamente, resultando em impactos maiores que o esperado; ou antagônicas, quando um estressor reduz ou neutraliza o impacto do outro (CÔTÉ *et al.*, 2016; JACKSON *et al.*, 2016). Essa variação dificulta a generalização dos efeitos ecológicos e reforça a necessidade de abordagens específicas e contextualizadas (ORMEROD *et al.*, 2010). Além disso, fatores como escala espacial e intensidade dos gradientes antrópicos exercem influência direta sobre as respostas das comunidades, contribuindo para padrões não lineares e de difícil previsão (VÖRÖSMARTY *et al.*, 2010).

Estudos ambientais de larga escala têm demonstrado como diferentes estressores interagem no espaço e no tempo, influenciando a estrutura das comunidades e a funcionalidade dos ecossistemas (CRAIN *et al.*, 2008). Contudo, tais abordagens, embora valiosas, muitas vezes não permitem isolar os efeitos individuais de cada estressor (O’BRIEN *et al.*, 2023, HE *et al.*, 2023). Por isso, abordagens experimentais controladas, realizadas em condições laboratoriais e em mesocosmos, têm ganhado destaque (FROST *et al.*, 2024; RODRIGUES-MACIEL *et al.*, 2025; ALMEIDA *et al.*, 2025), ao permitirem o controle rigoroso das variáveis e a avaliação direta de interações aditivas, sinérgicas ou antagônicas (CHRISTENSEN *et al.*, 2006; JACKSON *et al.*, 2016). A integração de dados observacionais e experimentais tem sido recomendada como uma abordagem robusta para identificar limites ecológicos e definir indicadores sensíveis a múltiplos distúrbios (PIGGOTT *et al.*, 2015a; 2015d; SCHINEGGER *et al.*, 2016; ALMEIDA *et al.*, 2025).

Nesse contexto, para compreender os efeitos de múltiplos estressores em diferentes níveis de organização biológica, diversos grupos têm sido empregados como modelos em estudos

experimentais e de biomonitoramento (HE *et al.*, 2023; BAO *et al.*, 2024). Entre esses, as comunidades bentônicas, especialmente macroinvertebrados (BONADA *et al.*, 2006; BAO *et al.*, 2024; ALMEIDA *et al.*, 2025) e microalgas (NARDELLI *et al.*, 2021; COSTA *et al.*, 2022; RODRIGUES-MACIEL *et al.*, 2025), ocupam posição central nestes estudos, devido à sua sensibilidade ambiental, relativa facilidade de amostragem e resposta rápida às perturbações (PASSY, 2007; ALMEIDA *et al.*, 2025; RODRIGUES-MACIEL *et al.*, 2025).

No contexto das comunidades algais, diversos estudos têm explorado os efeitos isolados e combinados de estressores, como carga orgânica, enriquecimento por nutrientes (MORIN *et al.*, 2008; PIGGOTT *et al.*, 2015b; RODRIGUES-MACIEL *et al.*, 2025), e sedimentos suspensos (MORIN *et al.*, 2008; RIDDLE *et al.*, 2009; RIMET *et al.*, 2019; RODRIGUEZ *et al.*, 2021; FROST *et al.*, 2024). Quando combinados, esses estressores podem ter efeitos sinérgicos, amplificando a dominância de espécies tolerantes e reduzindo a capacidade das comunidades de resistirem ou se recuperarem após distúrbios (GRONER *et al.*, 2014; GOTTSCHALL *et al.*, 2022).

No Brasil, embora estudos integrando múltiplos estressores em comunidades aquáticas, ainda sejam escassos, algumas iniciativas têm buscado preencher essa lacuna, com estudos experimentais (ALMEIDA *et al.*, 2025, RODRIGUES-MACIEL *et al.*, 2025) e observacionais, principalmente em abordagens de gradientes ambientais e uso da terra (TANIWAKI *et al.*, 2017; TANIWAKI *et al.*, 2019; CASSOL *et al.*, 2024; COSTA *et al.*, 2025). Esses estudos reforçam a importância de considerar a natureza interativa dos estressores na avaliação da integridade ecológica e no desenvolvimento de ferramentas de biomonitoramento mais sensíveis e realistas.

Apesar dos avanços alcançados, a compreensão das respostas de ecossistemas aquáticos à atuação simultânea de múltiplos estressores permanece limitada, em grande parte porque seus efeitos podem variar de forma não linear e dependem fortemente do contexto ambiental (HE *et al.*, 2023; DUDGEON, 2019; BAO *et al.*, 2024). Nesse cenário, abordagens integrativas como abordagens observacionais e experimentais tornam-se essenciais para captar essa complexidade. Esse desafio é ainda mais crítico em regiões tropicais, onde os estudos permanecem escassos e os efeitos combinados de estressores agrícolas e climáticos sobre microalgas bentônicas seguem pouco compreendidos.

IV. Espécies comuns e raras na estruturação de comunidades perifiticas: implicações ecológicas e taxonômicas

A estruturação das comunidades é influenciada por um conjunto dinâmico de fatores ambientais, espaciais e históricos, que atuam de forma integrada por meio de processos como seleção ambiental e interações bióticas, dispersão e estocasticidade demográfica (LEIBOLD *et al.*, 2004; HEINO *et al.*, 2015). Nesse contexto, as espécies comuns e raras desempenham papéis complementares e fundamentais para a dinâmica e funcionamento dos ecossistemas. As espécies comuns tendem a apresentar ampla distribuição e/ou elevada abundância local, influenciando fortemente o funcionamento dos ecossistemas e respondendo rapidamente a alterações ambientais (MAGURRAN; HENDERSON, 2003). Em contrapartida, espécies raras, embora apresentem baixa abundância ou distribuição restrita, contribuem para a diversidade, muitas vezes funcionando como componentes-chave na manutenção da resiliência ecológica (GASTON, 1994; MOUILLOT *et al.*, 2013).

O conceito de raridade, segundo Gaston (1994, 1997), vai além da simples baixa abundância, abrangendo também a distribuição geográfica limitada e a especialização ecológica das espécies. Por esse motivo, a raridade é de difícil mensuração, e frequentemente, negligenciada em estudos ecológicos. Quando se considera simultaneamente a abundância e a distribuição, observa-se que espécies raras não são apenas menos comuns, mas também tendem a ser mais especializadas, menos adaptáveis e mais vulneráveis a perturbações ambientais (GASTON, 1994). Em ecossistemas tropicais, onde a diversidade taxonômica tende a ser elevada e ainda insuficientemente descrita (LEITÃO *et al.*, 2016; REID *et al.*, 2019), distinguir entre espécies comuns e raras assume papel estratégico para a conservação e a compreensão das dinâmicas ecológicas. Muitas espécies raras pertencem a grupos endêmicos ou pouco conhecidas ecologicamente, possuindo elevado valor científico e conservacionista (LOBO *et al.*, 2016), e são geralmente as primeiras a desaparecer diante de múltiplas pressões, como degradação do habitat, poluição e mudanças climáticas (LEITÃO *et al.*, 2016). Em redes fluviais altamente conectadas, como observado por Stenger-Kovács et al. (2025), essas espécies podem indicar áreas de elevada qualidade ambiental, mostrando-se sensíveis a variações sutis no substrato, composição iônica ou regime hidrológico.

Espécies comuns como *Nitzschia palea*, *Navicula cryptocephala*, *Gomphonema parvulum* tendem a dominar ambientes eutrofizados, atuando como indicadores de poluição e degradação ambiental (WETZEL *et al.*, 2002; BERE; TUNDISI, 2010; 2011). Já algumas espécies como *Frustulia crassinervia* (Brébisson) Lange-Bertalot et Krammer, *Pinnularia*

latarea Krammer, *Sellaphora pupula* (Kützing) Mereschkowsky lato sensu, apresentam valores menores de saprobiedade (Lobo *et al.*, 2010), muitas vezes classificados como de baixa abundância, ocorrem em ambientes oligotróficos a mesotróficos e mostram-se sensíveis a pequenas variações ambientais (LOBO *et al.*, 2010; ZORZAL-ALMEIDA *et al.*, 2017). A presença dessas espécies em baixos números não diminui sua relevância ecológica, ao contrário, pois elas frequentemente respondem a filtros ambientais específicos e revelam padrões locais que poderiam passar despercebidos em análises focadas apenas nas espécies dominantes (MARQUARDT *et al.*, 2018, JUNQUEIRA *et al.*, 2023).

O reconhecimento dos papéis distintos entre espécies raras e comuns é essencial tanto em estudos de diversidade quanto na identificação dos principais filtros ecológicos e espaciais que moldam a estrutura das comunidades (ANDERSON *et al.*, 2011; HEINO; TOLONEN, 2017). Em comunidades de microorganismos, nas quais os desafios taxonômicos e as lacunas no conhecimento ainda são significativos, a atenção dedicada às espécies raras representa uma via promissora para ampliar o entendimento ecológico e taxonômico, além de aprimorar estratégias de monitoramento ambiental (STENGER-KOVÁCKS *et al.*, 2025). Assim, incorporá-las nas análises não é apenas uma escolha metodológica robusta, mas uma necessidade frente à complexidade e vulnerabilidade dos ecossistemas aquáticos tropicais.

V. Diatomáceas perifíticas como bioindicadores em ecossistemas aquáticos: respostas a múltiplos estressores e relevância taxonômica

As algas bentônicas estão entre os grupos mais diversos nos ecossistemas lóticos (MEYER, 2007), são as principais produtoras primárias (STEVENSON *et al.*, 1996; BATTIN *et al.*, 2003), e estão envolvidas efetivamente na dinâmica dos nutrientes e nos ciclos biogeoquímicos (STEVENSON, 1997; 2014), principalmente em ecossistemas aquáticos rasos. Além disso, apresentam características que as tornam excelentes bioindicadoras (LOWE; PAN, 1996; STEVENSON *et al.*, 1996; STEVENSON, 2014). Por serem sésseis ou de mobilidade limitada, respondem diretamente às condições ambientais locais (LOWE, 1996; STEVENSON, 1997; WU *et al.*, 2017; MASOURAS *et al.*, 2021). Seu tempo de geração, que varia de dias a meses, permite detectar tanto mudanças ambientais rápidas quanto graduais (JARLMAN, 1996; WHITTON, 2012). Cada espécie possui preferências e tolerâncias específicas a fatores como nutrientes, pH, salinidade e poluição orgânica, refletindo de forma precisa a química da água. Além disso, essas comunidades são altamente diversas, abrigando inúmeras espécies em pequenas áreas de substrato (LOWE; PAN, 1996).

As algas refletem a integridade ecológica principalmente por meio de sua composição taxonômica, uma vez que a estrutura da comunidade sintetiza respostas a gradientes ambientais, perturbações antrópicas e condições locais ao longo do espaço e do tempo (SOININEN 2007; DOLÉDEC; STATZNER, 2010; ZORZAL-ALMEIDA *et al*., 2017). Em comparação com macroinvertebrados e peixes, as algas respondem mais rapidamente a alterações locais e ao uso da terra, devido à sua posição basal nas cadeias tróficas e à baixa motilidade (JOHNSON *et al.*, 2006; RESH, 2008; MASOURAS *et al.*, 2021). Assim, a análise taxonômica e quantitativa das algas perificas parte da comunidade bentonica se faz necessária para compreender a estrutura da comunidade e interpretar as respostas ecológicas associadas às condições ambientais (STANCHEVA; SHEATH, 2016).

No entanto, apesar de sua sensibilidade e valor bioindicador, a resposta das comunidades de algas perifíticas, como parte da comunidade bentônica, pode ser complexa diante da atuação simultânea de múltiplos estressores ambientais (PRINGLE, 1990; WAGENHOFF *et al.*, 2013; PIGGOTT *et al.*, 2015a; DE CASTRO-CATALÀ, *et al.*, 2020; PISSARIDOU *et al.*, 2021; WU *et al.*, 2022) que agem em conjunto para estruturar a comunidade. Diversos estudos têm abordado o efeito de múltiplos estressores agrícolas sobre comunidades de algas perifíticas, avaliando efeitos sobre a composição de espécies (LANGE *et al.*, 2016; DE CASTRO-CATALÀ, *et al.*, 2020; YUAN *et al.*, 2022), diversidade (PIGGOTT *et al.*, 2015b; RODRIGUES-MACIEL *et al.*, 2025) e características funcionais (SALIS *et al.*, 2019; PISSARIDOU *et al.*, 2021).

Por exemplo, em um estudo experimental com mesocosmos na Nova Zelândia, Piggott *et al.* (2015b) manipularam estressores associados à agricultura (concentração de nutrientes e adição de sedimento) e às mudanças climáticas (aumento da temperatura da água). Os autores observaram que estressores individuais tendem a apresentar efeitos aditivos (ou previsíveis), enquanto estressores combinados frequentemente resultam em efeitos não aditivos, como sinergismos ou antagonismos. Especificamente, os efeitos combinados produziram respostas sinérgicas no nível populacional e antagônicas no nível da comunidade de algas perifíticas. (PIGGOTT *et al.*, 2015b). Por sua vez, outro estudo determinou que tanto nutrientes quanto sedimentos depositados tiveram um efeito detectável nas populações e comunidades de algas, mas que nutrientes e sedimentos juntos resultaram em um efeito aditivo simples devido a diferenças nos modos de ação (WAGENHOFF *et al.*, 2013). Em outro estudo, o efeito negativo da deposição de sedimento foi atenuado pelo maior efeito do enriquecimento de nutrientes (BAATTRUP-PEDERSEN *et al.*, 2020). Ainda, já foi demonstrado experimentalmente que o aumento no fluxo de água combinado com a entrada de sedimentos

leva à redução da biomassa e da diversidade de algas (SALIS *et al.*, 2019). No entanto, esse efeito na biomassa pode ser diminuído quando é combinada a entrada de sedimento com enriquecimento de nutrientes (PIGGOTT *et al.*, 2015b). A redução da velocidade de fluxo pode gerar uma maior riqueza de táxons (SALIS *et al.*, 2019), porém quando combinado sedimento e nutrientes a riqueza é afetada negativamente (PIGGOTT *et al.*, 2015b).

Entre os principais grupos que compõem as comunidades bentônicas, as diatomáceas perifíticas se destacam por serem amplamente reconhecidas como bioindicadoras sensíveis e eficazes na avaliação da qualidade ambiental de ecossistemas aquáticos continentais (LOBO *et al.*, 2004; SMOL *et al.*, 2010; BERE; TUNDISI, 2011; RIMET *et al.*, 2012), tanto em regiões tropicais (MORESCO *et al.*, 2015; LOBO *et al.*, 2020) quanto subtropicais (LOBO *et al.*, 2010). Sua ampla distribuição, elevada diversidade taxonômica e rápida resposta às alterações ambientais as tornam ferramentas valiosas para o monitoramento de estressores antrópicos (STEVENSON *et al.*, 2010; LOBO *et al.*, 2010; LOBO *et al.*, 2020), como poluição orgânica (nutrientes), alterações hidrológicas (MUNN *et al.*, 2018; RODRIGUES-MACIEL *et al.*, 2025) e mudanças no uso da terra (BERE; TUNDSI, 2011; MACDOUGALL *et al.*, 2017; MUNN *et al.*, 2018). Diversos estudos têm demonstrado também respostas consistentes de comunidades de diatomáceas a múltiplos estressores, tanto em riachos (MUNN *et al.*, 2018; RODRIGUES-MACIEL *et al.*, 2025) quanto em lagos (MACDOUGALL *et al.*, 2017). A poluição difusa por nutrientes, em especial, é apontada como um dos principais fatores responsáveis por alterações na estrutura dessas comunidades (MUNN *et al.*, 2018; RODRIGUES-MACIEL *et al.*, 2025). Por exemplo, Munn et al. (2018) observaram que o habitat e a concentração total de fósforo foram os principais responsáveis pelas variações nas composições de diatomáceas perifíticas, enquanto Rodrigues-Maciel et al. (2025) destacaram o enriquecimento em nitrato como o fator predominante que impulsiona mudanças na diversidade dessas comunidades.

Essas alterações na qualidade ambiental podem afetar diretamente os táxons mais sensíveis, que tendem a apresentar declínio diante da intensificação dos impactos (LOUHEED *et al.*, 2018; ZORZAL-ALMEIDA *et al.*, 2021). Embora algumas espécies possam, em alguns casos, persistir sob influência de um único estressor, sua tolerância é geralmente reduzida quando expostas a estressores mais intensos ou à ação simultânea de múltiplos fatores de perturbação (MUUN *et al.*, 2018). Como destacado por Rodrigues-Maciel et al. (2025), as interações entre diferentes estressores desempenham um papel determinante na estruturação das comunidades de diatomáceas, podendo resultar tanto na substituição de espécies quanto na redução da diversidade beta.

Comparações com outros grupos biológicos também evidenciam a sensibilidade de diatomáceas. Em um estudo abrangente, Hering et al. (2006), avaliaram 185 riachos europeus e compararam as respostas de diatomáceas, macrófitas, macroinvertebrados bentônicos e peixes a diferentes estressores ambientais, como eutrofização, poluição orgânica, uso da terra e degradação hidromorfológica. As diatomáceas apresentaram as correlações mais fortes com os gradientes de eutrofização, com 85% a 89% das métricas testadas mostrando respostas significativas, tanto em riachos de montanha quanto de planície. Em comparação, as métricas de macroinvertebrados mostraram 91% de correlação significativa nos riachos de montanha e 59% nos de planície (HERING *et al.*, 2006). Além disso, as diatomáceas responderam de forma consistente a mudanças no uso da terra e degradação hidromorfológica em escalas de microhabitat, destacando sua sensibilidade (HERING *et al.*, 2006). Dessa forma, a integração de análises multivariadas e métricas taxonômicas permitem uma compreensão mais abrangente das respostas das comunidades de diatomáceas a gradientes ambientais e múltiplos estressores (MÉNDEZ-ZAMBRANO *et al.*, 2024).

Não menos importante, estudos de sistemática são fundamentais para ampliar o conhecimento sobre a diversidade florística de diatomáceas. A taxonomia das diatomáceas continentais no Brasil desempenha papel fundamental para compreender a biodiversidade, a ecologia e os padrões biogeográficos dessas algas microscópicas em ambientes tropicais e subtropicais, mas ainda apresenta lacunas significativas. Apesar de avanços recentes, como o Catálogo de diatomáceas continentais da região Sul do Brasil (TAQUES *et al.*, 2024), que integra registros para Paraná, Santa Catarina e Rio Grande do Sul, o conhecimento taxonômico ainda é desigual entre os biomas e regiões (TREMARIN *et al.*, 2009; SILVA *et al.*, 2011; MENEZES *et al.*, 2015). Estudos pontuais recentes (RODRIGUES-MACIEL *et al.*, 2021; MOURA *et al.*, 2022; TAQUES *et al.*, 2024; BARROS; TORGAN, 2025) incluindo descrição de espécies novas (TUSSET *et al.*, 2024; JUNQUEIRA *et al.*, 2025; MARQUARDT *et al.*, 2025) revelam o potencial de descoberta de novos registros e novas espécies no país, destacando a necessidade de esforços sistemáticos e revisões taxonômicas em escala nacional. O sul do Brasil conta com um inventário relativamente abrangente da flora de diatomáceas, incluindo 1.248 táxons no Paraná, 729 no Rio Grande do Sul e 383 em Santa Catarina (TAQUES *et al*., 2024). Em contraste, a diversidade específica de diatomáceas no bioma Pampa segue amplamente subestimada, mesmo tratando-se de uma ecorregião reconhecida por sua elevada biodiversidade, com mais de 12.500 espécies descritas em diferentes grupos taxonômicos (ANDRADE *et al*., 2023). Nesse contexto, os registros de diatomáceas no Pampa ainda são pontuais e dispersos na literatura (e.g., JUNQUEIRA *et al*.,

2025; BARROS; TORGAN, 2025). Esses estudos reforçam que a integração de levantamentos regionais e catálogos estaduais são fundamentais para preencher as lacunas de conhecimento e consolidar uma flora diatomológica representativa dos ecossistemas continentais brasileiros (MENEZES *et al.*, 2015; BFG, 2021), levando em consideração seu potencial como bioindicadores de ambientes aquáticos continentais e que muitas espécies permanecem subdescritas ou não registradas, o que limita a eficácia do monitoramento ambiental e a compreensão das respostas às pressões regionais (MORESCO *et al.*, 2015). Portanto, ampliar os esforços taxonômicos e ecológicos é fundamental para aprimorar as ferramentas de avaliação ambiental e promover a conservação dos ecossistemas aquáticos nesses biomas.

Diante desse contexto, torna-se imprescindível aprofundar a compreensão dos efeitos de múltiplos estressores sobre as comunidades de diatomáceas perifíticas, especialmente em ambientes subtropicais brasileiros, onde os impactos antrópicos são crescentes e a informação ainda é limitada. Dessa forma, esta tese propõe-se a investigar os efeitos de múltiplos estressores ambientais sobre a composição, estrutura e diversidade das comunidades de diatomáceas em riachos, integrando uma abordagem experimental e observacional para avaliar suas respostas a gradientes antrópicos. Além disso, pretende-se contribuir para a caracterização taxonômica e o conhecimento florístico das diatomáceas no bioma Pampa, uma região subamostrada e de grande relevância ecológica.

Objetivos

Objetivo Geral

Esta tese visa investigar os efeitos de múltiplos estressores ambientais sobre a composição e diversidade das comunidades de diatomáceas em riachos subtropicais, combinando abordagens experimentais, realizadas em mesocosmos na Mata Atlântica, e observacionais, realizadas em riachos do bioma Pampa.

Objetivos Específicos

- No primeiro capítulo, investigamos de forma experimental, por meio de mesocosmos ao ar livre, os efeitos do enriquecimento com nitrato, da adição de sedimentos finos e da redução do fluxo sobre a biomassa de algas bentônicas (medida pela clorofila-*a*), assim como sobre os atributos de diversidade e composição das comunidades de diatomáceas bentônicas.

- No segundo capítulo, avaliamos como variáveis ambientais locais, de uso da terra e espaciais explicam a variação na riqueza de espécies e na estrutura de comunidades, com base em três conjuntos de dados: (i) todas as espécies que compõe a metacomunidade de diatomáceas, (ii) as espécies raras e (iii) as espécies comuns.

- No terceiro capítulo descrevemos uma espécie do gênero *Encyonema*, contribuindo para o avanço do conhecimento florístico e taxonômico das diatomáceas do bioma Pampa.

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CAPÍTULO 1:

Multiple stressors in tropical streams: nitrate, sediment and flow interactions mediate benthic biomass and diatom community responses in experimental streams

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Abstract

Human-induced impacts, such as deforestation and agricultural intensification, pose significant threats to tropical streams by promoting nutrient enrichment, fine sediment input, and altered flow regimes. This study employed outdoor stream mesocosms to assess how nitrate enrichment, fine sediment addition, and flow reduction influence benthic algal biomass (using chlorophyll-*a* as a proxy) and diatom community structure. Results showed that sediment addition decreased chlorophyll-a under ambient nitrate conditions but increased it at higher nitrate concentrations. Elevated nitrate levels reduced diatom species richness and evenness, increasing dominance, while changes in species composition were primarily driven by reduced flow and increased nitrate or sediment levels. These findings highlight the mechanisms by which agricultural stressors shape diatom community dynamics, providing critical insights for managing freshwater ecosystems under increasing agricultural pressure.

Keywords: Periphyton, Mesocosm, Freshwater, Eutrophication, Agriculture

Introduction

Freshwater ecosystems support high levels of biodiversity and provide essential ecosystem services to society (Sala et al. 2000; Reid et al. 2019). However, human-induced impacts pose a significant threat to these ecosystems (Dudgeon 2019; Albert et al. 2021; He et al. 2023). Tropical streams are especially vulnerable to deforestation and intensification of land use for agriculture (Dudgeon 2008; Barlow et al. 2018; He et al. 2023), and suffer from various stressors that will alter stream conditions, such as nutrient enrichment (Smith et al. 1999; Jackson et al. 2016); increasing loads of fine sediments (Jones et al. 2017; Weilhoefer and Pan 2022; Ferreira et al. 2023) and hydrological changes due to morphological alterations of watercourses, which in periods of drought can cause flow reduction (Dudgeon 2019). In addition, changes in the flow regime due to water abstraction for irrigation or impoundment (Dewson et al. 2007; Dudgeon 2019) have the potential to be exacerbated in many tropical regions due to predicted climate change (Gesualdo et al. 2019). These stressors stand out for having detrimental effects on freshwater species diversity and community composition (Jones et al. 2014; Tsoi et al. 2020); as well as on stream metabolism (Pérez-Calpe et al. 2021). With agricultural intensification expected to increase by around 50% in the tropics by 2050, landscape conversion and land use intensification pose major threats to tropical ecosystems (Godfray et al. 2010; Taniwaki et al. 2017). However, studies aimed at understanding the effects of multiple stressors on freshwater communities in the tropics remain scarce (He et al. 2023), despite being fundamental to developing strategies to mitigate the impacts of agricultural stressors and their interactions.

The individual effects of nutrient and sediment increases, and flow reduction are relatively well documented in the literature. Nutrient enrichment, particularly nitrogen and phosphorus released from industrial fertilizers and leached into waterways (Smith et al. 1999), typically results on subsidy-stress response gradients (Niyogi et al. 2007), in a manner that positive effects on biomass and species richness at low to moderate nutrient levels (e.g., Pringle 1990; Liess et al. 2009) can turn into adverse effects at higher concentrations (Townsend et al. 2008; Matthaei et al. 2010; Wagenhoff et al. 2013) and lead to detrimental changes in community composition (Biggs 2000; Shibabaw et al. 2021). This results in the dominance of eutrophic-tolerant species and the disappearance of sensitive species (Costa et al. 2022; Jin et al. 2024). Nitrate is efficiently utilized by diatom species, and accelerated growth rates can be observed under nutrient-rich conditions (Cloern and Dufford, 2005). For example, Donald et al. (2013) experimentally demonstrated that among the chemical forms of nitrogen, nitrate seems to be preferred by diatoms, increasing their biomass. Furthermore,

these microalgae are characterized by their ability to store nitrate intracellularly and use it for anaerobic respiration to survive in low-oxygen environments (Kamp et al. 2011; Merz et al. 2021). These studies highlight the dual role of nitrate as both a critical nutrient supporting diatom growth and a driver of ecological imbalance under excessive enrichment, emphasizing the need for careful nutrient management to maintain ecosystem health.

On the other hand, increased sediment load tends to have a negative linear effect on freshwater biodiversity. The deposition of fine sediments creates substrate instability (Jones et al. 2014), reduces habitat heterogeneity through clogging of interstitial spaces (Piggott et al. 2015b), and causes shading, burial, and scouring of benthic organisms (Jones et al. 2014). This leads to reduced algal biomass (Matthaei et al. 2010) and species richness of algae (Wu et al. 2019; Jin et al. 2024) and invertebrates (Matthaei et al. 2010). In addition, reduced streamflow reduces shear stress, which can result in increased algal biomass (Neif et al. 2017) and species turnover from communities with flow-resistant traits to those that prefer slow flows (Matthaei et al. 2010; Neif et al. 2017). However, low stream flows also reduce the diffusion of nutrients and gases, hampering the assimilation of resources by primary producers (Borchardt 1996) and thus decreasing their biomass (Matthaei et al. 2010) and leading to reduced diversity (Nemes-Kókai et al. 2023; Lin et al. 2024). These findings underscore the compounded negative effects of sedimentation and reduced streamflow on freshwater ecosystems, emphasizing the importance of mitigating these stressors to preserve biodiversity and maintain ecological balance.

Despite knowledge of their individual effects, there is a need to further understand how these multiple stressors simultaneously affect freshwater communities, as their interaction may lead to unexpected consequences (e.g., Matthaei et al. 2010; Piggott et al. 2015a; Piggott et al. 2015b; Taniwaki et al. 2017; Nemes-Kókai et al. 2023). In recent decades, studies have investigated the interactive effects of multiple stressors related to agricultural intensification and climate change. However, it remains challenging to identify general patterns regarding their interactive effects, particularly given the wide range of possible combinations of stressors related to agriculture and climate change that can occur in the environment (e.g., Piggott et al. 2015a; Salis et al. 2019; Hunn et al. 2024). Moreover, the same stressor can interact in different directions when combined with different stressors, resulting in either synergic (positive) or antagonistic (negative) interactions (Piggott et al. 2015a). On one hand, nutrient enrichment can, to some extent, reduce the negative effects of other stressors, such as the addition of fine sediments (Baattrup-Pederson et al. 2020). For example, the addition of sediment alone can reduce algal biomass, but this effect can be offset

by nutrient enrichment (Salis et al. 2019; Baattrup-Pederson et al. 2020). On the other hand, the interaction between stressors can result in unexpected responses. The interaction between nutrient and sediment addition weakened the effect of nutrient, which in isolation increased macroinvertebrate taxon richness (Piggott et al. 2015b). Moreover, this interaction also attenuated the effect of sediment, which when isolated increased algal community evenness (Piggott et al. 2015a). Another study showed a reduction on algal community evenness when sediment was added at slow flows, but not at fast flows (Salis et al. 2019). Given the complexity of these interactions, it is essential that studies are carried out to understand and predict the effects of multiple stressors on the aquatic communities of tropical streams. Such studies are essential for assessing and mitigating the impacts of multiple stressors (Taniwaki et al. 2017; Dudgeon 2019).

When addressing the complex interactions between agricultural stressors and climate change in aquatic ecosystems, it is important to recognize the importance of benthic algae as essential elements of these systems (Stevenson 1997; Wu et al. 2017). Benthic algae are the main primary producers in many streams and are effectively involved in the dynamics of biogeochemical cycles (Stevenson 1997; Wu et al. 2017). Among benthic algae, diatoms are typically the dominant group in streams (Round et al. 1990), showing a wide range of environmental tolerances among species and serving as biological indicators of ecosystem health (Tsoi et al. 2021; Costa and Schneck 2022). Therefore, assessing changes in diatom diversity and community composition can provide accurate data on the effects of multiple stressors related to agricultural intensification and climate change (e.g., Neif et al. 2017; Shibabaw et al. 2021; Mayombo et al. 2024).

Here, we used outdoor stream mesocosms to investigate the individual and combined effects of a nitrate enrichment gradient, fine sediment addition, and flow reduction on benthic algal biomass (using chlorophyll-*a* as a proxy), and on diversity attributes (species richness, evenness, and dominance) and species composition of tropical stream benthic diatoms. The use of stream mesocosms to investigate multiple stressors has the advantage to allow for full-factorial designs and the empirical testing of interactions, thus we tested three hypotheses related to the combined effects of the stressors: (i) nitrate enrichment would offset the negative effects of sediment addition and flow reduction on algal biomass; (ii) nitrate enrichment would increase the negative effects of sediment addition and flow reduction on the diversity attributes of diatom communities, also changing species composition towards taxa that tolerate such conditions; and (iii) the negative effects of reduced flow on algal biomass and diatom diversity attributes would be intensified by the addition of fine sediments.

Material and methods

Study site

We used outdoor experimental stream mesocosms to simulate a lotic system (ExStream; Piggott et al. 2015b, Piggott et al. 2015a) supplied with water from a second-order stream within the ESALQ/USP Experimental Station of Forest Sciences (EECFI), Itatinga, São Paulo, Brazil (23°10'S; 48°40'W). The landscape is predominantly covered by the exotic Eucalyptus saligna Sm., but riparian forests occur along the streams. The drainage area of the catchment where the experiment was carried out has an area of approximately 26 254 km² and an average flow of 243 m³ s-1 (Bezerril, 1990). According to the Köppen classification, the region has a Cwa climate, humid temperate with dry winters and hot summers, with an average annual temperature of 19.4 °C and average annual rainfall of 1 319 mm (Alvares et al. 2013). According to data collected at EECFI's weather station over the weeks during which the experiment was conducted (from January 5 to February 23; 2022), an average temperature of 23.5 °C was recorded, with a total rainfall of 176.75 mm.

Experimental design

The ExStream system (a structure 4.1 m high and 20 m long) contains four central polyethylene tanks (135 L capacity each) that feed the 64 mesocosms (3.5 L capacity, 25 cm outer diameter and central outflow 6 cm) equipped with a flow regulator valve (Fig. 1). The water was fed into the system via a 38.10 mm diameter pipe and a centrifugal pump (Schneider Single- stage BC–92S 1B 3 CV 143 MM). The pump was installed close to the bank and positioned inside the stream (Fig. 1), protected by a 4.5 mm metal mesh and another 3 cm mesh forming a double fence. There was also a barrier made of wood and metal mesh (opening 3 cm) inserted against the flow to prevent branches and leaves from clogging the pump (Fig. 1). These nets were cleaned twice a day with a shovel and brush. The pump was supplied by the three-phase electricity grid. It is worth noting that although there were interruptions in the power supply during the experiment, these never exceeded 2 hours in duration. In the system, water was conveyed to the four central tanks (controlled by a ball valve) by polyethylene pipes (diameter 19.05 mm) and transferred to the mesocosms by means of hoses with an internal diameter of 12.7 mm. The upper tanks were named block A, B, C and D and each block distributed water to 16 mesocosms. The mesocosms were filled with substrates (500 ml of 2 to 20 mm gravel and 16 to 20 mm surface stones) to simulate the small streams of the region.

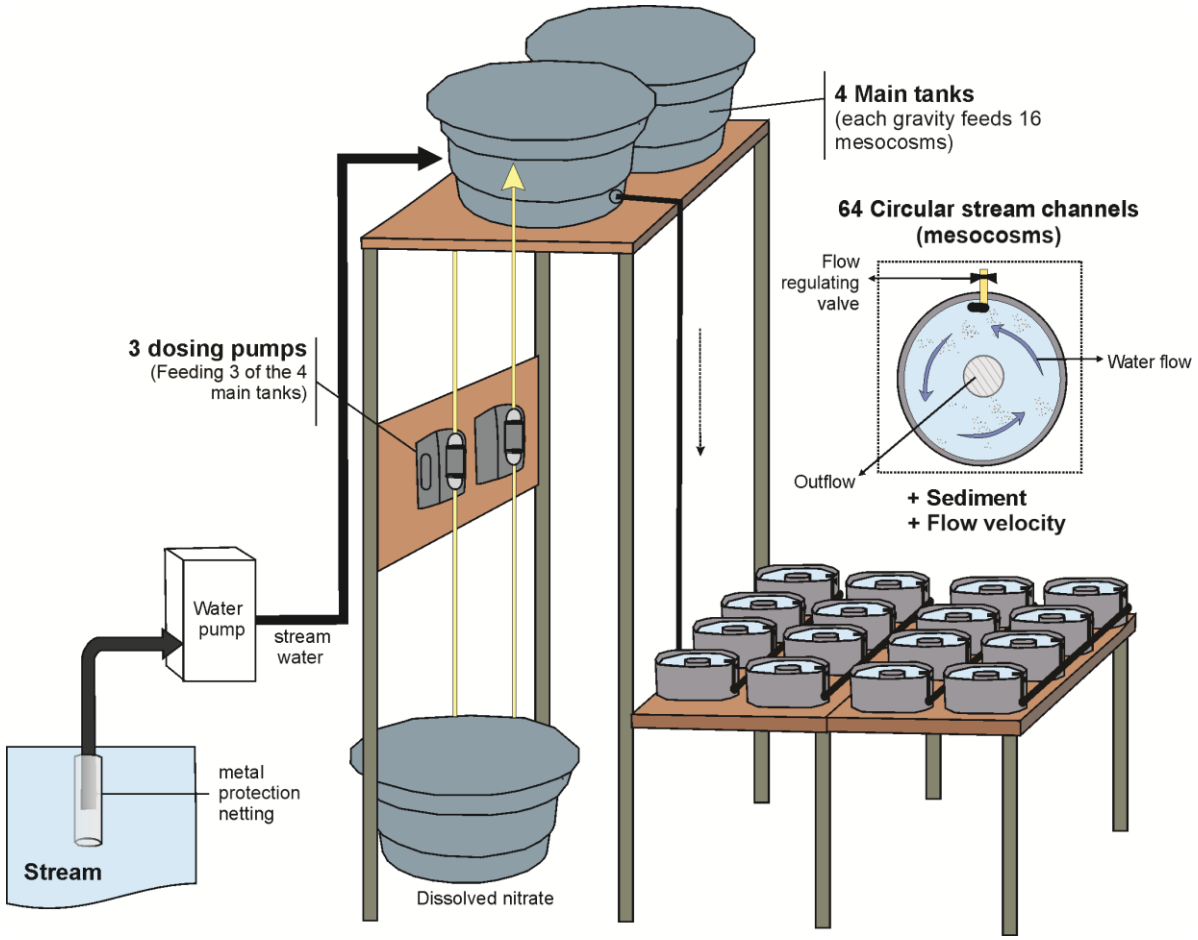

Figure 1. Schematic presentation of the structure of the ExStream experimental setup. Nitrate was manipulated at the main tanks; fine sediment and flow velocity were manipulated directly at the mesocosms.

Three stressors were manipulated: sediment (two levels), dissolved nitrate (four levels) and water flow (two levels) in a full factorial design. Within the four nitrate treatments, mesocosms were randomly distributed, with four replicates of each treatment combination (= 16 mesocosms in each nitrate block), totaling 64 mesocosms (4 nutrient × 2 sediment × 2 water flow × 4 replicates = 64; Fig. 2).

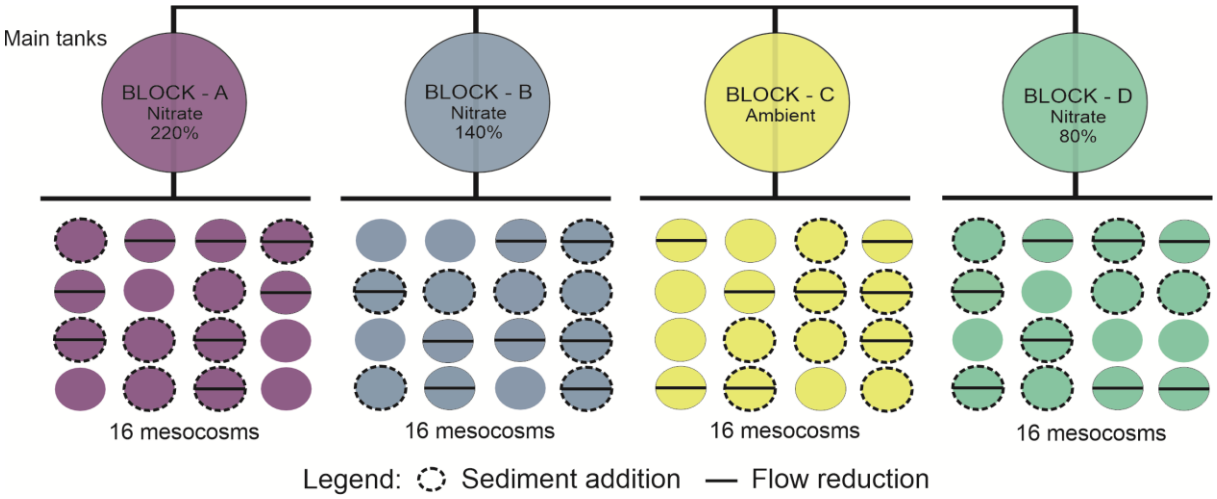

Figure 2. Schematic representation of the multifactorial experimental design (treatments: n = 4; mesocosms: n = 64).

Nitrate enrichment was based on the results presented by Taniwaki (2019), who found that nitrate concentrations in streams under the influence of sugarcane can be up to five times higher than in streams draining pastures, and up to eight times higher than in forested headwaters. We defined four treatments with different nitrate concentrations: (t1) no addition (ambient concentration, 0% increase in relation to ambient concentrations = ~5 mg L^{-1}, Block C, Fig. 2); (t2) ~ 2 × higher than ambient conditions (80% increase = ~ 9 mg L^{-1}, Block D, Fig. 2); (t3) ~ 2,5 × higher (140% increase = ~ 12 mg L^{-1}, Block B, Fig. 2); and (t4) ~ 3× higher (220%, increase = ~ 16 mg L^{-1}, Block A, Fig. 2). For this, we used potassium nitrate KNO_3 (YaraTera TM Krista TM K – composition: 13% water-soluble nitrogen, 43% water-soluble potassium (K_2O) and 1% water-soluble magnesium), which was dissolved in water in a polyethylene container with a capacity of 150 liters, following a ratio of 50 g of nitrate for every 100 liters of water. This solution was distributed to the mesocosms using pressure compensating drippers (Seko Kompact AML 200 with 0.52 ml/injection and 160 pulses min^{-1}) at different pressures to achieve the proposed nitrate enrichment treatments. Nitrate concentrations were measured twice a day, during the morning and afternoon periods in one randomly selected mesocosm of each experimental block, using a UV spectrophotometer (Model AJX–6100P6 double beam spectrophotometer) with wavelengths of 220 and 270 nm.

The effects of sediment were tested by adding fine sand (average grain size 0.2 mm) covering the mesocosms in two levels: (t1) ambient sediment; (t2) 80% coverage of the mesocosm (by adding 400 g of fine sand, Fig. 2). The sediment was obtained from local shops that extract sand from riverbanks in the region. The choice of grain thickness was defined

based on studies showing that the effects of sediment grain size on the benthic community had negative impacts when particle size is ≤ 0.2 mm (Blöcher et al. 2020) and the 80% increase in mesocosm coverage was based on Molina et al. (2017).

Finally, the flow rate changes were simulated in two levels: (t1) ambient (simulating the same water velocity in the adjacent stream, representing 1.5 L min^{-1} in the mesocosm), and (t2) reduction of ~ 66% (0.5 L min^{-1}) (Fig. 2). The flow rate in the system was measured twice a day in all the mesocosms using a digital flow meter (SEA1245) and regulated manually using the flow regulator valve attached to each mesocosm. We defined the reduced flow rate based on the projection of a 35–50% reduction in the flow of streams in the state of São Paulo in climate change scenarios (Gesualdo et al. 2019).

The experiment took place over 6 weeks during austral summer (January and February, 2022). After water circulation began, natural colonization by invertebrates, algae and other microorganisms took place for 21 days. During this period, the flow in the system remained constant at 1.5 L min^{-1}. The experiment was then manipulated for three weeks, with nitrate enrichment, addition of sediment and changes to the water flow rate beginning on day 0 and continued for 21 days. On the last day of the experiment, we used a multi-parameter probe (Hanna HI 9828) to measure the physical and chemical water parameters in the stream and in four mesocosms of each block (representing one of each treatment, Table 1).

Table 1. Physical and chemical water parameters measured in the study stream and in mesocosms of all treatments after 21 days. N0, ambient nitrate concentration; N80, N140, and N220, nitrate enrichment at 80%, 140% and 220% increase in relation to ambient concentrations, respectively; S, added sediment; F, reduced flow. Cond, electrical conductivity; Temp, water temperature; DO, dissolved oxygen; Sat, oxygen saturation.

Treatment	pH	Sat (%)	DO (mg L^{-1})	Cond (µS cm^{-1})	Temp (ºC)
Stream	6.20	78.9	6.64	53	23.3
N0	5.98	87.4	7.37	53	23.3
F	5.97	86.5	7.40	54	23.4
S	6.04	87.7	7.28	53	23.6
F × S	5.97	87.7	7.24	54	23.8
N80	5.95	90.6	7.63	59	23.3
F × N80	5.85	81.6	6.72	59	23.6
S × N80	5.88	86.2	7.21	59	23.7
F × S × N80	5.88	86.4	7.23	59	23.7
N140	6.12	90.3	7.56	65	23.3

F × N140	6.16	88.6	7.30	66	23.3
S × N140	6.20	85.1	7.20	66	23.3
F × S × N140	6.00	92.1	7.80	66	23.4
N220	6.07	85.6	7.37	73	23.3
F × N220	6.13	90.4	7.62	75	23.6
S × N220	6.05	82.3	6.98	74	23.9
F × S × N220	6.02	90.8	7.65	74	23.4

Algal sampling

On the last day of the experiment, we sampled the benthic biofilm from each mesocosm at five random locations on the sediment surface. For this we used a 5 cm inner diameter ring and aspirated the material inside the ring (algae and top 2 mm of sediment) with a pipette. The sampled material was stored in falcon tubes. We used a new pipette for sampling each mesocosm and the rings were rinsed with distilled water and washed with water from the mesocosms to avoid contamination between samples. Part of the material (10–15 ml) was used for chlorophyll-*a* analysis and part (40 ml) was fixed with formaldehyde 4% (Bicudo et al. 1990) for diatom community analysis.

For chlorophyll-*a* analysis, aliquots of 10–15 ml from each sample were immediately filtered using a syringe and glass microfiber filters (GF/C). The filters were stored in falcon tubes wrapped in aluminum foil and kept on ice until arrival in the laboratory. Chlorophyll-*a* was extracted in cold 90% acetone for 24 hours, centrifuged and measured by spectrophotometry (absorbance 665–750 nm) (Steinman et al. 2017). The formula used for the calculation was based on Moresco and Rodrigues (2013), which uses both the area sampled and the initial and final volume (amount filtered) of the sample.

To analyze the diatom communities, we used the Simonsen (1974) method for cleaning the frustules (potassium permanganate and hydrochloric acid), with a modification by Moreira-Filho and Valente-Moreira (1981), which includes a water bath to speed up the process. Permanent slides were then made and fixed with Naphrax® (refractive index=1.74). We counted 500 valves from each sample (i.e., mesocosm) using an optical microscope (Zeiss Primo Star®) with a digital camera attached at 1 000× magnification. All specimens were identified to the lowest practicable taxonomic level, usually species, using classic literatures as *Bibliotheca Diatomologica*; *Iconographia Diatomologica* and new publications (Krammer et al. 1991; Metzeltin et al. 1998, 2005, 2007; Krammer 2000; Lange-Bertalot et al. 2011; Costa et al. 2017; Krammer et al. 1997, 1985; Marquardt et al. 2017), as well as catalogs

available online for consulting valid names (Potapova et al. 2023; Silva 2023). We opted to quantify specimens from permanent slides instead of using Utermöhl chambers because of the highest precision of the first for the identification of diatom species. This decision was taken after observing that the distribution of viable cells (i.e., with chloroplast) did not differ among treatments (75.4% ± 5%; based on the quantification of 500 specimens from samples fixed with formaldehyde in two replicates of each treatment).

Data analysis

First, we analyzed the effects of individual predictor variables and their combinations on chlorophyll-*a* concentration and diversity attributes of diatoms (species richness, Pielou's evenness, and Simpson's dominance). We used generalized linear models (GLMs) with Gamma distribution for modeling chlorophyll-*a* and Gaussian distribution for species richness to meet the assumptions of normality and homogeneity of variance. For evenness and dominance, we used beta regressions for the dependent variable is continuous and restricted to the interval (0– 1; Cribari-Neto and Zeileis 2010). We then generated analysis of deviance tables based on likelihood-ratio chi-square (for GLMs) and chi-square statistics (for beta regressions) to obtain the P-values for each individual predictor and their combinations (Fox 2016). When effects were significant (considering alpha = 0.05) we performed post-hoc tests based on "estimated marginal means" and pairwise Tukey-adjusted comparisons. We also constructed species accumulation curves based on number of individuals by grouping samples according to the statistically significant predictors.

To assess changes in species composition among the treatments, we ran principal coordinates analyses (PCoA) using the Bray–Curtis (relative abundance data) dissimilarity coefficient (Legendre and Legendre 2012). To test whether treatments differ in species composition, we used distance-based RDA (db-RDA; Legendre and Anderson 1999). Abundance data were standardized using the Hellinger method (Legendre and Gallagher 2001) before PCoA and db-RDA. In addition, we used analysis of multivariate homogeneity of group dispersions (PERMDISP, Anderson 2006; Anderson et al. 2006), a test of differences in the 'amount' of variation, to assess whether communities subjected to different combinations of treatments differed in multivariate dispersion (i.e., beta diversity). This analysis allowed us to better understand some statistically significant interactions for which differences in community composition were not evident in the PCoA ordinations. Finally, we carried out an indicator species analysis (IndVal; Dufrêne and Legendre 1997) to search for

species strongly related to each treatment based on their abundance and frequency of occurrence.

We performed all the analyses in the R environment (R Core Team 2023). For GLMs and beta regressions we used packages “stats” (R Core Team 2023) and “betareg” (Cribari-Neto and Zeileis 2010). Models’ R^2 were obtained using package “performance” (Lüdecke et al. 2021), deviance tables using “car” (Fox and Weisberg 2019), pairwise comparisons using “emmeans” (Lenth et al. 2024), and species accumulation curves using “mobr” (McGlinn et al. 2021). We used “vegan” (Oksanen et al. 2017) for PCoAs, db-RDA, and PERMDISP, "labdsv" (Dufrêne and Legendre 1997) for IndVal, and “ggplot2” (Wickham 2016) for graphics construction.

Results

We recorded a total of 117 diatom species distributed in 31 genera. The most abundant species were *Achnanthidium tropicocatenatum* Marquardt, Wetzel and Ector; *Fragilaria fragilarioides* (Grunow) Cholnoky; *Nitzschia linearis* Smith; *N. subacicularis* Hustedt; and *Iconella delicatissima* (Lewis) Ruck and Nakov, all with a relative abundance of more than 5% in most of the treatments. Species accumulation curves showed that 500 cell counts were sufficient to capture the diversity in our local communities (Supporting Information, Fig. S1).

Contrary to our hypotheses regarding benthic algal biomass (as chlorophyll-*a*) and diversity attributes of benthic diatoms, we found evidence of interactive effects of the manipulated stressors only for chlorophyll-*a* concentrations (Table 2). Specifically, chlorophyll-*a* concentration depended on the interaction between nitrate and sediment (Table 2; Fig. 3a). As expected, the addition of sediments showed a tendency to reduce chlorophyll-*a* at ambient concentrations of nitrate by half (post-hoc comparison, $P = 0.05$), but this effect was reversed at the highest nitrate concentration, with the addition of sediment increasing chlorophyll-*a* concentrations by 2.25 times (post-hoc comparison, $P = 0.039$; Fig. 3a). There were no significant effects of water flow reduction individually or in combination with the other factors on chlorophyll-*a* (Table 2).

Table 2. Summary of the results for the effects of nitrate enrichment, sediment addition, and flow reduction on benthic algal chlorophyll-*a* concentrations, and three diversity attributes of stream benthic diatom communities: species richness, evenness, and dominance. Statistically significant P-values (< 0.05) are in bold. The R^2 of each model is shown. Test residuals = 48 for all analyses.

	df	Chisq	P-value	R^2
Chlorophyll-*a*				0.39
Sediment	1	2.35	0.125	
Flow	1	3.55	0.059	
Nitrate	3	6.58	0.087	
Sediment × Flow	1	<0.01	0.952	
Sediment × Nitrate	3	10.40	**0.015**	
Flow × Nitrate	3	0.27	0.965	
Sediment × Flow × Nitrate	3	0.49	0.922	
Species richness				0.52
Sediment	1	0.61	0.434	
Flow	1	0.03	0.868	
Nitrate	3	41.65	**<0.010**	
Sediment × Flow	1	2.23	0.136	
Sediment × Nitrate	3	1.72	0.633	
Flow × Nitrate	3	2.64	0.450	
Sediment × Flow × Nitrate	3	3.08	0.379	
Evenness				0.65
Sediment	1	0.95	0.329	
Flow	1	2.43	0.118	
Nitrate	3	83.41	**<0.010**	
Sediment × Flow	1	0.71	0.399	
Sediment × Nitrate	3	7.12	0.068	
Flow × Nitrate	3	5.90	0.116	
Sediment × Flow × Nitrate	3	5.16	0.160	
Dominance				0.63
Sediment	1	3.26	0.071	
Flow	1	2.47	0.116	
Nitrate	3	61.65	**<0.010**	
Sediment × Flow	1	0.60	0.436	
Sediment × Nitrate	3	5.10	0.164	
Flow × Nitrate	3	5.68	0.128	
Sediment × Flow × Nitrate	3	5.37	0.146	

Only nitrate concentration significantly affected the three diversity attributes evaluated, i.e. reduced species richness and evenness, and increased dominance (Table 2). As expected, species richness was higher in communities subjected to ambient concentrations than communities subjected to nitrate enrichment (Table 2; Fig. 3b; post-hoc comparisons for ambient × 80, 140, and 220% enrichment = $P \leq 0.002$). However, there were no statistically significant differences in species richness among the three enrichment treatments (post-hoc comparisons = $P > 0.05$). Similarly, nitrate enrichment decreased community evenness and increased dominance in comparison with communities subjected to ambient concentrations (Table 2; Figs. 3c, d; post-hoc comparisons for ambient × 80, 140, and 220% enrichment = $P < 0.001$). The species accumulation curves showed that all patterns were unlikely to be influenced by the 500-cell count, as all treatments showed similar asymptotic trends (Supporting information, Fig. S1).

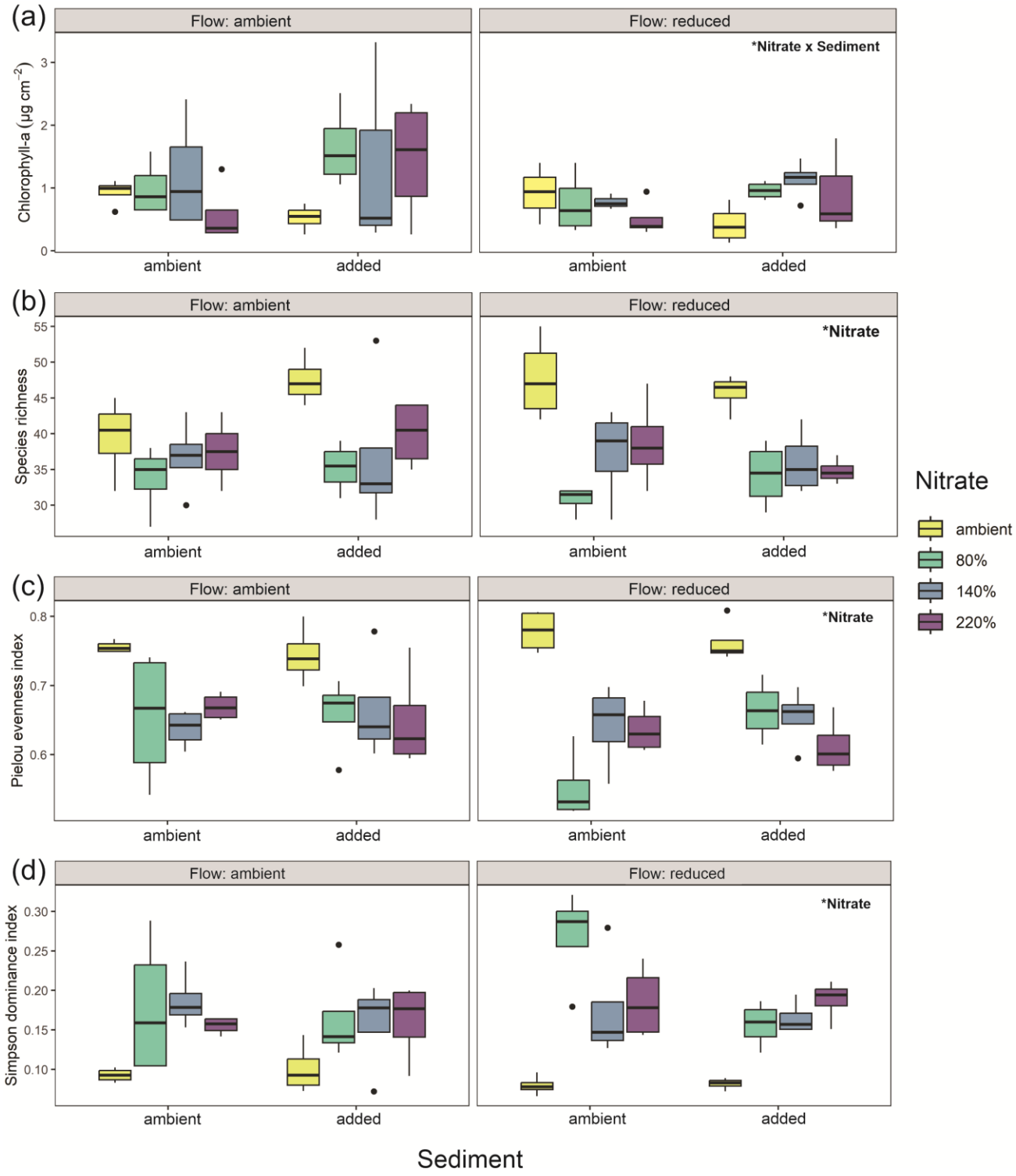

Figure 3. Effects of nitrate enrichment, sediment addition, and flow reduction on (a) benthic algal chlorophyll-a concentrations, and (b, c, d) the three measured diversity attributes of stream benthic diatom communities: (b) species richness, (c) evenness, and (d) dominance. The heavy horizontal line indicates the median, box ends are the first and third quartiles, whiskers indicate minimum and maximum values, and circles indicate outliers. "The main effects of the significant factors or interactions are indicated in the top right corner of each graph."

Community composition (relative abundance data) changed in response to the effects of all the three manipulated variables and their interactions (db-RDA; $R^2 = 0.29$; Table 3; Fig. 4). Specifically, the interactions between flow and nitrate and between sediment and flow were statistically significant (Table 3). Reduced flow did not affect community composition under ambient nitrate conditions, but in nitrate enrichment treatments there is a clear distinction between communities subjected to ambient and reduced flows (Fig. 4a, b). The differences in community composition resulting from the interaction between sediment and flow are subtler and not evident in the PCoA ordination plots (Fig. 4b, c). This is because the communities from distinct treatments differ in beta diversity, i.e., in the variation in species composition within treatments. Specifically, under ambient flow conditions, communities (i.e., mesocosms) subjected to the ambient and to the sediment addition treatments exhibited similar within-treatment amount of beta diversity (PERMDISP, $F_{1,30} = 0.586$; $P = 0.452$; Fig. 5a). However, under reduced flow conditions, the communities of the treatment with sediment addition exhibited greater similarity with one another than the communities without sediment addition (PERMDISP, $F_{1,30} = 5.238$; $P = 0.023$; Fig. 5b).

Table 3. Summary of the results of distance-based redundancy analysis (db-RDA) for the effects of nitrate enrichment, sediment addition, and flow reduction on community composition of stream benthic diatoms based on the Bray-Curtis dissimilarity coefficient (relative abundance data). Statistically significant P-values (<0.05) are in bold.

	df	Sum of squares	F-value	P-value
Sediment	1	0.12	2.63	**0.005**
Flow	1	0.11	2.46	**0.012**
Nitrate	3	0.77	5.58	**0.001**
Sediment × Flow	1	0.08	1.73	**0.033**
Sediment × Nitrate	3	0.18	1.28	0.100
Flow × Nitrate	3	0.20	1.48	**0.033**
Sediment × Flow × Nitrate	3	0.16	1.16	0.221
Residual	48	2.21		

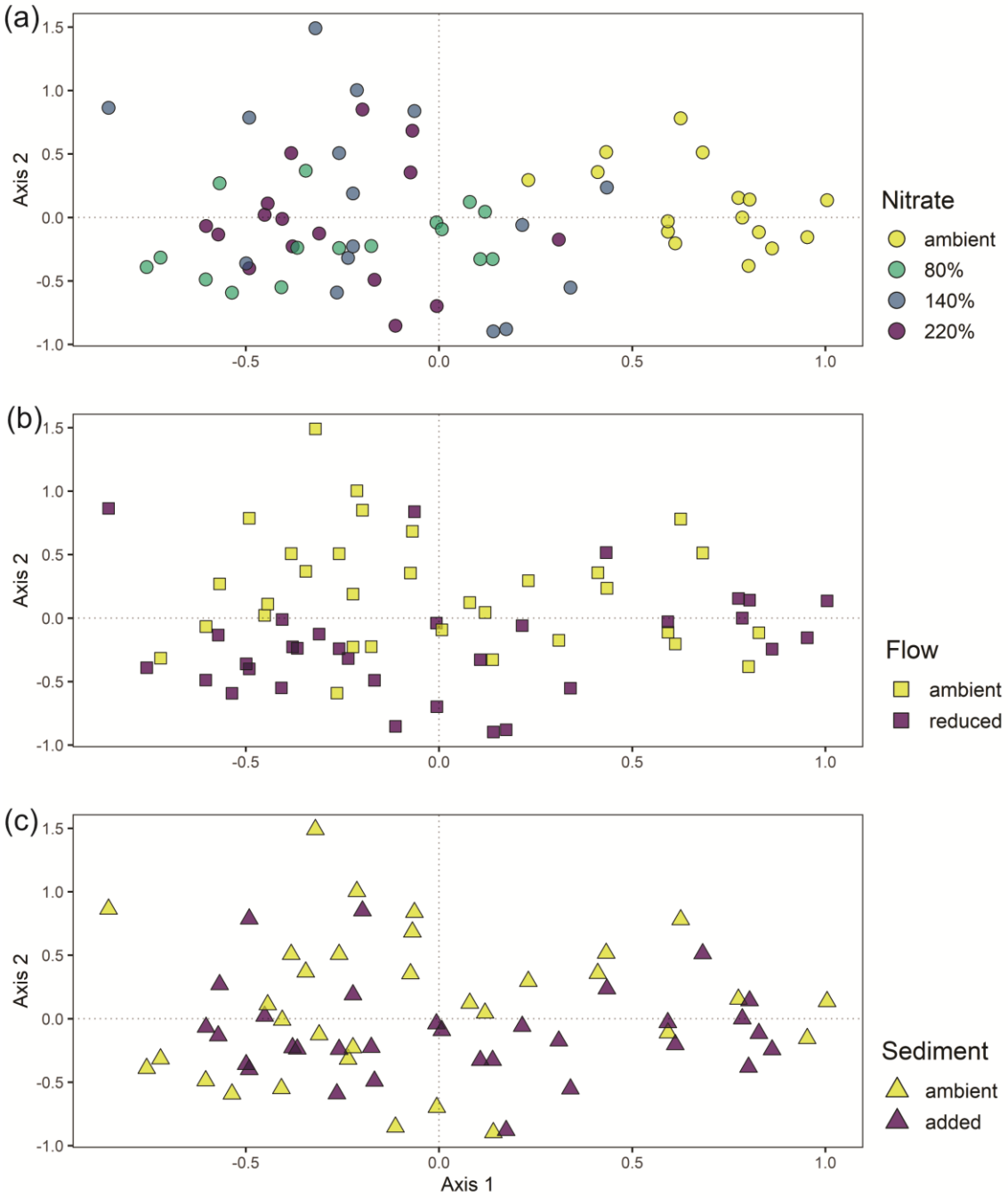

Figure 4. Principal coordinates analysis (PCoA) plots of stream benthic diatom communities subjected to (a) the nitrate treatments (ambient, 80%, 140% and 220% increase in relation to ambient concentrations), (b) the flow treatments (ambient and reduced), and (c) the sediment treatments (ambient and added). A single PCoA was performed, but factors were plotted separately for clarity.

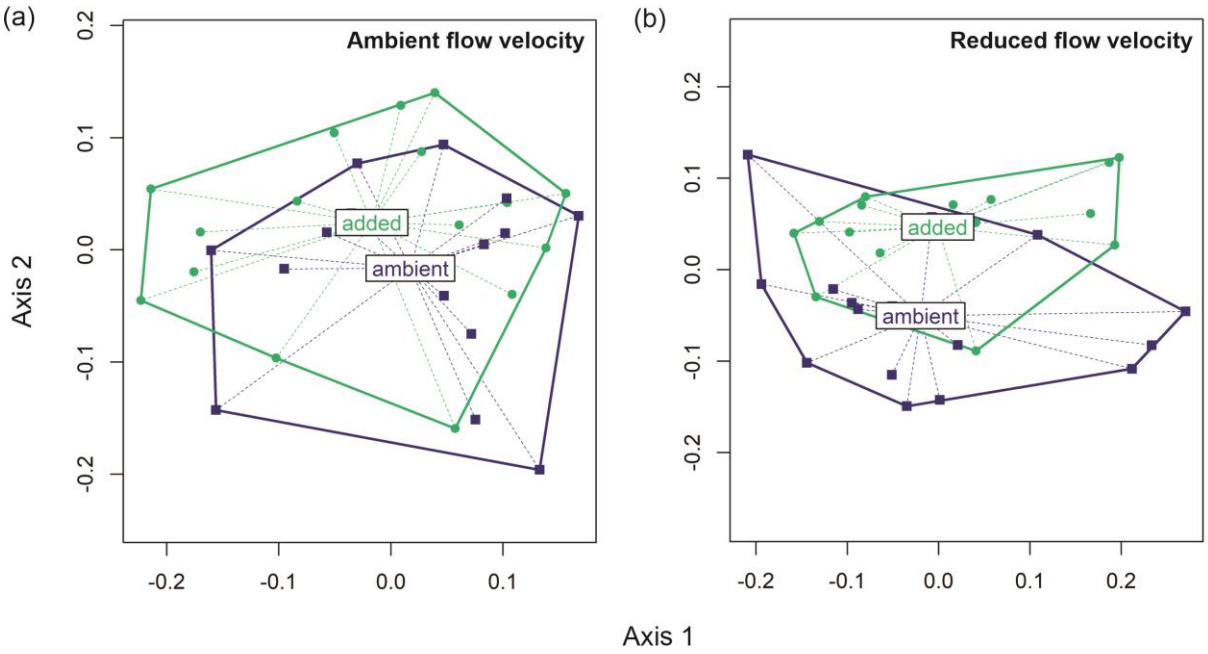

Figure 5. Multivariate dispersions based on Principal Coordinates Analysis (PCoA) of stream benthic diatom communities subjected to the sediment treatments (ambient and added) at (a) the ambient flow treatment and (b) the reduced flow treatment.

Finally, the IndVal analysis showed that 16 species had a significant association with one of the treatments, considering their abundance and frequency of occurrence (Table 4). Of these species, 11 were associated with reduced flow: six of them were associated exclusively to reduced flow conditions, two were associated with the combination of reduced flow and nitrate enrichment, and three with the combination of reduced flow and sediment addition.

Table 4. Indicator diatom species for the different treatments. N0, ambient nitrate concentration; N80, N140, and N220, nitrate enrichment at 80%, 140% and 220% increase in relation to ambient concentrations, respectively; S, sediment added; F, reduced flow. Only P-values < 0.05 are shown.

Species	Treatment	Indicator value	P-value
Achnanthidium minutissimum (Kützing) Czarnecki	F×N220	0.16	0.026
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	S	0.29	0.005
Diploneis elliptica (Kützing) Cleve	F	0.22	0.038
Eunotia bilunaris (Ehrenberg)	F	0.22	0.010
Eunotia intermedia (Krasske ex Hustedt) Nörpel and Lange-Bertalot	F	0.44	0.023
Gomphonema acidoclinatum Lange-Bertalot and Reichardt	F	0.18	0.008
Gomphonema lagenula Kützing	F×S	0.14	0.006

Halamphora normanii (Rabenhorst) Levkov	N0	0.50	0.047
Luticola mutica (Kützing) Mann	S	0.25	0.019
Navicula cryptotenella Lange-Bertalot	S	0.18	0.007
Navicula lohmannii Lange-Bertalot and U. Rumrich	F	0.23	0.004
Navicula wildii Lange-Bertalot	F×S	0.25	0.001
Neidium longiceps (Gregory) Ross	F	0.30	0.041
Nitzschia linearis Smith	N140	0.16	0.036
Nitzschia subacicularis Hustedt	F×N80	0.12	0.011
Sellaphora laevissima (Kützing) Mann	F×S	0.27	0.002

Discussion

The transformation of the natural landscape and the increase in land use for agricultural practices are among the main factors threatening tropical ecosystems, driving profound changes in their structure and functioning. Estimates suggest that agricultural intensification in tropical regions could increase by around 50% by 2050 (Godfray et al. 2010; Taniwaki et al. 2017), exacerbating problems such as soil degradation, biodiversity loss and water pollution due to the massive use of fertilizers and pesticides. Despite this, studies seeking to understand the effects of multiple stressors on aquatic community structure in the tropics have idiosyncratic results (He et al. 2023). Here, we experimentally examined the impacts of three stressors commonly associated with land use intensification on tropical stream diatom communities and algal biomass. Our results suggest that there is a strong relationship between sediment deposition and nitrate enrichment in determining benthic algal biomass (estimated using chlorophyll-*a* as a proxy), as well as nitrate enrichment being the main driver of change in diatom diversity metrics. Moreover, interactions between stressors played a key role in determining diatom community composition, which may respond to the stressors through species replacement or by reducing beta diversity.

Our first hypothesis was that nitrate enrichment would offset the negative effects of sediment addition and flow reduction on algal biomass. The results partially corroborated the initial hypothesis, as the negative effects of fine sediment addition on algal biomass were offset by nitrate enrichment during the experiment. Previous studies suggest that the deposition of fine sediment results in substrate instability, shading (Jones et al. 2014) and reduced habitat heterogeneity (Piggott, Townsend et al. 2015), generating negative effects on algal biomass concentrations (Matthaei et al. 2010). In experimental studies of the effects of sediment on algal communities, biomass decreased only in the short term after sediment addition and increased in the long term (after 2 to 3 weeks), with nutrient concentrations

associated with the increases in biomass (Izagirre 2009; Piggott, Towsend et al. 2015; Penk et al. 2024). In our study, we only assessed the community at the end of the experiment, and observed that the sediment and nutrient enrichment treatments showed a significant increase in biomass. This is probably related to the fact that nutrient-rich and relatively stable fine sediments are colonized by algae, particularly filamentous algae (Jones et al. 2014), which can result in an increase in biomass. Our results corroborate the findings of studies in both tropical (Baattrup-Pedersen et al. 2020) and temperate streams (Riddle et al. 2009), where the negative effect of sediment was attenuated by a greater effect of nutrient enrichment. Nevertheless, when examining the composition of the community, it becomes evident that the increased nutrient concentration did not serve to alleviate the detrimental effects of sediment addition. Instead, nutrient addition led to a replacement of species.

Our second hypothesis predicted that nitrate enrichment would impact negatively the diversity attributes of diatom communities, changing species composition towards tolerant taxa. The results showed that nitrate enrichment negatively impacted benthic diatom communities, reducing species richness and community evenness, increasing dominance, and changing composition. This finding is consistent with other studies suggesting that in nutrient-rich conditions, eutrophic-tolerant diatom species tend to dominate the community and thus reduce diversity (Jones et al. 2017; Tsoi et al. 2020). Furthermore, mobile species and those that live at the lower layer of biofilms (i.e. low-profile species) are closely related to increased nutrients (e.g., Passy 2007; Wagenhoff et al. 2013; Weilhoefer and Pan 2022). In our study, the mobile species *Nitzschia linearis* and *N. subacicularis* and the low-profile *Achnanthidium minutissimum* were abundant at all treatments, but mostly dominated at some of the nitrate-rich ones. These species are widely known to be tolerant of eutrophic conditions and in our study exhibited higher relative abundances and frequencies of occurrence at enriched nutrient concentrations than at ambient concentrations. These results suggest that competition between species plays an important role in structuring diatom communities in response to nutrient enrichment in aquatic environments (Passy 2007). Furthermore, it was evident from our results that high nitrate inputs are not necessary to affect species richness and community composition, as communities exposed to any increase in nitrate concentration differed from communities exposed to ambient concentrations for every community attribute evaluated.

Our third hypothesis predicted that the negative effects of reduced flow would be intensified by sediment addition. We found that despite not directly affecting diatom diversity metrics, the interaction between flow and sediment influenced community composition by

reducing the heterogeneity in species composition among mesocosms subjected to reduced flow and sediment addition. This is in accordance with other studies with lotic benthic diatoms that showed that the tolerance or sensitivity of species depends on the interaction of variables that characterize the process of environmental deterioration (Biggs 2000; Lobo et al. 2004; Bere and Tundisi 2011), causing a change in composition by favoring species adapted to such conditions. Although the reduction in flow decreases the exchange of nutrients in the water column (Borchardt 1996) and increases the concentration of nutrients in the environment (Nemes-Kókai et al. 2023), the absorption of these nutrients depends on the flow for a more efficient assimilation (Borchardt 1996). In our study, under low-flow conditions, a set of dominant species was observed: *Diploneis elliptica*; *Eunotia intermedia*; *E. bilunaris* and *Gomphonema acidoclinatum*. These species are capable of extending beyond the boundary layer above the substrate by forming long colonies and/or mucilage stalks, allowing them to exploit more favorable environments (Tuji 2000; Passy 2007). Previous studies have also observed that such characteristics favor the development of some diatom species in more stable flow conditions (Taniwaki et al. 2019; Várbíró et al. 2020). Moreover, in our study, the species *Luticola mutica* was associated with the sediment treatment, as expected (Jones et al. 2017). As deposited fine sediments are relatively unstable, they are not suitable for the settlement of attached and particularly chain-forming diatom species (Jones et al. 2014). However, reducing the flow rate provides a less unstable environment in which some species can develop (Jones et al. 2017). With reduced flow and the addition of sediment, water turbidity can be higher and such conditions tend to benefit medium to large species, such as *G. lagenula* and *G. parvulum* (Medeiros et al. 2022), and mobile species (Passy 2007; Jones et al. 2014). In our study the species *Gomphonema lagenula*; *Navicula wildii*; and *Sellaphora laevissima* showed a significant relationship with the conditions of reduced flow and sediment deposition. While high-profile species (e.g., *Gomphonema* spp.) produce mucilage stalks, a condition that favors their acquisition of resources above the periphytic mat, mobile species, such as those of the *Navicula*, *Luticola* and *Sellaphora* genera, can benefit by using high-profile species as an alternative substrate to reach resources (Tuji et al. 1999). Such changes in diatom communities through the abundance reduction or loss of sensitive species and the spread of species tolerant to degraded environmental conditions can result in the homogenization of biological communities (i.e., decreased beta diversity; Olden 2006; Siqueira et al. 2015), as was the case of the observed reduced heterogeneity among mesocosms subjected to flow reduction and sediment addition.

In a context where freshwater ecosystems face significant threats, our results help to

understand how diatom communities in tropical streams respond to multiple agricultural stressors. Our study showed that the effect of nitrate enrichment is the main driver of changes in diatom diversity metrics and that the negative effects of sediment deposition on algal benthic biomass can depend on the different concentrations of nitrate enrichment. Furthermore, two-way interactions between the stressors play a clear role in determining community composition through species replacement and biological homogenization. Given that benthic diatoms represent a significant proportion of primary producers in streams, it is crucial to understand whether and how observed alterations in diatom communities impact the functioning of stream ecosystems subjected to the effects of agricultural stressors. In terms of practical recommendations regarding agricultural management, our results indicate that the avoidance of fertilization of crops during the rainy season could prevent the effects of increased nutrient concentrations and sedimentation altogether, which would impact the primary producers in tropical streams. It is also important to maintain natural stream flows in order to reduce the negative effects of nutrient enrichment and sedimentation. Finally, the increase of riparian vegetation and the adoption of best agricultural practices (such as terracing) could mitigate the impact of these stressors on stream benthic algae, by reducing sedimentation and nitrate enrichment and improving the baseflow conditions during drought events. Furthermore, the implementation of a region-specific monitoring system, based on the use of diatom community composition as a bioindicator, is an effective tool to continuously assess the effectiveness of applied strategies, allowing dynamic adjustments in response to constant changes in environmental conditions.

Conclusion

In conclusion, our study provides critical insights into how tropical stream diatom communities respond to multiple stressors associated with agricultural intensification. We demonstrated that nitrate enrichment is the primary driver of changes in diatom diversity metrics, while the effects of sediment deposition on benthic algal biomass are moderated by nutrient concentrations. The interactions between stressors significantly influenced community composition through species replacement and biological homogenization, underscoring the complex dynamics in degraded freshwater ecosystems. Given the role of diatoms as key primary producers in streams, understanding their responses to agricultural stressors is vital for predicting broader ecosystem impacts.

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Supporting information

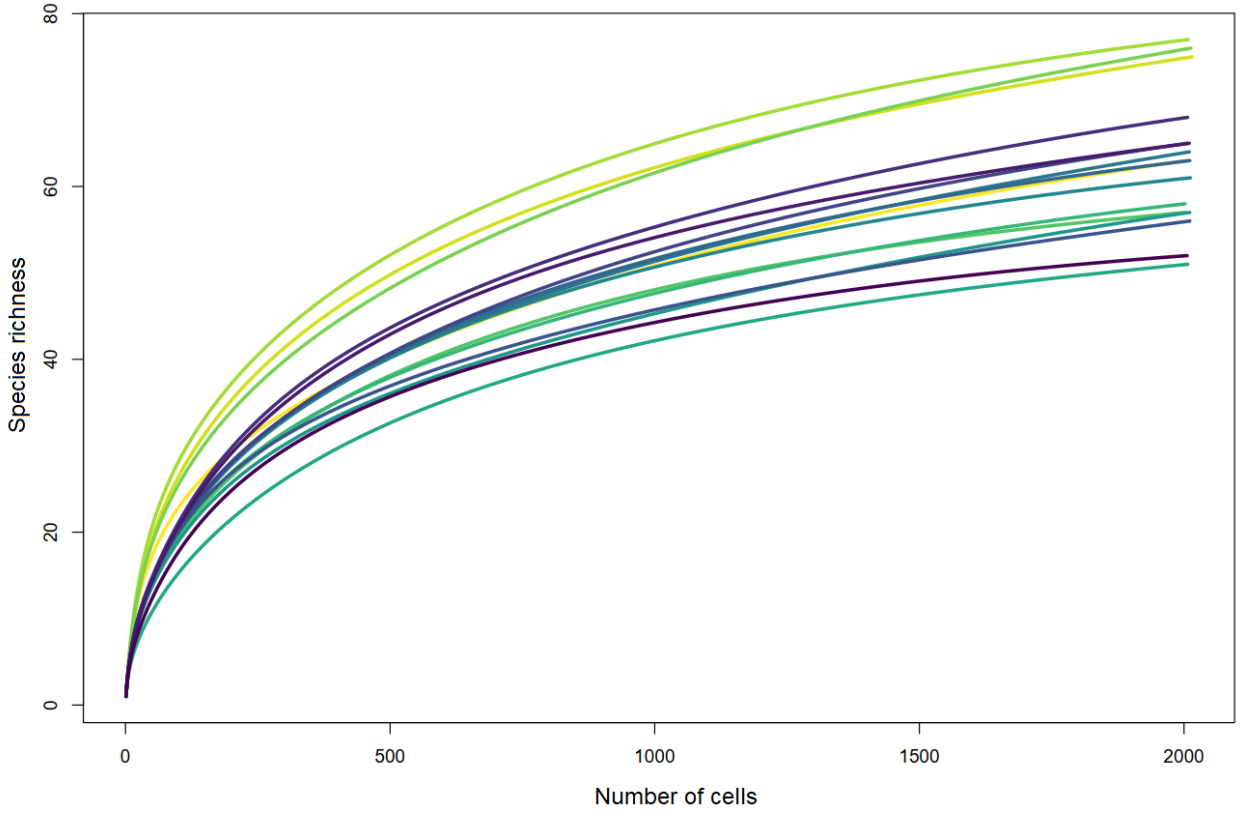

Figure S1. Species accumulation curves of stream benthic diatom communities subjected to each of the 16 treatments, i.e., combinations of nitrate (ambient, and 80%; 140%; and 220% increased concentrations); sediment (ambient and addition); and flow (ambient and reduction).

CAPÍTULO 2

The relative importance of local environmental, land use and spatial factors in explaining species richness and community composition varies for common and rare stream periphytic diatoms species

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Abstract

1. The relative importance of environmental selection, dispersal, and demographic stochasticity in structuring aquatic communities remains poorly resolved, particularly in diverse and human-impacted Neotropical regions. Using diatoms as a model, we assessed how local environmental conditions, catchment land use, and spatial factors shape community richness and composition in subtropical streams, and whether responses differ among common, rare, and very rare species.

2. Epilithic diatoms were sampled in 50 streams of 1st to 3rd order in the Ibicuí river basin (Pampa biome, southern Brazil). The species were identified to the lowest possible taxonomic level and classified as common, rare or very rare based on quartiles of abundance in the metacommunity. For each stream, we obtained local environmental variables, land use and land cover in the catchment and spatial predictors derived from PCNM. Species richness was modeled by multiple linear regression, while composition (Hellinger-transformed abundance data) was analyzed by pRDA and partitioning of variation between environmental, land use and spatial components.

3. Local environmental variables were the main predictors of species richness in all groups, with canopy openness standing out as a key filter. In contrast, community composition showed a strong spatial signature for the total assemblage and for common species, with large- and fine-scale PCNM explaining most of the explained variation, while the pure effect of land use was reduced. The composition of rare species was explained only by water velocity, while very rare species were structured exclusively by fine-scale spatial predictors.

4. These results show that the mechanisms that structure the diatom metacommunity depend on both the attribute evaluated (richness vs. composition) and the degree of rarity of the species. Environmental gradients govern richness in all groups, but spatial processes dominate compositional changes, especially for common and very rare taxa. Rare species are mainly filtered by hydrodynamic conditions, while very rare species reflect spatial configuration and dispersal limitation more than the environmental gradients measured. Although statistically significant, the pure spatial fraction explained a very small proportion of the variance in very rare species composition, indicating a weak but detectable spatial signal.

5. By integrating environmental, land use and spatial predictors and explicitly separating species groups, this study deepens our understanding of metacommunity organization in subtropical grassland streams. This study highlights how the relative influence of environmental and spatial processes varies with species rarity.

Keywords: benthic algae, land use, lotic environment, metacommunity, Pampa biome, pRDA.

Introduction

Despite the recognition that environmental selection and dispersal, in addition to demographic stochasticity, are the key processes underlying the spatial variation in species composition (Velend 2016; Leibold and Chase 2018), understanding their relative importance and the role of environmental factors operating at different scales remains challenging (Brown et al. 2011; Castillo-Escrivà et al. 2020). This is particularly true in regions with high species richness, such as the Neotropics (Liu et al. 2021; Valente-Neto et al. 2025), where challenges are further increased by increasing anthropogenic impacts, which can alter the composition of communities in diverse and often unpredictable ways (Socolar et al. 2016; Kramer et al. 2023). In this context, the organization of stream communities is affected by multiple ecological drivers at different scales, including local environmental conditions and catchment land use and cover (Petsch et al. 2021; Schneck et al. 2022). Land use and cover directly influence stream communities through hydrological, physical, and chemical processes (Paul and Meyer 2001; Allan 2004). For example, agricultural intensification and urbanization increase the input of sediments, nutrients and pollutants, and modify the flow regime and benthic substrate characteristics (Walsh et al. 2005; Jones et al. 2014; Polazzo et al. 2022).

Although it is clear that environmental conditions, at both local and landscape scales influence the structure of aquatic communities, determining the relative importance of each and how their effects are context dependent remains a significant challenge. This is especially true when considering how these drivers act across different groups of organisms, scales, and regions (see Heino et al. 2015; Xu et al. 2025). For example, the relative importance of these factors varies across biological groups, including macroinvertebrates (Wan et al. 2015; Castillejo et al. 2024), aquatic insects (Heino et al. 2008; Nicacio et al. 2020), macrophytes (Trindade et al. 2018), and diatoms (Marquardt et al. 2018; Castillejo et al. 2024). Even at broad geographic scales, local factors can explain a significant portion of variation in community composition (Gonçalves-Souza et al. 2014; Souffreau et al. 2015; Benito et al. 2018). Moreover, the relative importance of environmental and spatial processes can vary between common and rare species within a community (Siqueira et al. 2012; Wu et al. 2024).

Community structure depends not only on overall species composition but also on the relative dynamics between rare and common species. Rare species, often characterized by low abundance or restricted distribution (Gaston 1994; 1997), are more vulnerable to disturbance (Jain et al. 2013) and play key roles in resilience and regional diversity (Gaston 1994; van Nes et al. 2024). Conversely, common species, which are widely distributed and

abundant, exert a major influence on ecosystem functioning and energy flows (McGill et al. 2007; Magurran and Henderson, 2010). Studies show that rare and common species respond differently to environmental and spatial filters (Marquardt et al. 2018; Lengyel et al. 2020), highlighting the importance of analytical approaches that treat them separately. For example, Junqueira et al. (2023) found that although both common and rare diatoms were influenced by environmental variables (such as mesohabitat and substrate roughness), the specific impacts on community structure varied significantly between the two groups. In relation to land use factors, a greater tolerance to landscape degradation of common species was observed when analyzing plant communities (Faramarzi and Isselstein 2023). This pattern is related to the fact that common species are mostly generalists, adapted to a wide range of environmental conditions (Lennon et al. 2011). This allows them to disperse into newly available habitats more easily, enabling them to outcompete rare species (Benone et al. 2022), which are often specialists adapted to more specific conditions (Lennon et al. 2011). These specialists have narrower ecological niches (Lennon et al. 2011; Lengyel et al. 2020), making them more vulnerable to changes in environmental conditions (Wu et al. 2024). In this sense, rare species face greater vulnerability due to their specialized niche requirements and susceptibility to environmental filtering, while generalists benefit from heterogeneous habitats and are limited by dispersal, i.e. spatial constraints, with connectivity (Pandit et al. 2009; Siqueira et al. 2012; Stroud et al. 2015; Benone et al. 2022; Crisfield et al. 2024). Despite the well-known general patterns regarding common and rare species, there is a paucity of studies on the response of common and rare microorganisms to environmental selection and dispersal processes (Junqueira et al. 2023).

Diatoms (Bacillariophyceae) are especially suitable organisms for investigating the processes structuring communities in lotic environments. This is because of their high species diversity and rapid response to environmental change (Stevenson and Smol 2002; Costa and Schneck 2022). Thus, diatom-based community analyses offer a valuable approach for understanding how local and landscape environmental variables and spatial factors interact in shaping aquatic biodiversity in tropical and subtropical catchments (Marquardt et al. 2018; Medeiros et al. 2022). Among some environmental conditions, variables such as pH, electrical conductivity, nutrients (nitrogen and phosphorus), dissolved oxygen, and water temperature (Biggs 2000), as well as substrate characteristics and water velocity, have been identified as critical drivers influencing the attachment and productivity of primary producers (Passy 2007; Jones et al. 2014; Rodrigues-Maciel et al. 2025). Despite a large body of literature on aquatic metacommunities, the integration of local environmental, landscape, and

spatial factors, with a specific focus on the distinct responses of common and rare species, remains limited, specially in subtropical grassland streams and for microrganisms (Soininen 2007; Marquardt et al. 2018; Wu et al. 2024). Understanding the responses of common and rare species is fundamental to elucidating the mechanisms structuring aquatic biodiversity under increasing anthropogenic pressures.

In this context, the main objective of this study is to assess the relative contributions of local environmental, land use and cover within the catchment, and spatial factors on species richness and community composition of stream benthic diatom communities, with an emphasis on investigating whether rare and common species respond in distinct ways to these predictors. Our hypothesis is that the species richness and composition of diatom communities would be mainly influenced by local environmental and land use and cover variables, but the relative importance of these factors would differ between common and rare species, being more important for rare than for common species. Moreover, common species, being generalist, would show a relatively large effect of spatial constraints than rare species.

Material and methods

Study area

Samplings occurred within the Ibicuí River catchment (between 29°01′ S and 31°20′ S; 56°47′ W and 53°29′ W) located in the western portion of the state of Rio Grande do Sul, in southern Brazil (Fig. 1). We sampled 50 first- to third-order streams, during July and August 2023. Streams were distributed across the basin, with no direct hydrological connection among sampled reaches. The greatest distance between streams being was 125.7 km and the shortest was 0.51 km. The region has a subtropical climate, with annual precipitation ranging from 1,200 to 1,600 mm and a mean temperature of 18 °C (Alvares et al. 2013). This catchment is within the Pampa biome, characterized by landscape dominated by open grasslands, along with wetlands and sparse forest fragments (Pallarés et al. 2005; Overbeck et al. 2007). According to the classification proposed by Roesch et al. (2009), based on the distribution of tree species, the study area spans four ecoregions, reflecting marked heterogeneity in vegetation structure, particularly along riparian zones. Riparian vegetation ranges from grassland-dominated reaches with sparse shrubs and isolated trees (Savanna Steppe), through discontinuous riparian forest patches “capões” (Savanna) and xerophytic shrub formations including espinilho (Steppe), to denser deciduous and semi-deciduous riparian forests in transitional areas (Transition) (Renner et al. 2019). Over recent decades, the Brazilian Pampa has undergone extensive land-use change, primarily driven by the

expansion of agriculture and livestock production. According to MapBiomas (2024), approximately 41.6% of the biome is currently occupied by agricultural areas and planted pastures, while remnants of native vegetation comprise less than half of its original extent, reflecting a loss of nearly 30% since 1985. Despite its ecological importance, legal protection remains limited, with only 3.4% of the biome under conservation units (Joly et al. 2019; da Silva et al. 2025).

Diatom sampling

In each of the 50 streams, we sampled epilithic diatoms from the surface of stones by manually scraping them using brushes and jets of distilled water. We sampled 25 cm^2 from each of five stones, totaling a composite (integrated) sampling of 125 cm^2 per stream. Samples were stored frozen until laboratory processing. Frustule cleaning followed the protocol described by Simonsen (1974), using potassium permanganate and hydrochloric acid, with modifications proposed by Moreira-Filho and Valente-Moreira (1981), which included the use of a water bath to accelerate the reaction. Permanent slides were mounted using Naphrax® (refractive index = 1.74). For community analysis, 500 valves per sample were counted under a Zeiss Primo Star® light microscope at 1,000× magnification, with a coupled digital camera. Taxonomic identification was performed to the lowest possible level, usually at species level, based on specialized literature such as *Bibliotheca Diatomologica*, *Iconographia Diatomologica*, and classical publications (Krammer et al. 1985, 1991, 1997; Metzeltin et al. 1998; Krammer, 2000; Metzeltin et al. 2005, 2007; Lange-Bertalot et al. 2011; Marquardt et al. 2017), supplemented by updated online catalogs to verify valid nomenclature (Potapova et al. 2025; Silva, 2025). Also, when necessary, we used Scanning Electron Microscopy to confirm the identifications.

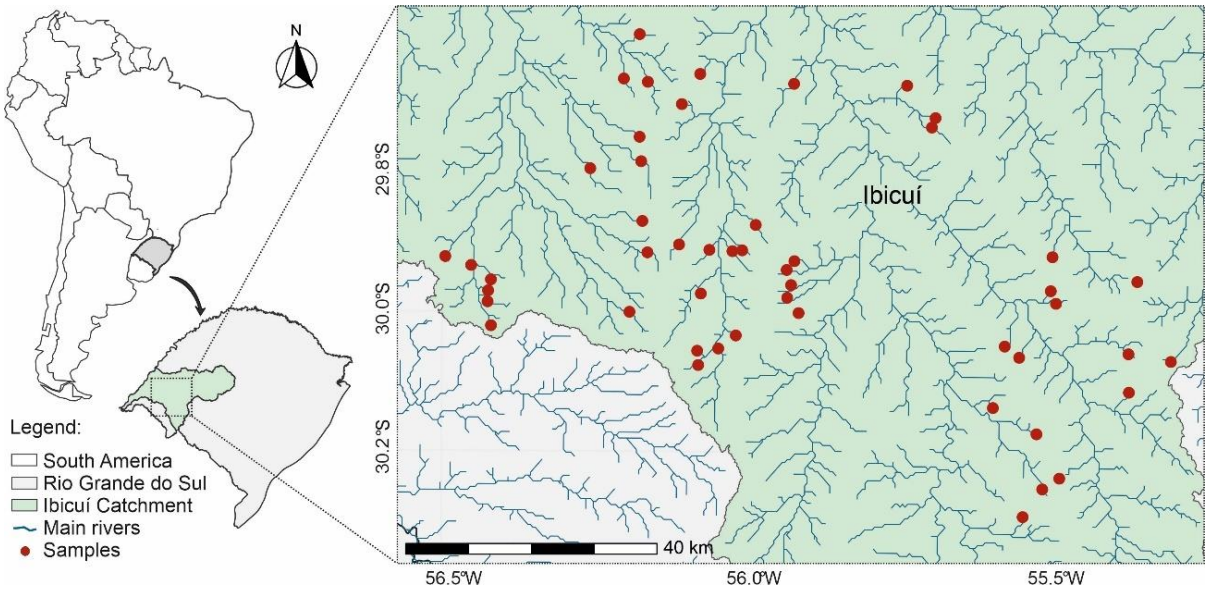

Figure 1. Geographic location of the sampling sites within the Ibicuí River catchment, southern Brazil. Red dots represent the 50 stream sampling sites (greatest distance between two streams 125.7 km; shortest distance 0.51 km). The inset maps show the location of Rio Grande do Sul within South America and the position of the Ibicuí River catchment. Main rivers are shown in blue.

Local environmental, landscape and spatial variables

A Horiba® multiparameter probe was used to measure in situ physical and chemical variables at each stream, including dissolved oxygen (DO; mg L^{-1}), oxygen saturation (Sat-O_2; %), pH, total dissolved solids (TDS; g L^{-1}), and electrical conductivity (COND; mS cm^{-1}). Water samples were also collected for the analysis of total nitrogen (TN; mg L^{-1}; Allen et al. 1974) and total phosphorus (TP; µg L^{-1}; Valderrama, 1981; Baumgarten & Rocha, 1996). The mean values of in-stream habitat characteristics were also measured to compose the environmental matrix, including stream width (m), canopy openness (%), depth (cm), and water velocity (Speed; cm s^{-1}). Canopy openness was visually estimated based on the proportion of canopy cover. All variables were recorded at five randomly selected points within each sampled stream.

At each sampling point, the substrate composition was visually estimated according to the substrate particle size, which were classified into: bedrock (>1 m), boulder (25.6 cm–1 m), cobble (6.4–25.6 cm), coarse gravel (1.6–6.4 cm), fine gravel (0.2–1.6 cm), sand (0.025–0.2 cm), silt/clay/mud (non-sandy fine sediment), consolidated clay (firm, fine substrate), and leaf packs, including small twigs (Cummis and Lauff 1969). For each class, an ordinal abundance score was assigned based on percentage cover within the sampling reach: 0 = absent (0%), 1 = sparse (<10%), 2 = moderate (10–40%), 3 = dense (40–75%), and 4 = very dense (>75%). Observations were made using ten replicate assessments per sampling point to

account for microhabitat heterogeneity. Additionally, substrate diversity was calculated using Simpson's index, considering the variety of substrate types present at each site.

The land use and land cover variables were obtained for each of the 50 streams within the catchment area from MapBiomas Collection 9 (MAPBIOMAS 2024). We used the 2023 annual mosaics covering the entire study area and its thematic classes. This database is derived from Landsat images (30 m resolution), made available free of charge by Google Earth Engine. For spatial processing, we used QGIS (version 3.28 Firenze) and ArcGIS Pro software, which made it possible to import the vector and raster layers, delimit the catchment and cut out the areas of interest. The values were obtained from pixels corresponding to the coverage classes and were then converted into hectares and expressed as percentages of the total area of each catchment. For analytical purposes, the original land use and cover classes were regrouped into broader categories, including Forest (Forest Formation), Agriculture (Silviculture, Land Use Mosaic, Soy, Rice, and Other Temporary Crops), Wetland (Flooded Grassland and Marsh Area), Grassland (Grassland Formation), Desertified Soil (Dune, and Sandy Area), Bare Soil (Other Non-Vegetated Areas and Rocky Outcrop), and Water (River and Lake). The categories were grouped according to the objectives of this study. The class 'desertified soil' refers to sampled areas undergoing desertification (Oliveira Júnior et al. 2006; Reichert et al. 2016).

Spatial variables were derived from the geographic coordinates of the sampling sites. The original point locations, recorded in the WGS84 geographic coordinate system (latitude and longitude in decimal degrees), were projected into the Universal Transverse Mercator (UTM) coordinate system, zone 21S (EPSG:32721). The UTM system provides a two-dimensional, orthogonal, metric framework (in meters), making it appropriate for the computation of Euclidean distance matrices, a key requirement for spatial modeling techniques such as Principal Coordinates of Neighbour Matrices (PCNM) analysis (Borcard et al. 2011).

Common and rare species

We classified diatom species as common or rare based on their abundance within the metacommunity using the quartile criterion, as proposed by Gaston (1994). According to this method, species were classified using the sum of their total abundances divided by quartiles: species with abundance values equal to or below the first quartile (25% less abundant species) were classified as *very rare*, those up to the second quartile (25.1% to 50% less abundant species) as *rare*, and those equal to or above the third quartile (75% most abundant species)

were classified as common species.

Data analysis

To evaluate the relative importance of local environmental, land use and cover, and spatial variables on species richness and diatom community composition, we used multiple linear regression and partial redundancy analysis (pRDA), respectively (Borcard et al. 1992; Legendre et al. 2005). The analyses were carried out with the complete set of diatom data and separately for common and rare species. Before pRDA, the three datasets were Hellinger-transformed. To meet the assumptions of multivariate analysis, the data on land use and cover were arcsine square root-transformed, as recommended for proportional variables (Legendre and Legendre 2012). The matrices of local environmental and land use and cover predictors used in the multiple linear regression and pRDA were constructed after excluding redundant variables based on the variance inflation factor (VIF < 3; Quinn and Keough 2002) (see supplementary material, Figure S1 and Figure S2 for Pearson correlations between all local environmental variables and between land use and cover variables). The final local environmental matrix included 10 variables: pH, conductivity, dissolved oxygen, water velocity, stream width, depth, canopy openness, total phosphorus, total nitrogen and substrate diversity. The final land use and cover matrix included the following classes: forest, agriculture, wetlands, desertified soil, bare soil and water. The spatial predictor matrix was constructed using Moran's Eigenvector Mapping (MEM), based on the coordinates of the sampled streams, through Principal Coordinates of Neighbour Matrices (PCNM; Borcard and Legendre 2002). The truncation threshold was established via the minimum spanning tree. PCNMs with higher eigenvalues capture broad-scale spatial structures, whereas those with lower eigenvalues reflect more localized, fine-scale variation (Griffith and Peres-Neto 2006).

Finally, we tested the global models using separately each of the three types of predictors: local environmental, land use and cover, and spatial. Only when the global model was statistically significant ($P < 0.05$), we proceeded with a forward selection of variables based on two criteria: (1) individual predictors had to be significant ($P < 0.05$), and (2) the adjusted R^2 of the final model had to be equal or lower to than that of the global model (Blanchet et al. 2008). We then partitioned the variation in species richness and community composition into independent components: [E] pure environmental effects, [LU] pure land use effects, and [S] pure spatial effects. Additional fractions included [E–LU] land-use structured local environmental effects, [E–S] spatially structured local environmental effects, [LU–S], spatially structured land-use effects, and [RES] unexplained residual variation

(Borcard et al. 1992). The significance of the pure fractions ([E], [LU], and [S]) was assessed using 999 permutations, and the proportion of explained variation was reported as adjusted R^2 (Peres-Neto et al. 2006).

All statistical analyses were performed in R (R Core Team 2025). For multicollinearity diagnostics, we used the *vif* function from the car package (Fox and Weisberg 2019). Variable selection was carried out with *forward.sel* from the adespatial package (Dray et al. 2025). For spatial eigenfunction analysis, constrained ordination, we employed the vegan package (Oksanen et al. 2025), using the *pcnm*, *rda*, and *varpart* functions. Graphical visualizations were generated using the *ggplot* function from the ggplot2 package (Wickham 2019). Spatial reprojections were performed using the sf package (Pebesma 2023). For mapping the streams, we used data from the HydroRIVERS v1.0 South America Shapefile (available at http://www.hydrosheds.org/products/hydrorivers) to extract the sub-basin area.

Results

Sampling sites were distributed along distinct environmental gradients. We recorded a total of 203 diatom species distributed across 41 genera, ranging from 11 to 66 species per stream, considering the total dataset (Figs. 2 and 3). The most abundant species were *Achnanthidium saprophilum* (H. Kobayashi & Mayama) Round & Bukhtiyarova and *Gomphonema parvulum* (Kützing) Kützing, with relative abundances of 14% and 7%, respectively, both occurring in 98% of the samples. Of the total species recorded, 52 species were classified as common (25.7%), 45 species as rare (22.2%), and 59 species as very rare (29.1%). Between 10 and 36 common species occurred per stream, whereas between two and 10 rare and between one and nine very rare species occurred per stream (Figs. 2 and 3). The analysis of species richness by group, based on abundance data, revealed contrasting patterns between common, rare and very rare species. There was clear spatial pattern in common and rare species distributions (Fig. 2).

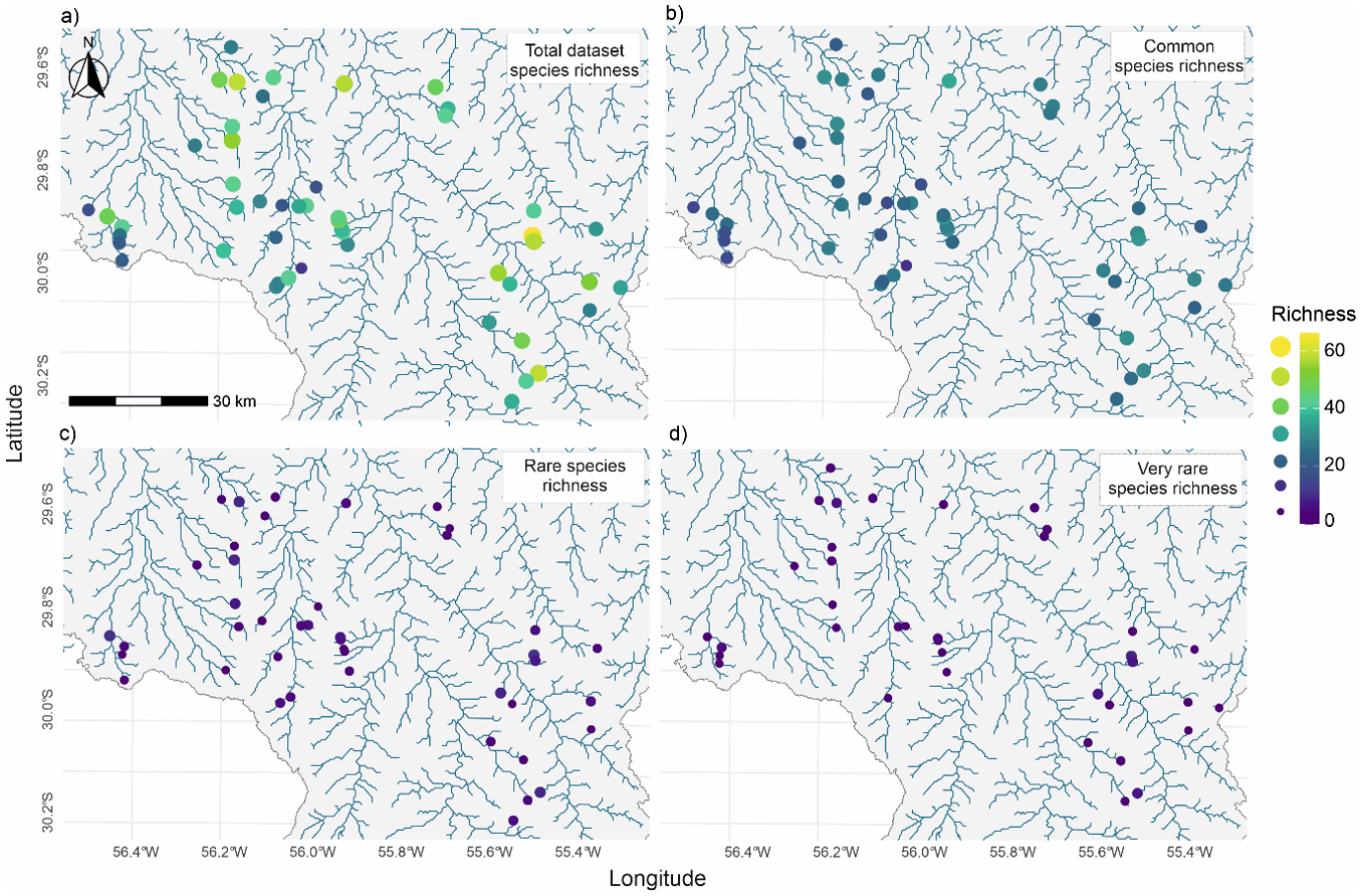

Figure 2. Spatial distribution map of diatom species richness in 50 streams in the western region of southernmost Brazil (Ibicuí river catchment): a) total species richness; b) common species richness; c) rare species richness; d) very rare species richness. The color and size gradient of the dots indicates the variation in the number of species in each dataset.

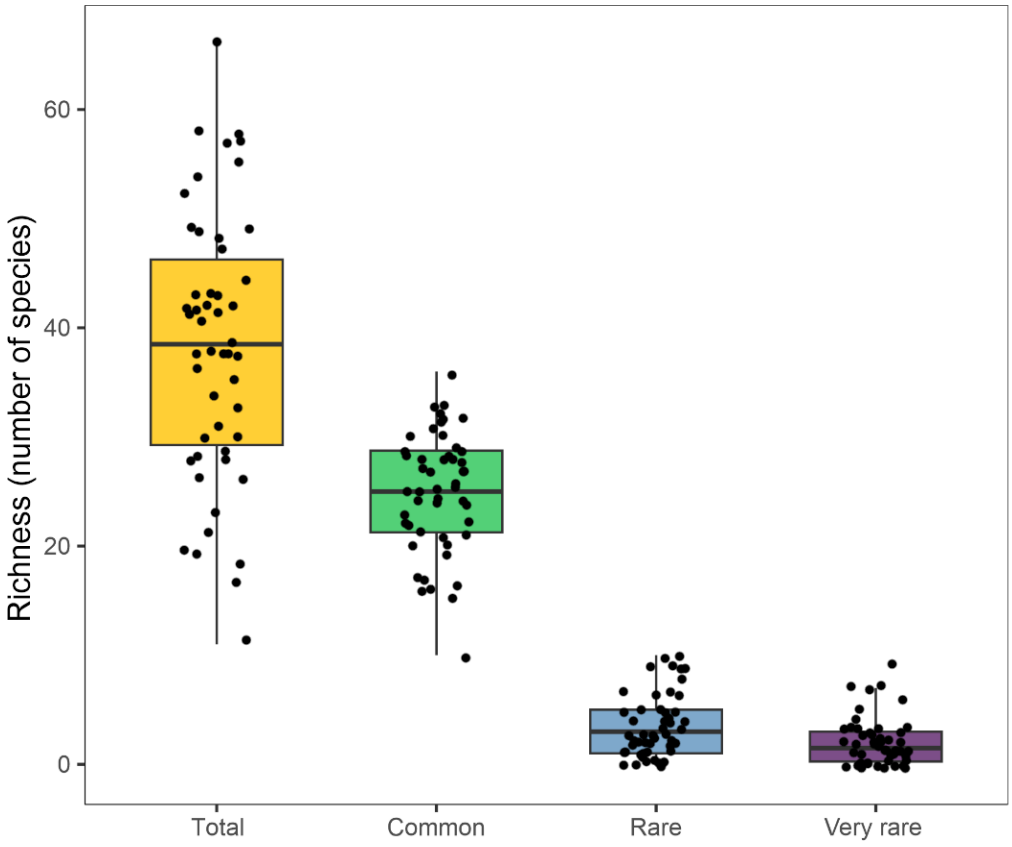

Figure 3. Stream benthic diatom species richness for the total dataset, common species, rare species, and very rare species. Each boxplot represents the median, the interquartile range (Q1-Q3). The dots indicate the values observed per sample. Richness was calculated as the number of distinct taxa per sample. Dataset were defined based on the total abundance of species, using the quartile criterion as proposed by Gaston (1994).

Variation in species richness was mainly explained by local environmental variables, while the global model on land use was significant only for the very rare species dataset and the global model on spatial variables was not significant at all (Table 1). For total richness, the selected local environmental variables were canopy opening, pH, total nitrogen, and water velocity, which explained 50.7% of the observed variation. A similar pattern was observed for common species with the local environmental fraction explaining 47.2% of variation, but with total phosphorus being selected instead of total nitrogen. For the rare species dataset, only canopy opening was selected, explaining 21.2% of the variation. Finally, for the dataset of very rare species, pure local environmental, pure land use and land-use structured local environmental effects explained together 21.2% of the variation in species richness. For this group, the selected local environmental variables were canopy opening, total nitrogen and conductivity, whereas forest was the only variable selected for the land use predictors (Table 1, Fig. 4a). The selected local environmental and land use variables affected species richness in the same directions, independently of the dataset: pH, canopy opening, water velocity and

conductivity showed a negative relationship with species richness, whereas total phosphorus, total nitrogen and forest positively affected species richness (Supplementary material, Figs. S3, S4, S5, S6).

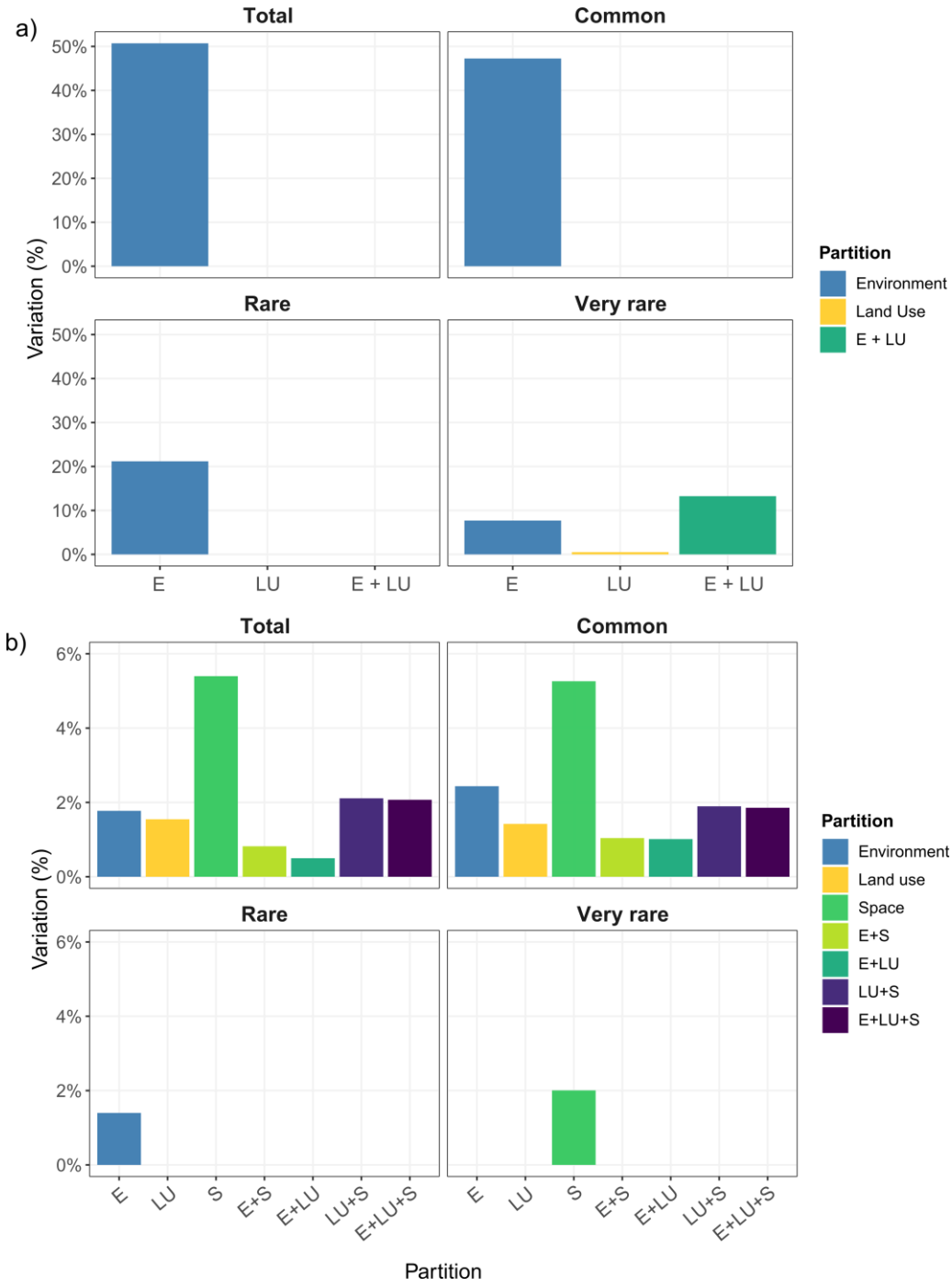

Figure 4. Variation partitioning results for (a) species richness and (b) community composition of stream benthic diatoms for the total, common, rare and very rare datasets. Percentage of explained variation attributed to local environmental, land use, and spatial predictors, as well as their shared effects, based on multiple linear regressions for species richness and partial Redundancy Analysis for community composition. Unexplained variation is not represented (See Table 1 for species richness and Table 2 for community composition). Note plots with different scales in the y-axis.

Considering community composition, distinct patterns were observed depending on the dataset. Variation in total community composition was partially explained by pure local environmental (conductivity and stream width), pure land use (forest and agriculture), and pure spatial variables (PCNM 1, 3, 6, 17 and 18), in addition to their shared fractions. Together, these variables accounted for 14.2% of the variation explained, of which more than one-third was attributable to the pure spatial fraction (Table 2, Fig. 4b). A similar pattern was observed for the community composition of common species, for which 14.8% of the variation was explained by the sum of all fractions. Once again, the pure spatial fraction was particularly important, while the pure land use fraction not significant (Table 2). For the rare and very rare species datasets, variation in community composition was explained by different sets of variables from those observed for common species. For rare species, only the pure local environmental fraction was significant, with water velocity explaining 1.4% of the variation in community composition. However, only spatial variables were important for very rare species, with PCNMs 19 and 25 explaining 1.1% of variation (Table 2, Fig. 4b). The relationship among all selected variables in the pure fractions can be seen in Supplementary Material Fig. S7.

Table. 1 Results of multiple regression analyses of local environmental, land use, and spatial models explaining benthic diatom species richness variation for the whole diatom community (all taxa) and for each dataset (common, rare and very rare species) in streams from southern Brazil.

	Local environmental variables	Land use	Spatial (PCNM)	*p*Global [E]	*p*Global [LU]	*p*Global [S]	Adj R^2 [E]	Adj R^2 [LU]	Adj R^2 [E-LU]	*p* [E]	*p* [LU]	*p* [S]
All taxa	Canopy opening, pH, TN, Speed	-	-	**<0.01**	0.12	0.16	0.507	-	-	**<0.01**	-	-
Common	Canopy opening, pH, TP, Speed	-	-	**<0.01**	0.41	0.48	0.472	-	-	**<0.01**	-	-
Rare	Canopy opening	-	-	**0.02**	0.32	0.14	0.212	-	-	**<0.01**	-	-
Very rare	Canopy opening, TN, Cond	Forest	-	**0.01**	**0.04**	0.28	0.077	0.003	0.132	**<0.01**	**<0.01**	-

Environmental, spatial and land use variables selected in the final model. Values of significance ($P < 0.05$) for the global environmental (*p*Global [E]), land use (*p*Global [LU]) and spatial (*p*Global [S]) models. Adjusted R^2 values for pure environmental (Adj R^2 [E]) and land use (Adj R^2 [LU]) fractions, and the shared fraction between environmental and land use variables (Adj R^2 [E- LU]). Values of significance ($P < 0.05$) for the environmental (*p* [E]), land use (*p* [LU]) and spatial (*p* [S]) fractions. TN: total nitrogen; Speed: water velocity.

Table. 2 Results of the partial redundancy analysis (pRDA) of local environmental, land use, and spatial models explaining variation in benthic diatom communities for the total community (all taxa) and for each dataset (common, rare and very rare species) in streams from southern Brazil.

	Local environmental variables	Land use	Spatial (PCNM)	*p*Global [E]	*p*Global [LU]	*p*Global [S]	Adj R^2 [E]	Adj R^2 [LU]	Adj R^2 [S]	Adj R^2 [E-LU]	Adj R^2 [E- S]	Adj R^2 [LU-S]	Adj R^2 [E-LU-S]	*p* [E]	*p* [LU]	*p* [S]
All taxa	Cond, Width	Forest, Agriculture	1, 3, 6, 17, 18	**<0.01**	**<0.01**	**0.02**	0.018	0.015	0.054	0.005	0.008	0.021	0.021	**0.04**	**0.05**	**<0.01**
Common	Cond, Width	Forest, Agriculture	1, 6, 17, 18	**<0.01**	**<0.01**	**0.03**	0.024	0.014	0.053	0.010	0.010	0.019	0.018	**0.02**	0.07	**<0.01**
Rare	Speed	-	-	**0.02**	0.13	0.15	0.014	-	-	-	-	-	-	**<0.01**	-	-
Very Rare	-	-	25, 19	0.09	0.25	**<0.01**	-	-	0.011	-	-	-	-	-	-	**<0.01**

Environmental, spatial and land use variables selected in the final model. Values of significance ($P < 0.05$) for the global environmental (pGlobal [E]), land use (pGlobal [LU]) and spatial (pGlobal [S]) models. Adjusted R_2 values for pure environmental (Adj R^2 [E]), land use (Adj R^2 [LU]) and spatial (Adj R^2 [S]) fractions, shared fraction between environmental and land use variables (Adj R^2 [E-LU]), between environmental and spatial variables (Adj R^2 [E-S]), between land use and spatial variables (Adj R^2 [LU-S]) and among environmental, spatial and land use variables (Adj R^2 [E-LU-S]). Values of significance ($P < 0.05$) for the environmental (*p* [E]), land use (*p* [LU]) and spatial (*p* [S]) fractions. Cond: Conductivity, Width: stream width, Speed: water velocity.

Discussion

We found that the factors driving the organization of benthic diatom communities in the studied Pampa streams vary depending on the community attribute (richness versus composition) and the degree of species rarity, showing different patterns on the relative importance of local environmental, land use and spatial filters. Specifically, the local environmental fraction explained most of species richness variation, while the spatial component was the most important in explaining the variation in community composition. Overall, our results partially corroborated the hypothesis that the benthic diatom metacommunity would be more strongly correlated with local environmental variables than with spatial variables. Furthermore, the deconstructed community approach corroborates previous studies showing that the relative importance of environmental selection and spatial processes varies between degrees of rarity (Siqueira et al. 2012; Marquardt et al. 2018; Wu et al. 2024).

Taxonomic richness

Our hypothesis that environmental variables (local and land use) would be more important than spatial variables was corroborated for taxonomic richness. This is because the local environmental variable was the most important factor in explaining the variation in taxonomic richness among the total, common, rare and very rare species groups. Land use was also an important factor for the very rare species, indicating that community structure responds strongly to the local physical and chemical conditions, and to the land use surrounding the streams to a lesser extent. This predominance may reflect not only the direct effects of local conditions, but also the cumulative influence of the physical properties of the catchment, since different land uses affect water quality and modify attributes of riparian and channel habitat (Bixby et al. 2009). The species *Achnanthidium saprophilum* and *Gomphonema parvulum* are widely tolerant to environmental variations, associated with nutrient availability and disturbance, which suggests that many of the streams sampled have mesotrophic to eutrophic conditions and some degree of anthropogenic pressure (Lobo et al. 2004; 2016). The fact that they dominate 98% of the streams is completely consistent with a system in which richness responds strongly to nutrients, shading and speed. Our findings reinforce the high sensitivity of diatoms to environmental gradients (Potapova and Charles 2002; Soininen 2007), such as nutrients, shading (Myrstener et al. 2023) and hydrodynamics (Passy 2007), as found here. Furthermore, the agreement between our results and previous

studies that point to the environment as the main driver of community structure (Potapova and Charles 2002; Siqueira et al. 2012; Algarte et al. 2014; Benito et al. 2018) suggests that, in subtropical Pampa streams, local environmental and land use gradients shape species richness with sufficient intensity to override potential spatial effects (Lennon et al. 2011). This is particularly relevant in systems influenced by large-scale human interventions, in which geomorphic changes resulting from land use affect habitat heterogeneity and result in degraded conditions with less diversity (Allan 2004), highlighting the importance of environmental management aimed at protecting local and landscape features for the conservation of benthic biodiversity.

Our results showed that canopy openness was the variable most consistently associated with diatom richness. The negative relationship observed between canopy openness and species richness may be related to the increase in luminosity in stretches with less riparian cover (and some open fields, in this study), a condition that favors the proliferation of filamentous algae (Sabater et al. 1997; Myrstener et al. 2023). Higher solar irradiation has been associated with increased richness of green algae (Chlorophyta) (Peres et al. 2017), which can intensify competitive interactions within the benthic biofilm. This excessive growth tends to reduce the availability of microhabitats and intensify competition for resources (Biggs 2000), leading to a decrease in diatom richness (Bixby et al. 2009; Blinn 2015). Furthermore, as the community increases in density, structural instability may occur, causing cells and larger portions of the biofilm to detach (Wetzel 2001). This process of 'sloughing' not only reduces biomass but also contributes to the loss of diatom species that are physically displaced from the substrate. Our findings reinforce that changes in canopy cover, common in agricultural landscapes, can trigger structural changes capable of reducing the diversity of diatom benthic communities. Related to canopy openness in the local scale, increased forest cover in the catchment increased richness of very rare species, indicating that the mainteinance of riparian forests as mosaics or near the banks of streams, is fundamental for stream biodiversity.

In streams, water velocity is another environmental attribute capable of influencing the diversity of benthic algae (Biggs and Hickey 1994; Passy 2001; 2007). In our study, water velocity emerged as a predictor that negatively affected the total richness and common species richness, suggesting that increasing velocity reduces the number of species able to persist in the biofilm. This pattern is in line with the role of hydrodynamics in favoring organisms that are more resistant to shear stress (Biggs and Hickey 1994). Thus, even in streams with broad environmental heterogeneity, the more intense water velocity acts as a

filter that limits local diversity (Sun et al. 2023). In addition, previous studies showed that water velocity regulates fundamental processes of community assembly of periphytic diatoms, modulating the balance between competition and disturbance (Passy 2007). At low velocities, competitive processes predominate and allow greater coexistence, while at high velocities disturbance intensifies and favors a few tolerant species (Passy 2007; Nemes-Kókai et al. 2023). Experimental evidence also shows that increases in speed can reduce richness and evenness and promote the replacement of periphytic diatoms by filamentous algae, reinforcing the disruptive role of intense flow (Sun et al. 2023). The agreement between our observational results and previous studies suggests that hydrodynamics acts as a robust mechanism capable of reducing diversity and limiting coexistence. Thus, the negative relationship between velocity and total and common richness indicates that higher water velocity conditions reduce the structural complexity of the biofilm and promote less diverse communities.

In addition to canopy cover and water velocity, variables such as total nitrogen, total phosphorus, pH and conductivity are also frequently pointed out as important predictors of variation in species richness of diatoms. In fact, intermediate concentrations of nutrients, such as nitrogen and phosphorus, can favor the growth and development of the community, reducing competition for resources (Biggs 2000), since the supply of resources increases biodiversity by allowing species that alternate between dominance and persistence to coexist (Passy 2007). Thus, it is clear that diatom richness is strongly related to environmental quality, decreasing as habitat conditions degrade (Zorzal-Almeida et al. 2017; 2021).

Community composition

Our hypothesis is that the species richness and composition of diatom communities would be mainly influenced by local environmental and land use and cover variables, but the relative importance of these factors would differ between common and rare species, being more important for rare than for common species. Moreover, common species, being generalist, would show a relatively large effect of spatial constraints than rare species.

Contrary to our initial hypothesis, environmental factors were not more important than spatial factors in explaining the variation in community composition for the total dataset and for common and very rare species. In fact, the pure fraction of space explained the greater variation in these datasets, being the only significant factor that explains the variation in the community composition of very rare species, indicating a strong influence of the spatial structure and the processes related to dispersion along the drainage network. The exception to

this observed pattern was the rare species group, for which the local environmental fraction was the only explaining the variation in community composition. Considering that rare species tend to have narrow niche ranges and to respond directly to environmental filtering, being structured mainly by local conditions, it is unexpected that very rare species did not follow the same pattern as rare species. In contrast, as we found, common species, which exploit a wider variety of habitats, tend to be more influenced by spatial processes related to dispersal limitation than rare species (Pandit et al. 2009; Siqueira et al. 2012; Stroud et al. 2015; Benone et al. 2022; Crisfield et al. 2024).

For the total community and for the set of common species, the selection of conductivity and stream width among the local environmental variables, together with the land use classes forest and agriculture, indicates that broad physicochemical and structural gradients, modulated by the landscape context, contribute to the organization of diatom communities (Soininen 2007; Heino et al. 2015). Conductivity, by reflecting the concentration of ions of geological or anthropogenic origin (e.g. agricultural effluents and erosion) (Carpenter and Waite 2000), acts as a direct selection mechanism on the cellular physiological performance of diatom species (Potapova and Charles 2002; Bere and Tundisi 2011). It is an important variable in determining the composition of the benthic diatom community (Bere and Tundisi 2011; Lobo et al. 2004). Stream width, in turn, integrates changes in light availability, channel morphology and habitat heterogeneity, factors recognized as determining the composition of benthic algae and competitive dynamics in the biofilm (Biggs 1996; Passy 2007; Stevenson et al. 2010). The significance of the forest and agriculture classes highlights the role of land use in regulating these local conditions, especially through riparian shading (Allan 2004), the modulation of nutrient and ion flows and the stability of the physical habitat (Stevenson et al. 2010). Together, these variables explain compositional variations associated with environmental filtering, even though their effects occur within a strongly spatially structured context.

The strong role of the spatial component in the composition of total and common species indicates that dispersal processes and the spatial organization of the catchment itself have a significant influence on these communities (Soininen 2007; Oliveira et al. 2020). This pattern suggests that processes related to hydrological connectivity and possible mass effect shape the composition of these communities, since species are able to maintain themselves in non-ideal locations because there is high dispersal between streams (Leibold et al. 2004), a mechanism which tend to influence mainly widely distributed taxa (Leboucher et al. 2020). Furthermore, the simultaneous selection of PCNMs associated with both broad spatial

gradients (e.g. PCNM1) and narrower spatial patterns (e.g. PCNM 18) reinforces that different spatial scales act together, so that dispersal limitation at large scales and mass effect at small scales may simultaneously shape stream diatoms community composition. Previous studies have also observed a strong effect of the spatial component in different freshwater ecosystems, such as floodplains (Bozelli et al. 2015), reservoirs (Marquardt et al. 2018), and rivers (Liu et al. 2013). For example, Heino et al. (2010) identified that space can be an important predictor of benthic diatom composition in boreal streams, whereas Passy (2012) highlighted the relevance of the spatial structure for microbial communities.

When we analyzed the rare and very rare species, we found different patterns. The variation in community composition of rare species were explained only by water velocity. This finding suggests that stream hydrodynamic exerts a strong physical control, acting as a key ecological filter. Previous studies indicate that water velocity and flow play important roles in the composition of diatom communities (Nemes-Kókai et al. 2023; Rodrigues-Maciel et al. 2025). For example, in stretches with strong flow, part of the biofilm may be removed, making it difficult for loosely attached species to remain, while strongly attached ones may be favored in such conditions (Biggs and Hickey 1994; Passy 2007). On the other hand, the composition of very rare species was explained exclusively by the pure spatial fraction, specificaly by fine-scale PCNMs (PCNM 19 and 25). This suggests that these species depend more on the organization of the streams in the hydrographic network than on the environmental conditions measured. These species occur in few locations because of dispersal limitation, local history or very specific conditions that have not been measured (Soininen et al. 2004). In general, this result suggests that the distribution of very rare species results from stochastic processes, such as rare colonization events and limited establishment, which is consistent with the interpretation of neutral models (Bell 2001; Arnan et al. 2015; Zarnetske et al. 2017).

Conclusions

Our results show that the distinction between sets of species defined by degrees of rarity is fundamental, as it reveals that common, rare and very rare species do not respond uniformly to environmental and spatial gradients. Taken together, our results reinforce that spatial configuration must be considered alongside environmental factors to understand how communities are organized (Heino et al. 2015). Our results provide strong evidence for the processes that structure the biodiversity of stream diatoms. First, they highlight that the key factors determining species richness and community composition may differ. Second, the key

factors explaining community composition change in terms of scale and nature, depending on species rarity, ranging from strong spatial control for common species and a weak but significant spatial signal for very rare species to highly specific environmental control for rare species. Finally, by integrating local environmental, land use and spatial predictors, and explicitly distinguishing common and rare species, this study advances the understanding of metacommunity organization in subtropical grassland streams.

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Author contribution statement: Conceptualization, developing methods: MGRM, FS. Conducting the research: MGRM, FS, APTC, CRTT, IMP. Data analysis: MGRM, IMP. Data interpretation: MGRM. Preparation figures and Tables: MGRM. Writing: MGRM, FS, APTC, RHT.

Declaration of conflict of interest: The authors declare that they have no conflict of interest.

Data availability statement: Data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting information

Figure S1. Correlation between local environmental variables. The matrix shows the Pearson correlation coefficients between physical, chemical and structural habitat variables. The symbol "*, **, ***" and latter bolder indicates significant correlations. The color scale ranges from -1 (negative correlation) to +1 (positive correlation). The strong positive correlation between TDS and Cond (0.92) and between Sat. DO and DO stand out. Abbreviations, TN: total nitrogen, Depth: stream depth, TP: total phosphorus, Width: stream width, Sat. O_2: oxygen saturation, DO: dissolved oxygen, TDS: total dissolved solids, Cond: conductivity, Div. sub = substrate diversity, Speed: water velocity.

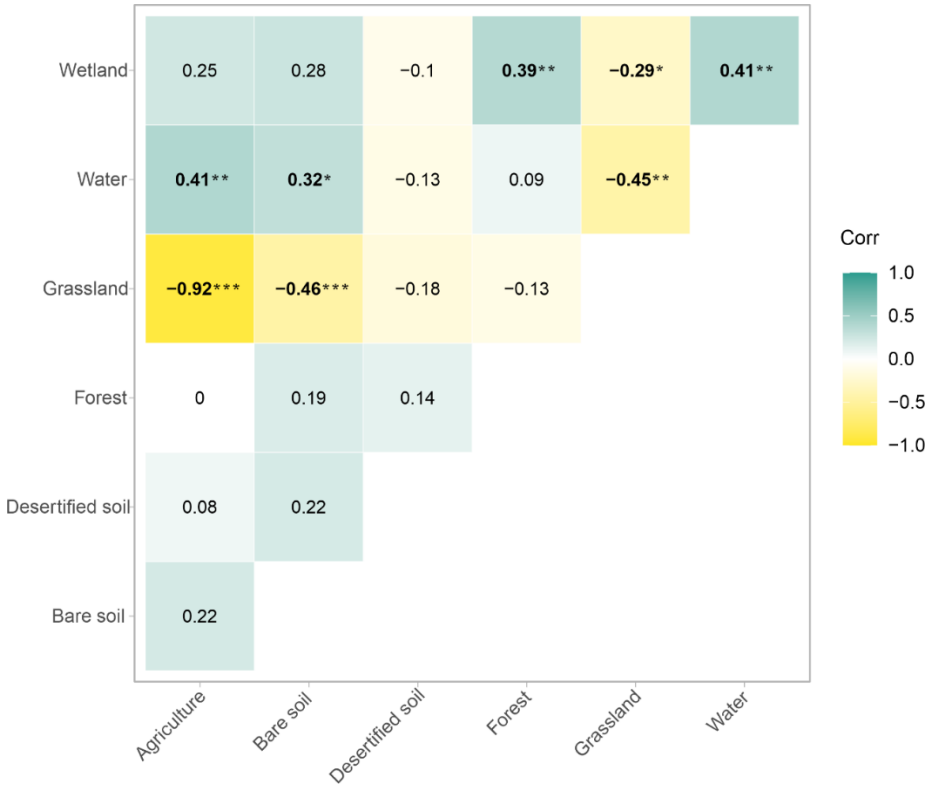

Figure S2. Correlation between land use and cover variables. The matrix shows the Pearson correlation coefficients between grassland, agriculture, water, forest, wetlands, bare soil and desertified soil. The symbol "*, **, ***" and latter bolder indicates significant correlations. The color scale ranges from -1 (negative correlation) to +1 (positive correlation). The strong negative correlation between grassland and agriculture (-0.91) stands out.

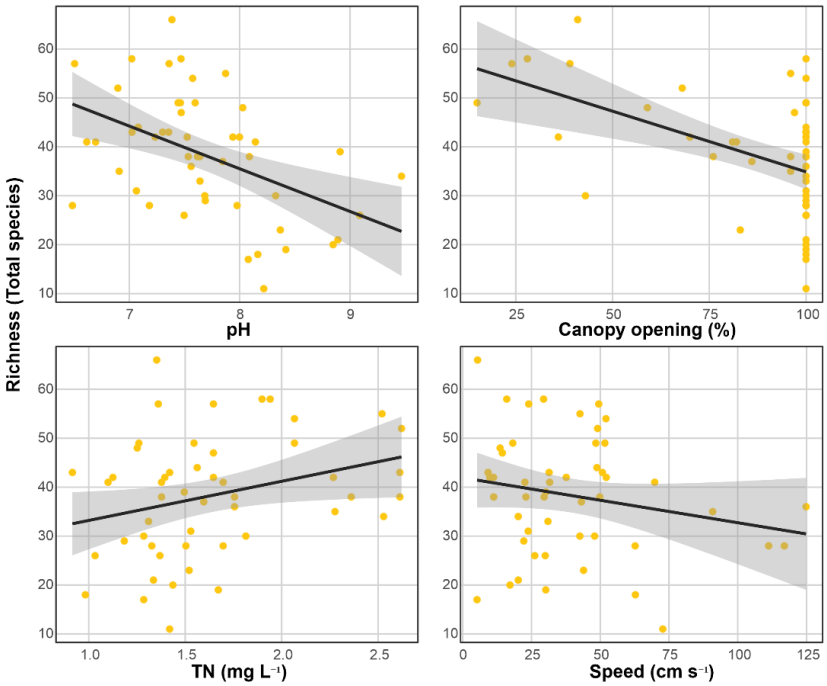

Figure S3. Relationship between total diatom species richness and the environmental variables selected by forward selection. Each point represents a sampling site; regression lines are shown for the models from Table 1. Speed: water velocity, TN: total nitrogen.

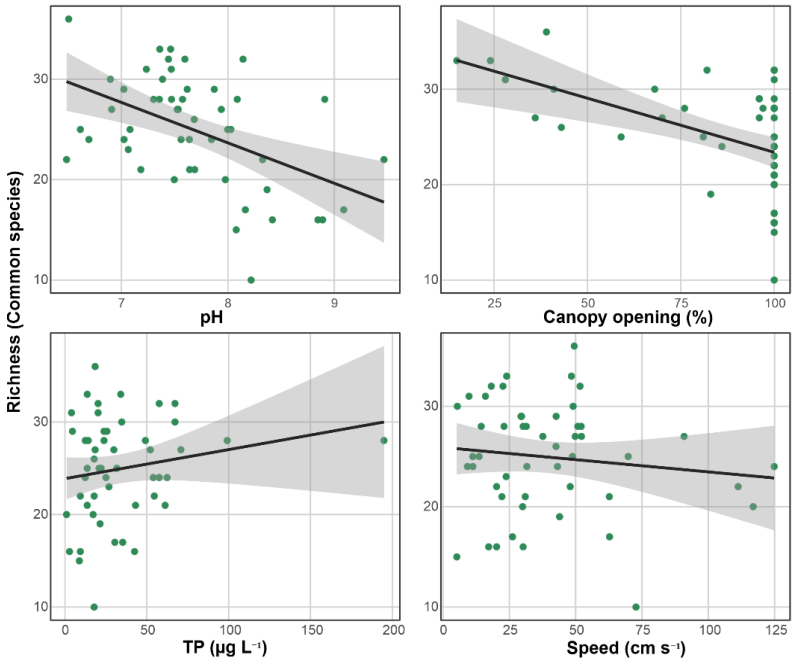

Figure S4. Relationship between richness of common species and the environmental variables selected by forward selection. Each point represents a sampling site; regression lines are shown for the models from Table 1. Speed: water velocity, TP: total phosphorus.

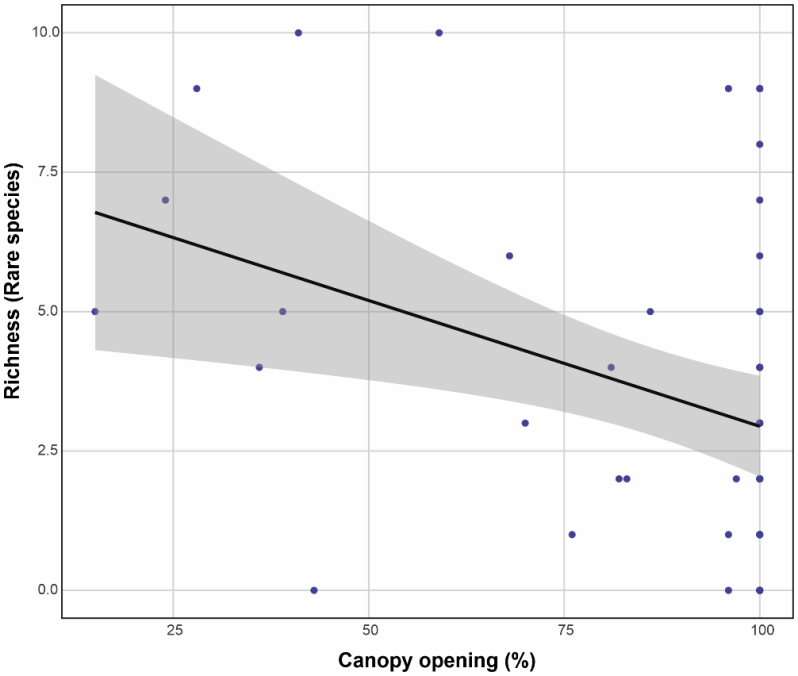

Figure S5. Relationship between richness of rare species and canopy opening, the only environmental variable selected by forward selection. Each point represents a sampling site; regression line is shown for the model from Table 1.

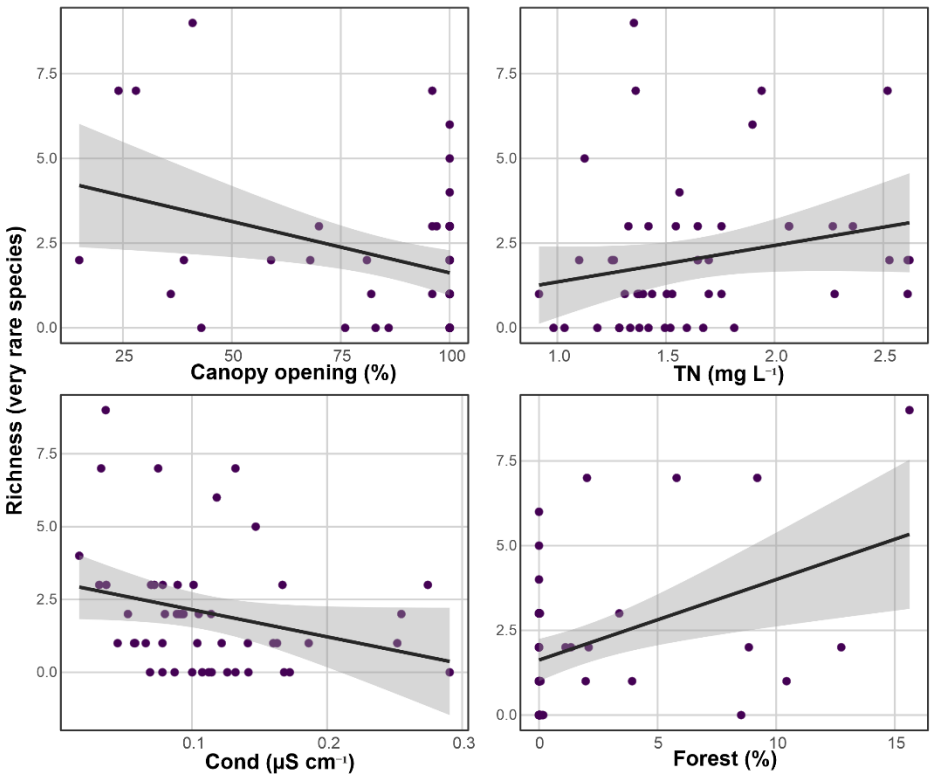

Figure S6. Relationship between richness of very rare species and the environmental variables selected by forward selection. Each point represents a sampling site; regression lines are shown for the models from Table 1. Cond: conductivity, TN: total nitrogen.

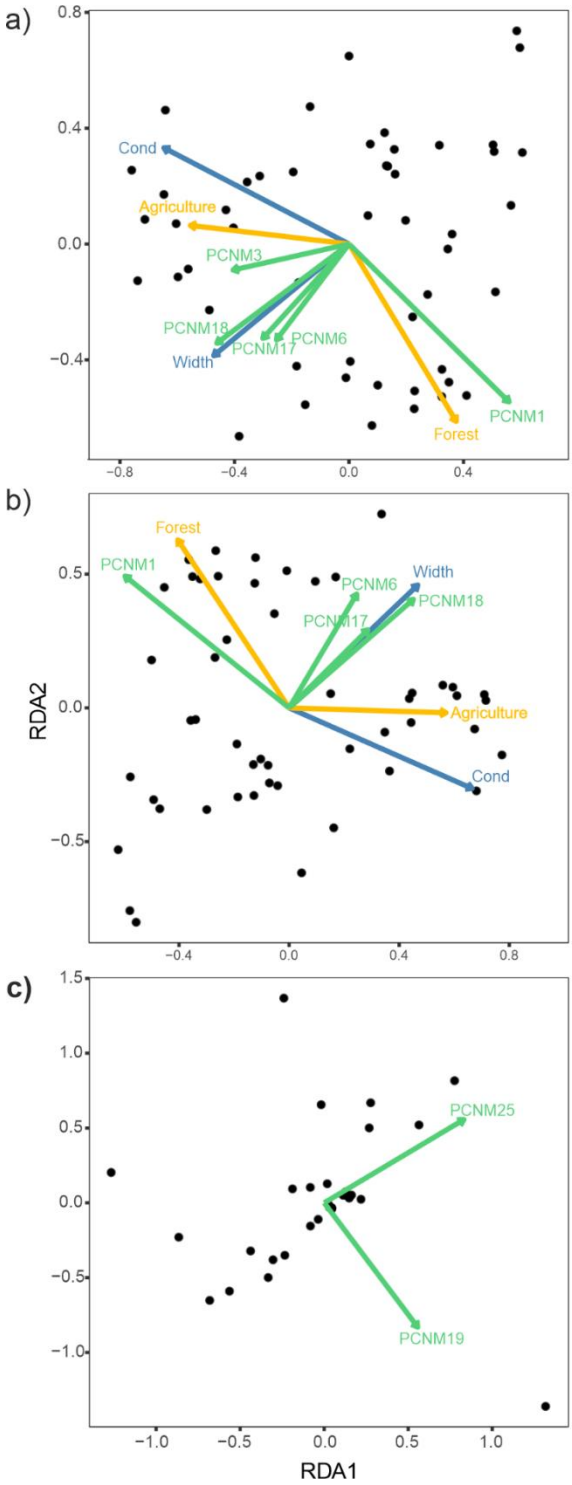

Figure S7. Redundancy Analysis (RDA) plots of the composition of stream benthic diatom communities and the influence of local environmental (green arrows), land use (orange arrows) and spatial variables (blue arrows) for: (a) the total dataset of species; (b) common species; (c) very rare species. Ordination plot of rare species is not presented because only canopy opening was selected as significant. The length and direction of the arrows indicate the magnitude and direction of the correlations between local environmental, land use and spatial gradients and community composition.

CAPÍTULO 3

A *Encyonema* species from the Brazilian Pampa biome

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ABSTRACT

This paper describes *Encyonema sp.*, collected from epilithic substrates in lotic environments of the Ibicuí river catchment (southern Brazil, Pampa biome). The material was examined with LM and SEM. Morphological analysis and PCA helped to separate from the most similar taxon: *Encyonema sprechmannii*. The new species can be differentiated from the others within the genus by a set of characteristics, which includes: by its valve outline, striae pattern, and absence intermission. The species is found in oligotrophic to mesotrophic environments, in streams with more stable flow. This finding contributes to the morphological understanding of *Encyonema* and emphasizes the taxonomic relevance of the underexplored Pampa biome.

KEYWORDS: Cymbellales, Encyonemataceae, freshwater, periphytic diatoms, South America.

INTRODUCTION

The genus *Encyonema* Kützing, traditionally placed in the order Cymbellales, has undergone important taxonomic revisions in recent years. Historically, *Encyonema* was considered part of *Cymbella* C. Agardh due to the shared dorsiventral shape of the valves, and was synonymized by Kirchner (1878). However, advances in morphological studies, especially with the use of scanning electron microscopy (SEM), have provided evidence that supported the independence of the two genera, leading to the reestablishment of *Encyonema* (Mann 1981, Krammer 1982, Round et al. 1990). This separation was later consolidated by Krammer (1997a, b), who revised the cimbeloid diatoms on a global scale and redefined Encyonema based on diagnostic morphological features, including marked valvular asymmetry and raphe with distal ends curved toward the ventral margin and proximal ends facing dorsally. These revisions have resulted in the description of numerous new species and varieties (Krammer 1997a, b, Silva & Menezes 2016, Van de Vijver et al. 2024).

More recently, molecular evidence has further refined the taxonomic positioning of the genus. Based on integrated morphological and molecular analyses, Mironov et al. (2024) erected the family Encyonemataceae, allocating *Encyonema* in a distinct monophyletic clade within Cymbellales. This family comprises genera characterized by apical asymmetry expressed in different degrees. In addition, Mironov et al. (2024) emphasized that valve asymmetry, when evaluated in conjunction with other essential features, such as raphe organization and the position of Voigt discontinuities, constitutes a robust criterion for distinguishing Encyonemataceae from Cymbellaceae. In Encyonemataceae, Voigt discontinuities occur on the ventral side of the valve, while in Cymbellaceae they are located on the dorsal valve.

Encyonema is commonly found in freshwater environments with low electrolyte conductivity (Round et al. 1990, Tremarin et al. 2011, Marquardt et al. 2016). It is recognized as a morphologically diverse genus with taxa described in different regions of the world (Blanco et al. 2019, Roy et al. 2020, Van de Vijver et al. 2024), encompassing around 442 including species as listed in the DiatomBase (Kociolek et al. 2024)Despite this diversity, their identification can be challenging due to high morphological variability (Bagmet et al. 2025), observed for example in widely distributed species such as *E. minutum* (Hilse) D.G. Mann and *E. silesiacum* (Bleisch) D.G. Mann (Vijver et al. 2024) or the scarcity of complementary data on valve

morphology and ultrastructure. In this context, complementary morphological and ultrastructural information (Bagmet et al. 2025), as well as detailed investigations in cosmopolitan species, have the potential to reveal new taxa (e.g. Marquardt et al. 2025).

One of the first records of the genus in Brazil can be found in the work of Patrick (1944), who described *Cymbella lineolatea* in southern Brazil. Considering the current taxonomic criteria that distinguish *Cymbella* from *Encyonema*, this species would probably be reclassified as *Encyonema*. Since then, several species and varieties of the genus *Encyonema* have been reported in Brazil, with significant contributions from the Amazon, Pará, Southeast and Central-West regions (e.g. Tremarin et al. 2011, Silva et al. 2013a, b, Silva & Souza, 2015, Marquardt et al. 2016, 2017, 2025, Sistema da informação sobre a Biodiversidade Brasileira 2025, Flora e Funga do Brasil 2025). In the north of the country, noteworthy records include the description of *Encyonema elginense* var. *stigmoideum* Krammer & Metzeltin, in the Tapajós River (Metzeltin & Lange-Bertalot 1998), an important tributary of the Amazon River, and *E. paratropicum* Metzeltin & Lange-Bertalot in the Arapiuns River, Pará (Metzeltin & Lange-Bertalot 2007). The Southeast hosts the largest number of described species in the country. Marquardt et al. (2017) described four new species of the *E. angustecapitatum* complex: *E. acquapurae* Wengrat, Marquardt & C.E. Wetzel, *E. sparsistriatum* Marquardt, Wengrat & C.E. Wetzel, *E. tenue* Marquardt, Wengrat & C.E. Wetzel and *E. paradisiacum* Marquardt, Wengrat & C.E. Wetzel, and recently *E. coloniense* Marquardt & C.E. Wetzel (Marquardt et al. 2025a) broadening our understanding of the morphology and distribution of the group in the of São Paulo State. Previous records from this region also included *E. angustecapitatum* Krammer and *E. ponteanum* Krammer (Marquartdt et al. 2017) and *Encyonema wojtalae* Metzeltin (Metzeltin & Lange-Bertalot 1998), although with limited geographical documentation. Additional examples of Brazilian taxa are *E. menezesiae* W.J. da Silva & M.G. M. de Souza and *E. candangense* W.J. Silva & M.G.M. Souza (Silva & Souza 2015) described in the Central-West region, as well as *E. aquasedis* Marquardt, A. Rocha & C.E. Wetzel (Marquardt et al. 2016) and *E. capixabense* Marquardt, Morais & Zorzal-Almeida described more recently in the Rio Doce, in the of Espírito Santo State (Marquardt et al. 2025b).

In contrast, southern Brazil remains comparatively underrepresented in taxonomic studies on *Encyonema*. Until recently, only two species had been described for this region: *E. exuberans* P.I. Tremarin, C.E. Wetzel & T.A. Veiga Ludwig,

recorded in lotic systems in Paraná (Tremarin et al. 2011), and the historical record cited by Patrick (1944). In Rio Grande do Sul, ten species of the genus *Encyonema* were recorded in previous Brazilian studies (Taques et al. 2024), of which six occur in the Pampa biome: *E. mesianum* (Cholnoky) D.G. Mann, *E. silesiacum* (Bleisch in Rabenh.) D.G. Mann, *E. brevicapitatum* Krammer, *E. exuberans*, *E. neomesianum* Krammer and *E. sprechmannii* Metzeltin, Lange-Bertalot and García-Rodríguez. The other species were recorded in transition zones and in the Atlantic Forest portion of the state. *Encyonema sprechmannii*, originally described for the Uruguayan Pampa, also has records in the Brazilian Pampa, as well as in transition areas and Atlantic Forest in Rio Grande do Sul (Lobo et al. 2010, Bes et al. 2012, Silva et al. 2017).

Despite the advances achieved in other regions of the country, significant gaps persist in knowledge about the diversity of *Encyonema* in the Pampa biome, located in the extreme south of Brazil (Zorzal-Almeida et al. 2022). This region, recognized for its high biodiversity and unique floristic composition (Andrade et al. 2023), remains little explored from the taxonomic perspective of diatoms, highlighting the need for systematic documentation efforts (Taques et al. 2024). In this context, the present study describes *Encyonema sp.*, collected in a lotic environment in the Pampa biome, contributing to the reduction of gaps in regional floristic knowledge.

In addition to taxonomic aspects, recent studies have highlighted the morphological variability and ecological preferences of Encyonema species (Silva & Souza 2015, Zorzal-Almeida et al. 2017), emphasizing its relevance for understanding the relationship between species distribution and environmental conditions, as well as for its application in water quality indices (Lobo et al. 2014). In this sense, the accurate description of new species is essential for the improvement of ecological assessments.

MATERIAL AND METHODS

Study area

The Pampa biome, located in southern Brazil, covers approximately 2.3% of the national territory and is confined to the state of Rio Grande do Sul, where it occupies around 63% of the state's area (MAPBIOMAS 2024). It is characterized by open grasslands with grasses, shrubs, and sparse trees, along with wetlands, forest fragments, and coastal vegetation (Pallarés et al. 2005, Overbeck et al. 2007, Robaina et al. 2020). Despite its ecological importance, legal protection remains limited, with only 3.4% of its area under conservation units (Joly et al. 2019, da Silva et al. 2025). The region hosts

intensive agroindustrial activities, where agricultural irrigation represents the main use of water resources (ANA 2015, Joly et al. 2019). The climate is subtropical, with a mean annual temperature of 18 °C and rainfall ranging from 1,200 to 1,600 mm, irregularly distributed throughout the year (Alvares et al. 2013). Within this context, the Ibicuí River catchment is located in the southwestern part of the state, between latitudes 29°01′ S and 31°20′ S and longitudes 56°47′ W and 53°29′ W. Encompassing an area of approximately 47,740 km², it forms part of the broader Uruguay River catchment. The region is predominantly composed of sandy soils and has experienced a substantial expansion of irrigated rice cultivation since the 1950s, an activity that has significantly influenced water availability and contributed to localized variations in humidity and vegetation cover (Dias et al. 2013, Aimi et al. 2020).

Diatom sampling

The material analyzed in this study was obtained during three field sampling campaigns carried out between July and August 2023, austral winter. Epilithic samples were collected at four distinct sites distributed across the municipalities of Quaraí, Alegrete, and Rosário do Sul, encompassing different micro-catchments within the Ibicuí catchment (Figure 1, Table 1). The samples were obtained by manual scraping of rock surfaces, subsequently preserved and stored under freezing conditions until laboratory analysis.

Table 1. Sampling sites in Rio Grande do Sul, Brazil, with coordinates, stream identification, HURG registration, and average ecological data of the water column obtained by a multiparameter probe (Horiba U50), except for TP and TN that were measured using standard protocols in the laboratory. Abbreviations: Altitude (m); Temp. = temperature (°C); pH; Cond. = electrical conductivity (µS cm^{-1}); TP = total phosphorus (µg L^{-1}); TN = total nitrogen (mg L^{-1}).

Nº	HURG	Geographical coordinate	River	City	Altitude (m)	Temp (ºC)	pH	Cond (µS·cm^{-1})	TP (µg·L^{-1})	TN (mg·L^{-1})
1	8123	30°04.177'S 56°04.257'W	Arroio Lagoão da Cruz	Alegrete	153.62	15.3	7.68	0.17	17.83	1.29
2	8124	30°03.803'S 56°03.988'W	Arroio Lagoão da Cruz affluent	Alegrete	149.05	15.5	7.69	0.11	61.19	1.18
3	8125	30°03.094'S 56°02.578'W	Arroio Inhanduí	Quaraí	162.15	14.8	7.94	0.19	52.16	1.39
4	8096	30°10.204'S 55°30.903'W	Sanga do Salso	Rosário do Sul	117.65	12.0	7.60	0.07	67.20	1.55
5	8101	29°46.987'S 56°10.356'W	Arroio Guaçu-Boi	Alegrete	99.67	9.2	7.57	0.10	23.86	2.07

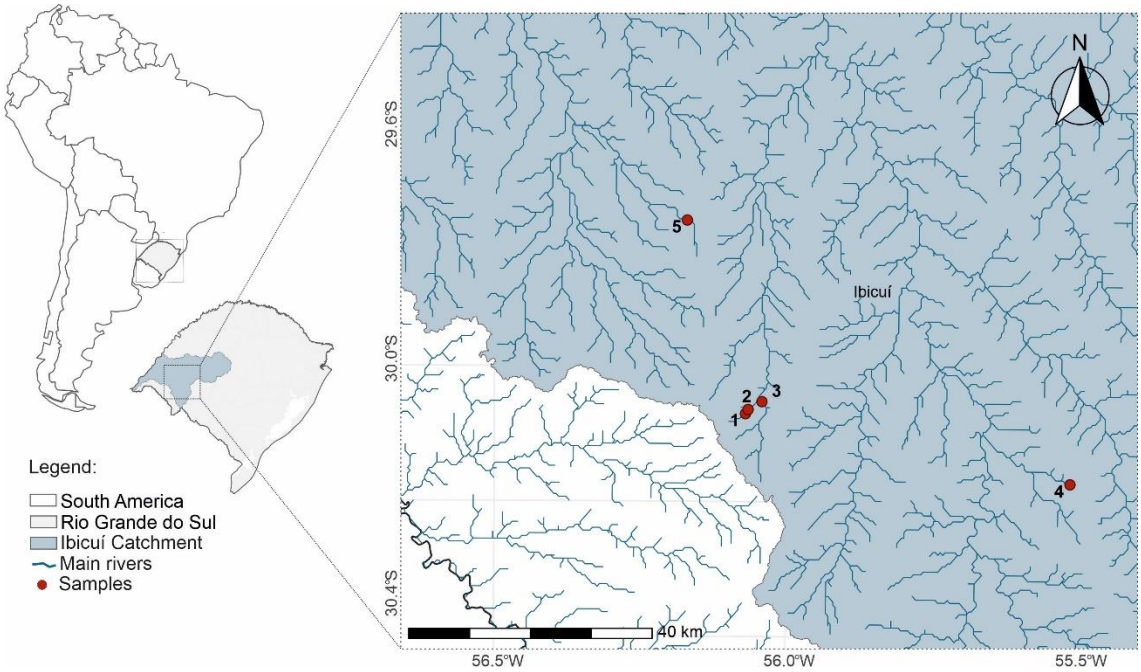

Figure 1 South America, Rio Grande do Sul, Ibicuí catchment. The red dots represent the sampling points (numbered following Table 1).

Aliquots of the samples were oxidized following the method of Battarbee et al. (2001), using concentrated hydrogen peroxide (H_2O_2) and heated in a sand bath for approximately 24 hours to remove organic material. After digestion, the residue was decanted, thoroughly rinsed with deionized water, and permanent slides were prepared using Naphrax® mounting medium (refractive index = 1.74) for qualitative analysis under light microscopy.

The diatom community was examined using a Zeiss Axio Imager A2 light microscope (LM) equipped with Differential Interference Contrast (DIC), a 100× oil immersion objective, and an AxioCam MR5 digital camera. For scanning electron microscopy (SEM), parts of the oxidised suspensions were filtered with additional deionised water through a 3 µm Isopore™ polycarbonate membrane filter (Merck Millipore), pieces of which were affixed on aluminium stubs after air–drying. Filters were coated with a 12 nm platinum layer using a Leica EM ACE600 sputter coater (Leica Microsystems GmbH, Germany). SEM analysis was performed with an ultra-high-resolution analytical field emission microscope (FE-SEM Hitachi SU-70, Hitachi High-Technologies Corporation, Tokyo, Japan) at 5 kV and a 10 mm working distance, using the lower secondary electron (SE-L) detector. Micrograph plates combining LM and SEM images were assembled using CorelDRAW Graphics Suite 2022.

Quantitative morphometric analysis was conducted using the SHERPA software (Shape Recognition, Processing and Analysis) (Kloster et al. 2014), which allows for the extraction of geometric and shape descriptors from processed diatom valve images. High-resolution micrographs of the valves were first pre-processed to enhance contrast and remove background noise. Subsequently, shape metrics such as triangularity, circularity, and elongation were computed. These descriptors were then subjected to Principal Component Analysis (PCA) to evaluate morphological differences among taxa, particularly between *Encyonema sprechmannii* Metzeltin, Lange-Bertalot & García-Rodríguez and *E. sp.*, based on the material collected in this study.

All oxidized samples and permanent slides are deposited in the Herbarium of the Universidade Federal do Rio Grande (Herbário Universidade do Rio Grande HURG), Brazil, and the Luxembourg Institute of Science and Technology (LIST), Luxembourg. Morphological terminology followed Krammer (1997a), and Vijver et al. (2024). Measurements were taken from at least 30 valves per species. Slide and material are deposited at the Herbarium of the Federal University of Rio Grande (HURG) (Table 1).

RESULTS AND DISCUSSION

Encyonema sp. Rodrigues-Maciel, M.G. & Wetzel, C.E. (Figs. 03–29)

Light microscopy (LM) (Figs. 03–23): Valves strongly dorsiventral. Dorsal margin broadly arched. Ventral margin nearly straight to slightly concave. Apices narrow, widely rounded, not protracted. Length 20.3–44.0 μm. Width 4.6–8.6 μm. Length/width ratio 2.3-9.0 μm. Mantle width in valve view 3.9–4.1 μm (Figs. 15–16). Axial area narrow, linear, and eccentric, slightly widened toward the center. Central area absent. Raphe filiform, positioned close to the ventral margin. Proximal raphe ends weakly expanded, slightly curved toward the dorsal margin. Terminal raphe fissures strongly deflected toward the ventral margin. Dorsal striae radiate, 9–10 in 10 μm, becoming slightly denser toward the apices (up to 10 in 10 μm in the apical region). Ventral striae nearly parallel to slightly radiate, 8–9 in 10 μm, up to 11 in 10 μm at the poles. Areolae indistinct. Isolated pore not visible.

Scanning Electron Microscopy (SEM) (Figs 24–29): External view: The external raphe slit is slightly sigmoid toward the apical end (Figs. 24–27). The proximal ends are drop-shaped and slightly curved dorsally (Figs. 24–27). The terminal ends of the raphe are dorsally positioned, then strongly hooked toward the ventral side (Figs. 24, 26–27)., terminating proximal on the valve mantle (Fig. 27). The axial area is narrow, linear, and eccentric, becoming slightly wider towards the dorsal margin. At the poles, the ventral side is slightly more expanded, while the dorsal side narrows (Figs. 24–26). Towards the central region, the axial area widens very subtly, with both sides showing slight enlargement (Fig. 25). The striae are uniseriate, arranged parallel to each other in the median region, becoming radiated near the extremities, and continuing in the mantle (Fig. 25). The areolae are elongated in the apical direction, generally presenting a slit-like shape; at the apices, they may look less elongated and/or like dots (Figs. 24, 26), especially near the mantle (Fig. 27); around the axial area the striae are also less elongated and may look slightly "C" shaped, or one or the other may be "Y" shaped (Figs. 24, 26). Numbering ca. 32 in 10 μm in the dorsal region, 40 in 10 μm in the ventral region, and ca. 45 in 10 μm at the apices. The cingulum is composed by 2 or 3 open non-ornamented bands (Fig. 27). Internally, areola foramina slit-like, located in long, shallow, transapical grooves (Figs 28-29). Areolae separated by short, siliceous, incomplete struts have no visible occlusions, indicating absence or corrosion of the hymens (Fig. 28). Externally, the raphe fissure looks like a linear fissure, widening slightly in the center in the shape of a drop (Figs. 24-26). Internally, terminal raphe endings terminating onto small helictoglossae (Fig. 28). The raphe fissure is uninterrupted, absence of intermission (Fig. 29). Long transapical slit at the end of the central stria, an isolated pore visible only internally (Fig. 29).

Locality: Brazil. Rio Grande do Sul, Ibicuí catchment, Quaraí, Arroio Inhanduí, epilithic substrate (periphyton). Collected by M.G. Rodrigues-Maciel, 21 August 2023. Coordinates 30°03.094'S, 56°02.578'W.

Taxonomic remarks: *Encyonema sp.*, described in this study, presents a set of morphological characteristics that clearly distinguish it from other species of the genus *Encyonema*. The valve outline is strongly dorsiventral, especially in smaller specimens, with a linear-elliptic to elliptic-lanceolate shape, separating it from morphologically similar taxa. Species similar to the new specie are *E. chebalingense* Zhang & Blanco, *E.*

stigmoideum Krammer, *E. distinctepunctatum* Krammer, *E. keshrii* Roy, Radhakrishnan, Taylor, Kulikovskiy & Karthick and *E. sprechmannii.*

Comparatively, *E. chebalingense* Zhang & Blanco exhibits more lanceolate and less dorsiventral valves, and the axial area differs because it is ventrally inclined, with 1-2 short ventral striations, in addition to the striae pattern being radiating and not parallel (Huang et al. 2021). *E. sigmoideum* differs mainly by the presence of a stigma (isolated pore) and by its more strongly convex dorsal margins (Krammer, 1997). *E. sp.* also differs from *E. distinctepunctatum* Krammer and *E. keshrii* Roy, Radhakrishnan, Taylor, Kulikovskiy & Karthick by its more pronounced dorsiventral valve margins and its narrow, linear, and eccentric axial area, which slightly widens toward the center without forming a distinct central area (Krammer 1997, Roy et al. 2020). This pattern contrasts with that of *E. venezolanum* Krammer and *E. alpiniforme* Krammer, which exhibit lanceolate and more expanded axial areas in the central region (Krammer 1997). The valve ornamentation of *E. sp.* follows a transapical striae pattern, with central dorsal striae arranged parallel or slightly radiate. This configuration differs from the radiate striae of *E. chebalingense* Zhang & Blanco (Huang et al. 2021) and the indistinctly punctate transapical striae observed in *E. venezolanum* Krammer (Krammer 1997). The raphe is continuous (Fig. 29), showing no interruptions, in contrast to species such as *E. alpiniforme* Krammer, and *E. distinctepunctatum* Krammer, in which noticeable interruptions are present in the raphe structure (Krammer 1997).

In her study on diatoms from southern Brazil, Patrick (1944) described *Cymbella lineolatea*, a taxon that, based on current taxonomic frameworks, would likely belong to the genus *Encyonema*. Although *C. lineolatea* and *Encyonema sp.* share general morphological similarities, they can be distinguished by several key features. *C. lineolatea* is smaller, measuring 16.8 µm in length and 4.6 µm in width (vs 20.3-44 and 4.6 – 8.6), with a narrow axial area and apices that appear slightly deflected dorsally. In contrast, *E. sp.* exhibits a broader size range (20.3–44 µm), a narrow axial area that widens in the central region, and rounded apices that may be slightly elongated. Despite these distinctions, *C. lineolatea* appears to be morphologically more similar to *Encyonema sprechmannii*, another small-sized species (18–34 µm in length; 6–8 µm in width).

Additional samples collected during the same expedition revealed a high abundance of *Encyonema sprechmannii* (see Figures Figs. 30–35). This species was originally described from Uruguay Pampa (Metzeltin & García-Rodríguez 2003),

however, later records indicate its occurrence also in the Pampa of Rio Grande do Sul (Silva et al. 2017) and in transition regions between the Atlantic Forest and the Pampa of Rio Grande do Sul (Lobo et al. 2010; Bes et al. 2012). This species differs from the new species in that it presents displays slightly dorsiventral symmetry, shorter valve length (18–34 µm vs 20.3–44 µm), converging striations, similar to a *Navicymbula* Krammer and mainly by the position of the raphe that is displaced almost in the central position of the valve surface, while in *E. sp.* is displaced ventrally. It also differs by the intermission "interrupted raphe" (see Fig. 33), a structure absence in *E. sp.* (see Fig. 29). This difference was evident not only through traditional morphological analysis (Table 2) but also through the use of complementary morphometric tools. The results of the Principal Component Analysis (PCA), based on the measurements obtained by analyzing the images in the SHERPA software (triangularity, compactness and roundness) showed a clear separation between the two species (see Fig. 2). *E. sp.* exhibited higher triangularity values, while *E. sprechmannii* presented greater compactness and roundness (Fig. 2). *E. sprechimannii* also differed in relation to environmental variables of the streams in which it occurred, *E. sp.* occurs in streams with higher pH variations and greater substrate heterogeneity (Table 2).

Table 2. Means and standard deviation of local environmental variables measured in the studied streams.

Stream	Occurrence of species		pH	Div. Sub. (%)
1	*E. sp.*	*E. sprechimannii*	7.68 ± 0.05	0.59 ± 0.08
2	*E. sp.*		**7.69 ± 0.03**	**0.62 ± 0.07**
3	*E. sp.*		**7.94 ± 0.03**	**0.65 ± 0.09**
4	*E. sp.*	*E. sprechimannii*	7.60 ± 0.41	0.39 ± 0.21
5	*E. sp.*	*E. sprechimannii*	7.57 ± 0.23	0.14 ± 0.19

Ecology: Specimens of *Encyonema sp.* were recorded in lotic environments of the Brazilian Pampa region, occurring at altitudes between 327 and 532 meters a.s.l. (abose sea level). The sampling sites showed a wide range of environmental conditions, with water temperatures ranging from 9.2 °C to 15.5 °C, slightly alkaline pH values (7.57 to 7.94), low electrical conductivity (0.07 to 0.19 $\mu S \cdot cm^{-1}$), and low total phosphorus (17.83 to 67.20 $\mu g \cdot L^{-1}$) reflecting oligotrophic to mesotrophic conditions and suggesting a preference for nutrient-poor waters (Table 1). The *E. sp.* was found in association with other epilithic diatoms such as *Gomphonema bourbonense* E. Reichardt, *G. brasiliensoides* Metzeltin, Lange-Bertalot & García-Rodríguez, *Planothidium*

frequentissimum (Lange-Bertalot) Lange-Bertalot, and *Nitzschia amphibia* Grunow. The co-occurrence of these species likely reflects communities adapted to stable hydrologically conditions and firm substrates, a characteristic observed in the five streams sampled in this study, but not typical of small streams in the Pampa biome, which usually have sandy streambeds.

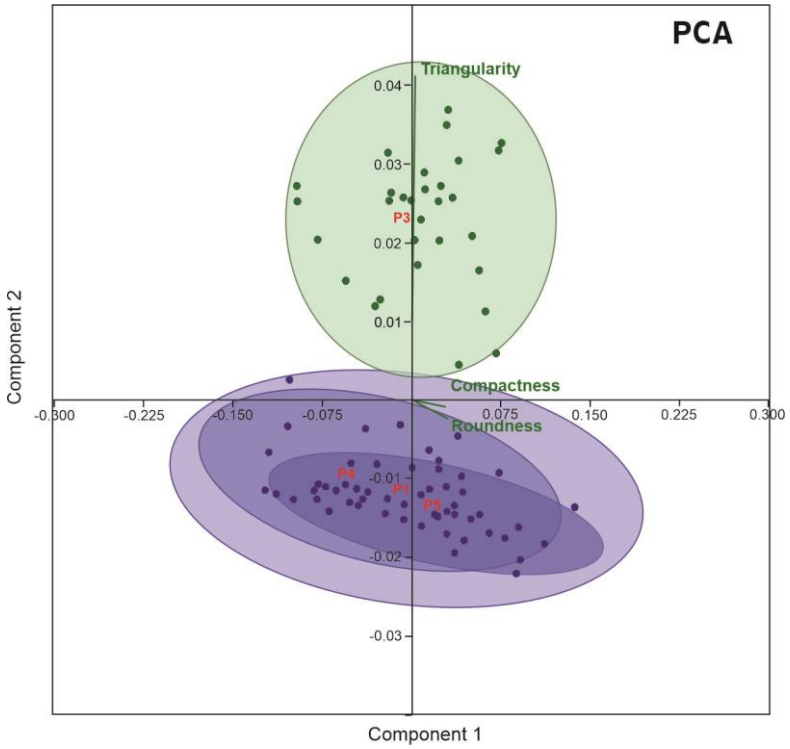

Figure 2. Principal Component Analysis (PCA) based on morphometric descriptors extracted using SHEPA software for populations of *Encyonema sp.* (green dots) and *Encyonema sprechmannii* (blue dots). The principal components were derived from three shape variables: triangularity, compactness, and roundness. The clear separation between clusters highlights significant morphological differences between the two taxa, with *E. sp.* showing higher triangularity and *E. sprechmannii* exhibiting greater compactness and roundness. Ellipses indicate 95% confidence intervals around the group means. The red numbers indicate the samples submitted for analysis.

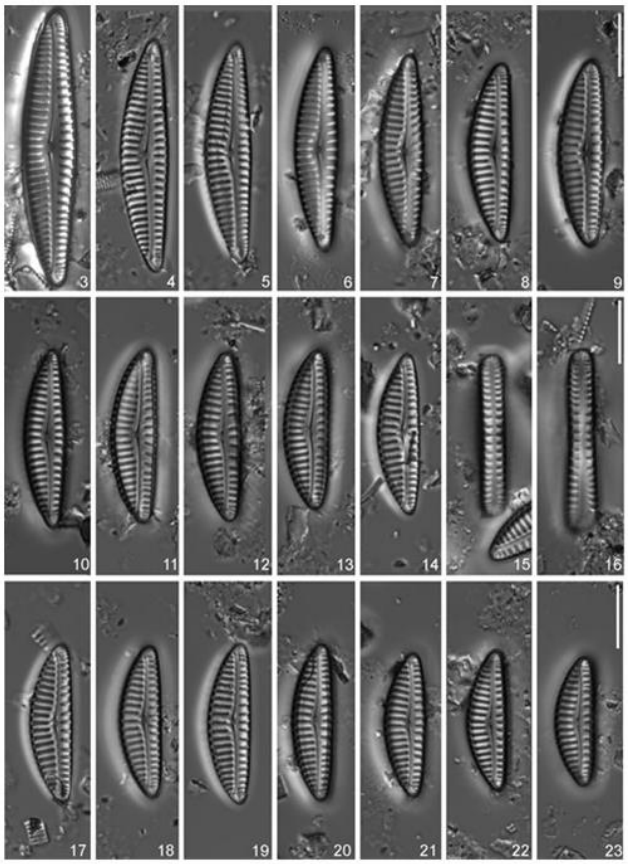

Figure 3–23 Material of *Encyonema sp.*, Light microscopy (LM), all images obtained from the population (HURG–8125). Observe the view of the mantle (15–16). Scale 10 μm.

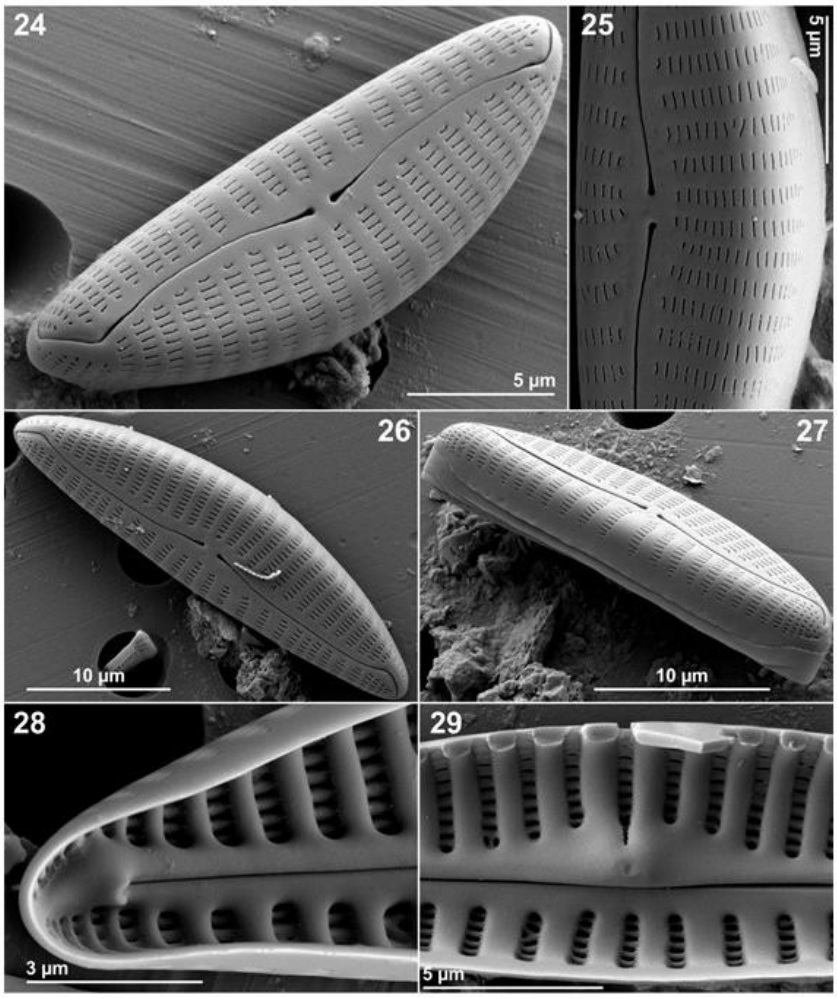

Figure 24–29. Material of *Encyonema sp.*, scanning electron micrographs. All images were taken from the population (HURG–8125). **24–27.** External SEM views of the entire valve; **24, 26–27.** Show the structure of the distal and proximal raphe, as well as the striae and areolae; **25.** Shows the external detail of the central area with higher magnification, demonstrating the subtle undulation of the raphe; **27.** Detail of the cingulum, composed by 2 or 3 open non-ornamented bands. **28–29.** Internal SEM details of the areolae and raphe; **28.** Detail of the helictoglossa, also note detail of the striae marks interrupted by prominent internal siliceous struts, absence of visible occlusions; **29.** Detail of the central area with the continuous raphe, and isolated pore.

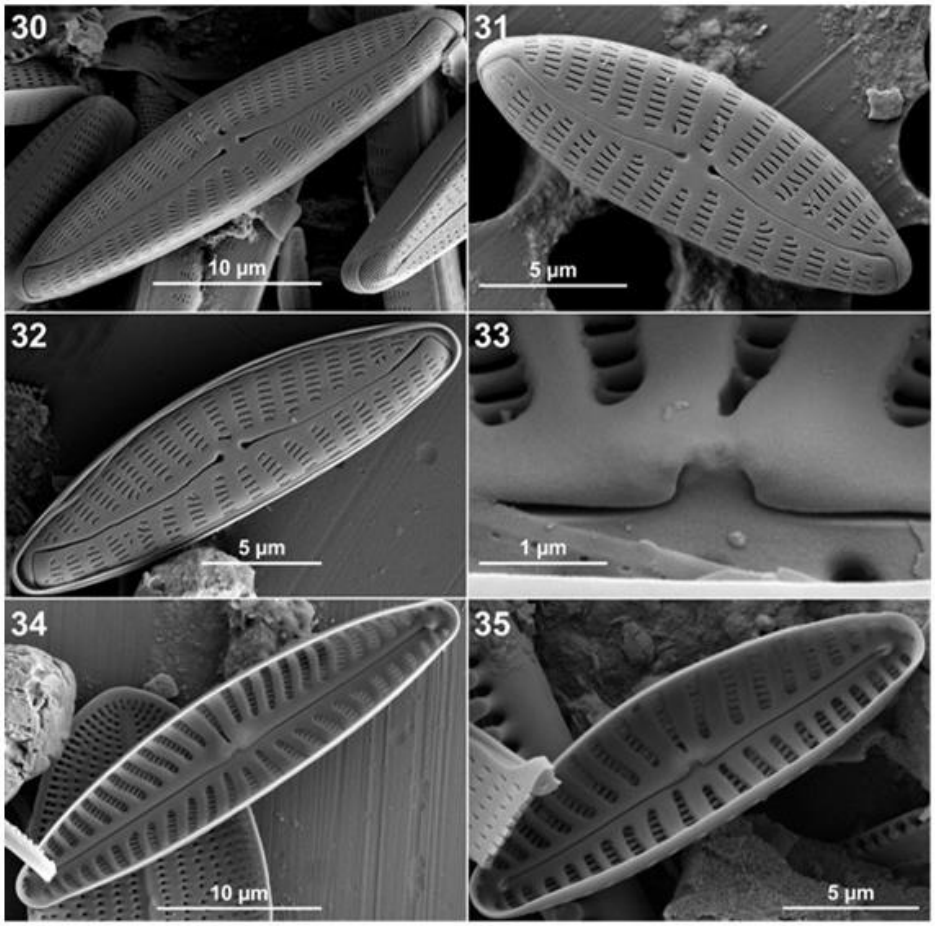

Figure 30–**35.** Material of *Encyonema sprechmannii*, scanning electron micrographs, all images obtained from the population (HURG–8123): **30–32.** External SEM views of the entire valve showing the structure of the raphe and striae. **33–35.** Internal SEM views; **33.** detail of the intermission "interrupted raphe" (the main detail that distinguishes it from the *E. sp.*), also note the rib-like striae.

Table 3 Morphological comparison of *Encyonema sp.* with morphologically similar taxa.

Species	***E.* sp.**	***Cymbella lineolatea***	***E. chebalingense***	***E. sprechmannii***	***E. keshrii***	***E. alpiniforme***	***E. stigmoideum***	***E. venezolanum***	***E. distinctepunctatum***
Literature	This study	Patrick (1944)	Huang et al. (2021) Zhang, & Blanco, S. (2021)	Metzeltin, Garcia-Rodrigues (2003)	Roy et al. (2020)	Krammer (1997)	Krammer (1997)	Krammer (1997)	Krammer (1997)
Valve length (µm)	20.3 – 44	16.8	17.8–26	18–34	15.3–30.8	14–37	18–41	16–29	16–32
Valve width (µm)	4.6 – 8.6	4.6	5.2–6.7	06–08	3.50–4.67	06–09	6.4–8.5	4.5–07	05–07
Valve shape	dorsiventral, linear-elliptic to elliptic-lanceolate	semilunate	strongly dorsiventral, semi-elliptic to semi-lanceolate	slightly dorsiventral, lanceolate	dorsiventral, lanceolate to linear-lanceolate, slightly heteropolar	dorsiventral, elliptic-lanceolate	strongly dorsiventral, semi-lanceolate	dorsiventral, slightly swollen near centre	strongly dorsiventral, semi-lanceolate, larger valves slightly swollen near centre
Ventral margin	slightly concave to straight	slightly concave							slightly concave to straight
Dorsal margin	convex to strongly arched	—	clearly arched	convex	—	convex	strongly convex	strongly convex	convex
Axial area	narrow, linear, eccentric, slightly widening towards the centre	narrow, distinct, no wider than the central node	narrow, ventrally inclined, with 1-2 short ventral striations	narrow, lanceolate, indistinct central area	narrow, lanceolate, slightly widening towards the centre	narrow, central area absent	narrow, lanceolate, widening towards the central node	very narrow, indistinct area	narrow, lanceolate, widening towards the central node
Striae (in 10 µm)	10–11 (terminal), 8–10 (central)	10–12	10–11 (terminal), 8–10 (central)	9–11	13–14	9–11	10–12	11–13	11–14
Areolae per stria	32–45 (indistinct under LM)	—	40–45 (indistinct under LM)	Indistinct under LM.	31–41 (indistinct under LM)	26–32	28–32	29	23-26
Striae pattern	transapical, distinctly linear, parallel or slightly radiating towards the apices	—	radiate	transapical, distinctly linear, parallel or slightly radiating towards the apices	—	slightly radiating towards the apices	transapical, distinctly linear, slightly radiating towards the apices	transapical indistinctly punctate, parallel or slightly radiating towards the apices	transapical distinctly linear, slightly radiating towards the radiating apices

Raphe	slightly ventrally inclined, nearly straight; proximal ends slightly curved dorsally; distal fissures conspicuously curved ventrally	—	slightly inclined towards the ventral margin, almost straight and linear, proximal ends slightly curved dorsally; distal fissures noticeably curved towards the ventral margin	filiform, proximal ends curved dorsally; distal fissures ventrally curved	weakly lateral, with proximal ends slightly curved towards the dorsal margin, rounded and pore-like; distal fissures sickle-shaped, curved towards the ventral margin	filiform, with proximal ends deflected dorsally and distal fissures ventrally curved	eccentric, filiform to slightly broadened; ventral margin slightly curved; proximal ends deflected dorsally; distal fissures ventrally curved	eccentric, filiform; ventral margin slightly curved; proximal ends deflected dorsally; distal fissures ventrally curved	eccentric raphe, ventral margin slightly broad and gently curved; proximal ends deflected dorsally; distal fissures ventrally curved
Isolate pore	not visible, in MEV, only internally	—	absent	present; not visible in MEV.	absent	absent	present; dorsal side, clearly visible	not visible	present; dorsal side
Apices	narrow, widely rounded, not protracted	—	rounded or slightly protracted, gradually narrowed,	obtusely rounded, not prolonged	rounded to acute, never	narrow, widely rounded, not protracted	—	rounded or slightly protracted, gradually narrowed,	obtusely rounded, not prolonged
Intermission	absent	—	—	present	—	present	present	—	—
Geographic distribution	Brazil (RS)	Brazil (RS)	China	Uruguay	India	South Africa	Venezuela	Venezuela	Venezuela

CONSIDERATIONS

The description of *Encyonema sp.* reinforces the importance of taxonomic research in regions that remain underexplored, such as the Pampa biome. Although southern Brazil has a relatively well documented diatom flora with 1,248 taxa recorded in Paraná, 729 in Rio Grande do Sul, and 383 in Santa Catarina (Taques et al. 2024), the specific diversity of the Pampa remains underestimated. Despite being part of a highly biodiverse ecoregion, with over 12,500 species recorded across various taxonomic groups (Andrade et al. 2023), diatom records in the Pampa are still scarce and fragmented (e.g. Junqueira et al. 2025, Barros & Torgan 2025).

In this context, the discovery of a endemic species highlights the region's untapped taxonomic potential and underscores the urgency of expanding sampling and research efforts in the lotic environments of the Pampa biome. Future studies are essential not only to document existing diversity but also to support conservation strategies and improve our understanding of the distribution and ecology of diatoms in this important Brazilian biome.

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DECLARATION OF CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Nesta tese, investiguei como múltiplos fatores estruturam comunidades de diatomáceas bentônicas, integrando duas abordagens complementares. A primeira foi um experimento conduzido em riachos de Mata Atlântica (SP), no qual avaliei os efeitos combinados de estressores associados à intensificação do uso da terra sobre a comunidade de algas bentônicas. A segunda abordagem foi observacional, envolvendo análises taxonômicas e ecológicas de comunidades de diatomáceas bentônicas em riachos de pequena ordem localizados na região sudoeste do bioma Pampa (RS). Nessa etapa, examinei como variáveis ambientais locais, variáveis espaciais e o uso da terra contribuem para a variação da estrutura da comunidade.

A proposta central da tese foi avançar o entendimento sobre os impactos do uso da terra em ecossistemas lóticos neotropicais, considerando que estressores frequentemente atuam de forma conjunta (TANIWAKI *et al.*, 2017; HE *et al.*, 2023) e que processos ambientais e espaciais apresentam papeis importantes na determinação da composição das comunidades (HEINO *et al.*, 2015). Para isso, organizei bancos de dados correspondentes aos diferentes tratamentos experimentais e ao estudo observacional, incluindo variáveis ambientais locais e preditores espaciais na escala de bacia hidrográfica. A partir de diferentes abordagens analíticas e estatísticas, investiguei a contribuição relativa desses fatores para os padrões de diversidade taxonômica das comunidades de diatomáceas em riachos neotropicais.

No primeiro capítulo, investiguei experimentalmente, por meio de mesocosmos ao ar livre, os efeitos do enriquecimento com nitrato, da adição de sedimentos finos e da redução do fluxo sobre a biomassa de algas bentônicas (medida pela clorofila-a), assim como sobre os atributos de diversidade e composição das comunidades de diatomáceas bentônicas. Dentre os principais achados estão:

(1) A adição de sedimentos reduziu a concentração de clorofila-a sob condições de nitrato ambiente, mas esse efeito foi revertido em altas concentrações de nitrato, nas quais a clorofila-a aumentou 2,25 vezes, indicando que o enriquecimento por nitrato compensou o impacto negativo do sedimento.

(2) Apenas a concentração de nitrato afetou significativamente os três atributos de diversidade avaliados. O enriquecimento por nitrato diminuiu a equitabilidade e a riqueza de espécies nas comunidades, e aumentou a dominância em comparação com concentrações ambientais.

(3) A composição da comunidade respondeu tanto aos efeitos individuais quanto às interações entre as três variáveis manipuladas. As mudanças na composição de espécies foram principalmente associadas à redução do fluxo, seguida pelo aumento das concentrações de nitrato ou pelo acréscimo de sedimento.

(4) As interações entre fluxo e nitrato, assim como entre fluxo e sedimento, apresentaram efeitos estatisticamente significativos sobre a composição da comunidade.

(5) A interação entre fluxo reduzido e adição de sedimento influenciou a composição da comunidade, resultando em menor heterogeneidade (ou seja, reduziu a diversidade beta) entre os mesocosmos.

Esses achados destacam que o enriquecimento por nitrato é o principal fator que impulsiona as alterações nas métricas de diversidade das diatomáceas, e que as interações entre os estressores são cruciais na determinação da biomassa algal bêntica e da composição da comunidade. Em termos de recomendações práticas para a gestão agrícola, o estudo indica que a mitigação dos estressores pode impactar positivamente os produtores primários nos riachos tropicais. Algumas recomendações são importantes em vista desses resultados, como: melhorar as práticas agrícolas, evitando a fertilização das culturas durante a estação chuvosa; manter a vegetação ripária e manter o fluxo natural, são ações que podem ajudar a prevenir o enriquecimento de nutrientes e reduzir os impactos da sedimentação e nesses ecossistemas. Além disso, a implementação de um sistema de monitoramento específico da região, baseado na comunidade de diatomáceas como um bioindicador, seria uma ferramenta eficaz para avaliar continuamente a eficácia das estratégias aplicadas.

No segundo capítulo, investiguei os fatores que estruturam as comunidades de diatomáceas bentônicas em 50 riachos subtropicais na Bacia do Rio Ibicuí, no Bioma Pampa, no sul do Brasil. O objetivo principal foi avaliar as contribuições relativas de variáveis ambientais locais, uso e cobertura da terra na bacia hidrográfica e fatores espaciais (dispersão), com foco nas respostas distintas de espécies comuns, raras e muito raras. O estudo revelou uma distinção no controle da metacomunidade, com os principais fatores variando dependendo do atributo da comunidade (riqueza vs. composição) e do grau de raridade, sugerindo que o filtro que determina a composição taxonômica muda em escala e natureza dependendo do grau de raridade da espécie.

(1) A riqueza de espécies, predominantemente, foi explicada por variáveis ambientais locais. Espécies totais e espécies comuns obtiveram maior parte da variação explicada pela fração ambiental pura (50,7% e 47,2%, respectivamente). Para

espécies raras a fração ambiental pura, explicou 21,2% da variação. Para espécies muito raras a riqueza foi explicada pela combinação de efeitos ambientais locais puros, uso da terra puro e seus efeitos compartilhados (21,2% no total). A variável mais consistente, selecionada por todos os grupos de raridade, incluindo a comunidade como um todo foi abertura do dossel, o que evidencia um resultado semelhante ao de Zorzal-Almeida et al. (2017b), onde as diatomáceas de reservatórios tropicais responderam principalmente ao gradiente trófico e de disponibilidade de luz, o que implica também na seleção na variável floresta para espécies muito raras, evidenciando o quão importante são essas zonas próximas aos riachos, no bioma Pampa.

(2) A composição da comunidade foi dominada pelo componente espacial. A fração espacial pura foi o componente mais importante na composição da comunidade, explicando a maior porção da variação explicada (5,4% e 5,3%) no total, para espécies totais e espécies comuns, respectivamente. Isso sugere uma forte estruturação espacial, possivelmente relacionada à dispersão, devido à natureza dos riachos amostrados (micro-bacias independentes). Espécies raras: A variação foi explicada apenas pela fração ambiental local pura (1,4%), com a velocidade da água como a única variável selecionada. Isso corrobora a hipótese de que espécies raras, sendo especialistas de nicho, são mais sensíveis a filtros ambientais locais (controle físico estrito) (WU *et al.*, 2024). Espécies muito raras: A composição foi explicada exclusivamente pela fração espacial pura (1,1%), sem contribuição significativa de fatores ambientais ou de uso da terra. Isso é consistente com a interpretação de processos estocásticos ou modelos neutros (eventos raros de colonização e estabelecimento limitado) (LEIBOLD, *et al.*, 2004).

Nossos resultados mostram que distinguir espécies por raridade é crucial, visto que táxons comuns, raros e muito raros respondem de maneira distinta a gradientes ambientais e espaciais, reforçando a ideia de que a organização das comunidades é moldada pela interação entre configuração espacial e condições ambientais (Heino et al., 2015). Nesse contexto, este estudo aprofunda a compreensão da organização de metacomunidades de diatomáceas perifíticas ao demonstrar que os mecanismos estruturantes são heterogêneos. Enquanto a riqueza foi predominantemente explicada por filtros ambientais locais, a composição apresentou um controle crescente de processos espaciais, culminando em uma estruturação essencialmente espacial para espécies muito raras. Esses resultados refinam modelos clássicos de organização de comunidades ao indicar que processos associados à dispersão e à

estocasticidade não atuam de maneira uniforme em toda a comunidade, mas tornam-se mais relevantes à medida que a raridade aumenta (ver LEIBOLD, *et al.*, 2004). Ao integrar preditores ambientais, de uso da terra e espaciais e ao separar explicitamente os grupos de espécies, nossos achados ampliam o entendimento sobre a dinâmica de metacomunidades de diatomáceas em riachos subtropicais do bioma Pampa, com implicações diretas para o monitoramento e a conservação de sistemas lóticos em paisagens dominadas por pastagens.No terceiro capítulo descrevemos uma espécie do gênero *Encyonema*, contribuindo para o avanço do conhecimento florístico e taxonômico das diatomáceas do bioma Pampa. Os principais insigths:

(1) *Encyonema sp.* é distinguida de táxons similares (como *E. sprechmannii*, *E. chebalingense* e *E. stigmoideum*) por sua forma valvar, posição da rafe, padrão de estrias e a ausência de intermissio. Entre as espécies semelhantes, *E. sprechmannii* é a mais semelhante e descrita também para o Bioma Pampa, mas na porção Argentina. O uso da Análise de Componentes Principais (PCA) com o software SHERPA mostrou uma clara separação morfométrica entre *E. sp.* e *E. sprechmannii*. *E. sp.* exibiu maior triangularidade, e *E. sprechmannii* maior compatacidade e arredondamento.

(2) A *E. sp.* foi encontrada em ambientes oligotróficos a mesotróficos, com baixa condutividade elétrica e baixos níveis de fósforo total, sugerindo preferência por águas pobres em nutrientes.

Este achado destaca a relevância de pesquisas taxonômicas em regiões consideradas subamostradas, como o bioma Pampa (ANDRADE *et al.*, 2023), apontando para um potencial taxonômico ainda pouco explorado. Ele também sugere a necessidade de ampliar esforços de amostragem e investigação em ambientes lóticos desse bioma. Nesse sentido, estudos futuros podem ser importantes não apenas para documentar a diversidade já existente, mas também para subsidiar estratégias de conservação e aprofundar a compreensão sobre a distribuição e a ecologia das diatomáceas na região.

Como um todo, minha tese demonstra que comunidades de diatomáceas são estruturadas por múltiplos estressores que atuam de forma integrada, e que a resposta da comunidade depende do atributo analisado e do grau de raridade das espécies. Ao integrar experimentação e observação em escala de bacia, mostro que riqueza, composição e raridade respondem a mecanismos distintos, combinando filtros ambientais e limitação de dispersão. Esses resultados avançam a teoria de metacomunidades, refinam estratégias de biomonitoramento e indicam que a conservação em riachos subtropicais exige ações locais e

regionais combinadas, com foco na manutenção de processos ecológicos e conectividade da paisagem.

Para finalizar, neste estudo aponto caminhos para futuras investigações que aprimorem a compreensão dos mecanismos de estruturação da metacomunidade de diatomáceas. As principais sugestões de pesquisa referem-se à incorporação de variáveis que não foram contempladas nos capítulos anteriores (1 e 2), como por exemplo, interações bióticas e fator temporal. Incluir a análise de interações bióticas, como a herbivoria, um processo chave que pode controlar a composição e distribuição das diatomáceas bentônicas (SOININEN 2007; SOININEN *et al.*, 2025), poderia tornar os resultados mais robustos. Uma vez que, estressores como a abertura do dossel (que aumenta a temperatura) podem controlar a atividade herbívora (ANDERSON *et al.*, 2001; HILLBRAND *et al.*, 2009), sendo, portanto, importante capturar essa variação na comunidade. Diversos estudos têm destacado que a dimensão temporal pode modificar a importância relativa de fatores ambientais e espaciais na estruturação de comunidades de diatomáceas (LIU *et al.*, 2013; MARQUARDT *et al.*, 2018; SOININEN *et al.*, 2025). Assim, investigar essa variação ao longo do tempo no bioma Pampa seria relevante. A inclusão de uma abordagem temporal nas coletas poderia capturar oscilações sazonais em variáveis ambientais e no regime de fluxo, aspectos que influenciam diretamente a composição da comunidade. Além disso, a força dos filtros ambientais e espaciais pode variar sazonalmente, alterando o peso relativo desses fatores na determinação da estrutura das comunidades de diatomáceas (LIU *et al.*, 2013; SOININEN *et al.*, 2025). Portanto, incorporar séries temporais permitiria uma compreensão mais completa da dinâmica ecológica nesses riachos. Reconhece-se, portanto, que modelos que integrem a estrutura da paisagem e as interações bióticas, são bem-vindos para o avanço das estratégias de manejo e conservação em um contexto de rápidas mudanças ambientais (ZARNETSKE *et al.*, 2017).

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ANEXOS

Anexo 1: Publicações extra doutoramento, entre 2022 e 2025

1. **RODRIGUES-MACIEL, M. G. R.;** CAVALCANTE, K.; LUDWIG, T. V. Unraveling centric diatoms from the Caatinga: Coscinodiscophyceae and Mediophyceae in northwestern Ceará, Brazil. **Rodriguesia**, v. 73, 2022. DOI: https://doi.org/10.1590/2175-7860202273087

2. MACIEL, D. R.; **RODRIGUES-MACIEL, M. G.;** LIMA, J. S.; SÁ, D. M. A. T. et al. Plantas medicinais alimentícias que contribuem para o aumento da imunidade: uma revisão sistemática. In: **Gestão da Qualidade e Segurança dos Alimentos**. v. 3, p. 253–267, 2023. DOI: 10.35260/54210751p.253-267.2023.

3. TUSSET, E. A.; TREMARIN, P. I.; **RODRIGUES-MACIEL**, M. G.; CAVALCANTE, K. P.; LUDWIG, T. A. V.; CARDOSO, L. S. Three new *Simonsenia* species (Bacillariophyta) from Brazil. **Fottea**, v. 24, n. 1, p. 61–72, 2024. DOI: https://doi.org/10.5507/fot.2023.009

4. SANTOS, N. P.; **RODRIGUES-MACIEL**, **M. G.;** GUIMARÃES, P. S.; TRINDADE, C. R. T.; SCHNECK, F. Negative effects of cigarette butt leachate on freshwater phytoplankton communities. **Ecotoxicology**, v. 33, p. 884–892, 2024. DOI: https://doi.org/10.1007/s10646-024-02787-3

5. PEREIRA, J. N.; FERREIRA, M. M.; AZEVEDO, V. A. N.; MACIEL, M. G. R.; **RODRIGUES-MACIEL, M. G**. et al. Desafios na atuação do biólogo em cidades do interior do Ceará: um relato de experiência. In: **V Congresso Brasileiro de Ciências Biológicas**, 2024. DOI: 10.51189/conbracib2024/35124.

6. ALMEIDA, G. S. S.; SAITO, V.S.; SARTORI, M.; SAULINO, H. H. L.; PENHA, L. O. da; MIRANDA, P. S. C. T.; MORILLA, M.; **RODRIGUES-MACIEL, M. G.;** COLLYER, G.; MORETTI, Marcelo S.; SCHNECK, F.; PIGGOTT, J. J.; PIMENTEL, I. M.; MATTHAEI, C. D.; FERRAZ, S. F. B.; TANIWAKI, R. H. Experimental effects of multiple agricultural stressors on diversity and size structure

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7. **RODRIGUES-MACIEL, M. G.;** COSTA, A.P.T.; PINZON, I.; WETZEL, C.E.; SCHNECK F. Inventário morfológico e taxonomia de diatomáceas em riachos do Bioma Pampa, RS. *Caderno de resumos*, **1º encontro Brasileiro de diatomologia** (orgs. Cavalcante, K.P.; Talgatti, D.M.; Faria, D.M.; Barroso, H.S.; Almeida, S.Z.; Silva, W.J.), 2024.

8. CAVALCANTE, K. P; TREMARIN, P. I.; **RODRIGUES MACIEL, M. G.;** TUSSET, E. A.; FARIA, D. M.; LUDWIG, T. A. V. *Gomphonema acidoclinatiforme* (Gomphonemataceae, Bacillariophyta) in Brazil: novelties on fine morphology and distribution of a rarely reported diatom. **Diatom Research**, p. 1–38, *no prelo.*

Anexo 2: Artigos da tese publicados

1. RODRIGUES-MACIEL M. G.; TANIWAKI, R.H.; SAITO, V.S.; MORETTI, M.S.; P.S.C.T.; MORILLA, M.; DA PENHA, L.O.; SARTORI, M.; ALMEIDA, G.S.S.; PIGGOTT, J.J.; SCHNECK, F. (2025). Multiple stressors in tropical streams: nitrate, sediment and flow interactions mediate benthic biomass and diatom community responses in experimental streams. *Inland Waters*, 15(1): 2475683. https://doi.org/10.1080/20442041.2025.2475683

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Multiple stressors in tropical streams: nitrate, sediment, and flow interactions mediate benthic biomass and diatom community responses in experimental streams

Maria Gabrielle Rodrigues-Maciel, Ricardo Hideo Taniwaki, Victor S. Saito, Marcelo S. Moretti, Paulo Sergio de Camargo Teles Miranda, Mariana Morilla, Lyandra Oliveira da Penha, Milena Sartori, Gabrielle Segatti Soares Almeida, Jeremy Piggott & Fabiana Schneck

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Anexo 3: Imagens dos locais de estudo.

Figura 1. Estrutura ExStream: Pulsador responsável pela distribuição das concentrações de nitrato para os tanques principais; estrutura completa mostrando os tanques principais; mesocosmos simulando pequenos riachos exibidos com mais detalhes; riacho ao qual a estrutura da bomba foi instalada para abastecer os mesocosmos. (Fotos: equipe ExStream BR).

Figura 2 Alguns procedimentos realizados durante e ao final do experimento: Medição das concentrações de nitrato; nitrato sendo dissolvido; delimitação da área no mesocosmos a ser coletada e tubo falcon para coleta; Parte das amostras fixadas com formol (4%); e parte filtrada para análise de clorofila. (Fotos: equipe ExStream BR).

Figura 3 Alguns pontos de coleta no Pampa para observação da diversidade de habitats nos quais as amostras foram coletas. Seixos e delimitação do seixo também são mostrados. (Fotos: Rodrigues-Maciel, M.G.).