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LUCAS ABBADI EBLING

Efeitos da variabilidade ambiental sobre as comunidades de hifomicetos e invertebrados aquáticos associados à decomposição foliar em riachos Subtropicais

ERECHIM, DEZEMBRO DE 2022.

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Regional Integrada do Alto Uruguai e das Missões como parte dos requisitos para obtenção do Título de Mestre em Ecologia.

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Dedico este trabalho

Aos meus pais que me amam e me proporcionam grandes oportunidades para voar

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“Um dia me disseram
Que as nuvens não eram de algodão
Sem querer eles me deram
As chaves que abrem essa prisão

Quem ocupa o trono tem culpa
Quem oculta o crime também
Quem duvida da vida tem culpa
Quem evita a dúvida também tem”

(Somos quem podemos ser – Engenheiros do Hawaii)

“She said ‘Luck is what you make it

You just reach out and take it

Now let's dance a while’

She said ‘nothing ever happens

If you don't make it happen

And if you can't laugh and smile’

But after a while

You realize time flies

And the best thing that you can do

Is take whatever comes to you

'Cause time flies’”

(Porcupine Tree – Time Flies)

Efeitos da variabilidade ambiental sobre as comunidades de hifomicetos e invertebrados aquáticos associados à decomposição foliar em riachos Subtropicais

Lucas Abbadi Ebling

Profa. Dra. Rozane Maria Restello e Prof. Dr. Luiz Ubiratan Hepp

14 de Dezembro de 2022

Resumo

O avanço da urbanização e das práticas agrícolas nas áreas de drenagem vem modificando as condições naturais dos riachos. A variabilidade ambiental gerada nos riachos, em decorrência destas práticas antrópicas, pode afetar as comunidades que habitam estes ambientes, e consequentemente, comprometer os processos ecossistêmicos. O objetivo desta dissertação foi avaliar o efeito da variabilidade ambiental sobre a estruturação de comunidades de hifomicetos e invertebrados aquáticos associados a detritos em decomposição. Estudamos 10 riachos de pequena ordem localizados ao norte do Rio Grande do Sul. Quantificamos os usos e cobertura da terra da área de drenagem desses riachos por meio de técnicas de geoprocessamento. Mensuramos as variáveis físico-quimicas da água dos riachos. Incubamos folhas senescentes da espécie *Nectandra megapotamica* em 30 *litter bags* de malha fina e 30 *litter bags* de malha grossa. Após 30 dias de experimento, retiramos os *litter bags* e identificamos os hifomicetos e invertebrados aquáticos associados aos detritos. Agrupamos os invertebrados aquáticos em Grupos Tróficos Funcionais (GTF). Identificamos 10 espécies de hifomicetos e 4068 invertebrados aquáticos, distribuídos em 41 táxons e cinco GTF. As comunidades de hifomicetos e invertebrados não responderam de maneira similar a variabilidade ambiental observada nos riachos. A comunidade de invertebrados, se mostrou mais sensível as variáveis vegetação na zona ripária e temperatura da água. A variabilidade ambiental esteve relacionada com a abundância de invertebrados, fragmentadores e coletores. Observamos que a presença da vegetação na zona ripária proporciona melhores condições de integridade ecológica aos riachos, assim, atuando na proteção das comunidades e do processo ecossistêmico da decomposição de detritos nestes ambientes. Nossos resultados podem auxiliar na tomada de decisão para ações de gestão e manejo dos recursos hídricos.

Palavras-chave: Ecologia de comunidades. Processos ecossistêmicos. Vegetação ripária. Usos e cobertura da terra. Grupos tróficos funcionais.

Effects of environmental variability on hyphomycetes and aquatic invertebrate communities associated with foliar decomposition in Subtropical streams

Lucas Abbadi Ebling

Profa. Dra. Rozane Maria Restello e Prof. Dr. Luiz Ubiratan Hepp

December 14th, 2022

Abstract

The advance of urbanization and agricultural practices in the drainage areas has been modifying the natural conditions of the streams. The environmental variability generated in streams, as a result of these anthropic practices, can affect the communities that inhabit these environments, and consequently, compromise ecosystem processes. The aim of this dissertation was to evaluate the effect of environmental variability on the structuring of communities of hyphomycetes and aquatic invertebrates associated with decomposing debris. We studied 10 small order streams located in the north of Rio Grande do Sul. We quantified the uses and land cover of the drainage area of these streams through geoprocessing techniques. We measured the physicochemical variables of the water in the streams. We incubated senescent leaves of the species Nectandra megapotamica in 30 fine mesh litter bags and 30 coarse mesh litter bags. After 30 days of the experiment, we removed the litter bags and identified the hyphomycetes and aquatic invertebrates associated with the debris. We group aquatic invertebrates into Functional Trophic Groups (GTF). We identified 10 species of hyphomycetes and 4068 aquatic invertebrates, distributed in 41 taxa and five GTF. Hyphomycete and invertebrate communities did not respond similarly to the environmental variability observed in streams. The invertebrate community was more sensitive to the variables vegetation in the riparian zone and water temperature. Environmental variability was related to the abundance of invertebrates, shredders and collectors. We observed that the presence of vegetation in the riparian zone provides better conditions for the ecological integrity of streams, thus acting to protect communities and the ecosystem process of decomposition of debris in these environments. Our results can help in decision-making for management actions and management of water resources.

Key-words: Community ecology. Ecosystem processes. Riparian vegetation. Uses and land cover. Functional feeding groups.

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1. INTRODUÇÃO GERAL

1.1 Riachos como ambientes dinâmicos e complexos

Os ecossistemas de águas continentais compreendem todas as águas interiores, fazendo parte deste tipo de ambiente os rios, riachos, arroios, lagoas e banhados, cobrindo aproximadamente 1% da superfície do planeta Terra (ALBERTONI e PALMA-SILVA, 2010; HÄDER et al., 2020). Estes ecossistemas podem ser divididos em lênticos, de águas paradas (lagos e lagoas) e lóticos, de águas correntes (riachos e rios) (CARPENTER et al., 2011). Estão classificados em: riachos de cabeceiras (1ª a 3ª ordem), rios de trecho médio (4ª a 6ª ordem) e grandes rios (7ª ordem ou superior) (SILVEIRA, 2004).

Os ecossistemas aquáticos continentais desempenham importante papel ecológico, pois abrigam as mais variadas formas de vidas, com a biodiversidade formada por peixes, insetos aquáticos, crustáceos, macrófitas e microrganismos (BRANDIMARTE et al., 2004). Estes ecossistemas contribuem para o fornecimento de diversos serviços ecossistêmicos, como abastecimento de água potável, recreação, ecoturismo, entre outros (DODDS et al., 2013; HALLOUIN et al., 2018).

Os ecossistemas aquáticos representam um dos mais variados no planeta terra (AGRA et al., 2019). Assim, Vannote et al. (1980) apresentaram o Conceito do Rio Continuo (*River Continuum Concept*) descrevendo que o rio apresenta um fluxo de matéria e energia em um gradiente longitudinal, com base na origem da matéria orgânica e na variedade de invertebrados bentônicos. Apesar de esta teoria ser aceita, muitos pesquisadores sugerem acrescentar aspectos que este modelo não abordou, para assim, ter uma maior compreensão do funcionamento dos rios e riachos (LARSEN et al., 2019). Como por exemplo, o modelo relata a heterogeneidade ambiental como a principal condutora na composição da estrutura das comunidades bióticas, mas não é abordada a questão acerca da capacidade de dispersão destes organismos (DORETTO et al., 2020). Outro aspecto que os críticos ao modelo sugerem que foi negligenciado na teoria é a questão das intervenções e impactos humanos (POOLE, 2002; LARSEN et al., 2019).

Um dos fatores bióticos mais importantes nos riachos é a presença da vegetação ripária nas margens destes ambientes. A vegetação ripária representa a conexão entre os ecossistemas terrestres e aquáticos (BREDA et al., 2020; JUNK et al., 2022). A estabilidade da margem do riacho, fornecimento de energia e regulação térmica, constituem apenas algumas das inúmeras funções ecossistêmicas desempenhadas por estas zonas de transição (PASTORE e HEPP, 2021). A vegetação ripária atua filtrando os poluentes e pesticidas agrícolas escoados, advindos das áreas de entorno dos riachos, e impede que estas substâncias cheguem ao curso d’água e venham a contaminar estes ambientes (NOVOA et al., 2018). Geralmente, os riachos de cabeceira apresentam intensa vegetação ripária a qual limita a incidência de luz solar (JUNK et al., 2022). Com isso, reduzindo a produtividade primária autóctone e tornando estes ecossistemas dependentes de fontes de matéria orgânica alóctone da vegetação terrestre, como recurso energético e alimentar (ABELHO, 2001).

1.2. As modificações na paisagem pelas atividades antrópicas geram variabilidade

Podemos compreender o termo paisagem como sendo resultante de fatores dinâmicos, dependentes de elementos físicos, biológicos e antrópicos, onde ocorrerá a integração destes fatores, tornando estes ambientes um conjunto único e indissociável em constante evolução (SILVA et al., 2017). Consequentemente, a paisagem é formada por espaços compostos por variados ecossistemas com diferentes usos e cobertura da terra (METZGER, 2001). Em uma visão ecológica, a paisagem é vista como um mosaico de variados hábitats, sujeitos a processos diferentes de perturbação (naturais ou antrópicas) (METZGER et al., 2007). É necessário e indispensável estudos que considerem o ser humano, a sociedade e o meio físico juntamente com os seus fatores químicos e físicos (NUCCI, 2007).

Atualmente, é crescente a preocupação referente com o manejo dos recursos naturais, com isso, gerou uma demanda por investigações científicas que possam suprir aos problemas referentes à disposição territorial e a gestão espacial destes recursos (FRANÇA et al., 2019). Principalmente em um país com grandes dimensões territoriais como é o caso do Brasil, o estudo da paisagem torna-se imprescindível. Dessa forma, possibilitaria a obtenção de informações relativas à estrutura e a dinâmica das alterações nestes ambientes com o objetivo de estabelecer estratégias e ações para a conservação da biodiversidade (RIBEIRO et al., 2019).

Com a produção de conhecimentos acerca da caracterização e estrutura da paisagem, será possível traçar ações de intervenção frente às mudanças impostas nestes ambientes (SILVA et al., 2017; FRANÇA et al., 2019). Ferramentas de planejamento ambientais tais como: licenciamento ambiental, definições de corredores ecológicos e unidades de conservação, dentre outras ações de intervenções, podem ser implementadas através do conhecimento acerca da estrutura das paisagens (FRANÇA et al., 2019).

Atualmente, com a intensiva pressão antrópica sobre os ecossistemas, através da substituição das paisagens naturais por usos e cobertura da terra, como a agricultura e a urbanização (CALABONI et al., 2018; GARRETT et al., 2018). Dessa forma, resultando em problemas para o meio ambiente afetando a qualidade e a disponibilidade dos recursos naturais (VALENTE e VETTORAZZI, 2002; LUSTIG et al., 2015).

Nos ecossistemas aquáticos continentais, as pressões das atividades antrópicas, acabam modificando os usos e cobertura da terra e afetam as características físicas e químicas da água e a composição da biota desses sistemas hídricos (HEPP e SANTOS, 2009; PAIVA et al., 2021). Essas pressões antrópicas afetam processos ecossistêmicos, como a decomposição de detritos foliares em riachos (ENCALADA et al., 2010). E alterações no processo de decomposição nos riachos, impulsionado por estes usos e cobertura da terra, modificam potencialmente o fluxo de energia e a ciclagem de nutrientes (FERREIRA et al., 2015; CORNEJO et al., 2020).

Estas modificações impulsionadas pelas atividades antrópicas, alteram a variabilidade ambiental. A variabilidade ambiental nos ecossistemas, pode ser caracterizada pelo pH, sazonalidade, temperatura da água, incidência de luz solar e pelos usos e cobertura da terra das áreas de drenagem e entre outras variáveis (TÓTH et al., 2019; GERHARD et al., 2022). A variabilidade é inerente a todos os ecossistemas naturais, porém, as alterações nos padrões da variabilidade existente nos ambientes, em decorrência das pressões antrópicas, ainda são incipientes (GERHARD et al., 2022). As propriedades de um ambiente resultam de processos dinâmicos, como os efeitos observados nas paisagens em decorrência da variabilidade ambiental (OLIVEIRA-JUNIOR et al., 2019). E os ecossistemas aquáticos, estão submetidos a severas pressões que alteram os seus componentes naturais, modificando a variabilidade ambiental observada, e estas pressões são impulsionadas pelas atividades humanas (BORGWARDT et al., 2019).

A dinâmica da variabilidade induzida por fatores naturais e antropogênicos pode gerar padrões na variabilidade ambiental e podem ser caracterizados por diferentes componentes nos ecossistemas (GERHARD et al., 2022). Por exemplo, no contexto dos riachos, estas modificações têm grandes implicações para a biodiversidade e para os processos ecossistêmicos que ocorrem nestes ambientes (CRABOT et al., 2020; BECQUET et al., 2022).

Mensurar e interpretar a variabilidade ambiental nos ecossistemas, é de fundamental importância para entender como as comunidades são afetadas, uma vez que estas, respondem as condições ambientais (CID et al., 2020; CRABOT et al., 2020). O conhecimento gerado acerca dos impactos da variabilidade ambiental sobre as comunidades, pode influenciar positivamente o processo de decisão em programas de conservação (OLIVEIRA et al., 2019). Para avançar nesse campo, é necessário integrar diferentes abordagens para chegar ao entendimento preciso das mudanças globais presentes e futuras (GERHARD et al., 2022).

1.3. Entrada de material alóctone nos riachos e o processo de decomposição

As zonas ripárias representam um ecótono entre os ecossistemas terrestres e aquáticos (NAIMAN et al., 2008; LIU et al., 2021). Dessa forma, as zonas ripárias representam ambientes de alta complexidade (SINGH et al., 2021). E com a presença da vegetação ripária margeando os corpos hídricos, a produtividade primária nestes ecossistemas é limitada em decorrência da baixa incidência da luz. Dessa forma o material alóctone (serapilheira) proveniente desta zona ripária, torna-se a principal fonte de matéria e energia (VANNOTE et al., 1980; MEDEIROS et al., 2015; ABELHO e DESCALS, 2019). A matéria orgânica alóctone é depositada no leito do riacho de forma vertical, lateral ou estoque bentônico (FRANÇA et al., 2009; BAMBI et al., 2022).

O processo de decomposição nos corpos hídricos ocorre através dos estágios de lixiviação, condicionamento e fragmentação, que em suma apresentam conceitos distintos, mas, contudo, podem ocorrer de forma simultânea (ABELHO, 2001). No momento em que este material orgânico entra no corpo hídrico, provoca uma série de complexos processos ecológicos (LOUREIRO et al., 2015). Esta matéria orgânica particulada grossa, folhas e detritos, necessitam ser processadas para enfim poder ser assimiladas em sua totalidade pelo sistema hídrico (HEPP e GONÇALVES-JÚNIOR, 2015). Este processo envolverá a mudança deste detrito por meio da atuação de diversos fatores bióticos e abióticos (GIMENES; CUNHA-SANTINO; BIANCHINI-JÚNIOR, 2010; YUE et al., 2018). Neste sentido, os microrganismos e invertebrados aquáticos, desempenham importante papel, que através de modificações estruturais da folha, convertem a matéria orgânica particulada grossa em matéria orgânica particulada fina (GRAÇA et al., 2015).

A primeira etapa da decomposição do material orgânico alóctone é a lixiviação, que ocorre no momento em que o detrito é imerso na água. Nesta fase a principal característica é a remoção dos compostos hidrossolúveis, em decorrência do contato deste material vegetal com a água do riacho (MORA-GÓMEZ et al., 2020). Compostos orgânicos como proteínas, aminoácidos, carboidratos, lipídeos, compostos fenólicos, e compostos inorgânicos como K, Ca, Mg e Mn são lixiviados dos detritos durante está etapa (GONÇALVES-JÚNIOR et al., 2014).

A perda de massa foliar durante o processo de lixiviação pode alcançar ou ultrapassar 30% da massa inicial das folhas (BÄRLOCHER, 2020). Este processo geralmente inicia durante as primeiras 48 horas de imersão e este tempo, pode ser prolongado conforme a composição química destes detritos (SILVA et al., 2018). Fatores como a temperatura e a correnteza da água, podem atuar na fase de lixiviação, acelerando a remoção dos compostos, resultando em um aumento do potencial de solubilidade destas substâncias (GIMENES et al., 2010; GRAÇA et al., 2016).

Em seguida decorre o processo de condicionamento ou catabolismo, etapa em que ocorre à colonização destes detritos por microrganismos (GIMENES et al., 2010; HEPP et al., 2020). Neste processo, ocorrem modificações químicas e estruturais dos detritos, provocados pelas enzimas hidrolíticas dos microrganismos, resultando no aumento da palatabilidade e na qualidade nutricional deste material orgânico, para os invertebrados (GONÇALVES-JÚNIOR et al., 2014). Sendo a comunidade microbiana a colonizadora destes detritos, formada essencialmente por fungos e bactérias (GULIS e SUBERKROPP, 2003; GONÇALVES-JÚNIOR et al., 2006; GRAÇA et al., 2015). No entanto, os hifomicetos aquáticos contribuem predominantemente, consumindo cerca de 14% da massa dos detritos (DUARTE et al., 2006). Está perda de massa pode ser de forma direta, por meio da maceração com o metabolismo, e a incorporação destes detritos para a produção secundária, ou de forma indireta, elevando a qualidade nutricional dos detritos para os invertebrados aquáticos (GRAÇA et al., 2015).

A última fase é denominada fragmentação, onde este processo pode ocorrer de forma física em decorrência do desgaste destes detritos pela água, ou biologicamente, através do consumo deste material por invertebrados aquáticos pertencentes ao grupo trófico funcional fragmentador (GONÇALVES-JÚNIOR et al., 2014; HEPP et al., 2020).

1.3.1. Hifomicetos aquáticos

Os hifomicetos aquáticos também conhecidos por fungos ingoldianos ou fungos anfíbios, são fungos anamórficos e formam um grupo filogeneticamente heterogêneo (PASCOAL et al., 2005; BARROS e SEENA, 2022). Estes fungos que comumente habitam detritos vegetais em decomposição, realizam a sua esporulação submersa na água (BÄRLOCHER, 1992; WONG et al., 1998). Estes organismos são identificados com base na morfologia dos seus conídios, que basicamente podem apresentar-se como ramificado, sigmóide, tetrarradiado ou multirradiado (FIUZA et al., 2017).

Riachos apresentando vegetação ripária, com águas limpas e bem oxigenadas representam o habitat propicio para o desenvolvimento desses organismos (KRAUSS et al., 2011; GONÇALVES et al., 2014). Mudanças nos usos e cobertura da terra, eutrofização, poluição e as mudanças climáticas, representam os principais fatores que podem afetar a diversidade e as funções ecológicas dos hifomicetos aquáticos (CANHOTO et al., 2015; BARROS e SEENA, 2022).

Com distribuição cosmopolita, os hifomicetos aquáticos compreendem aproximadamente 300 espécies (SHEARER et al., 2007; BASCHIEN et al., 2013). No Brasil, a Amazônia conta com 19 registros, a Mata Atlântica com 53, a Caatinga com 39 e o Cerrado com 21 espécies (FIUZA et al., 2017). Porém, com o avanço de estudos envolvendo taxonomia, filogenia e diversidade funcional dos hifomicetos aquáticos, o numero de espécies tende a aumentar em escala global (DUARTE et al., 2016).

Os hifomicetos aquáticos são extremamente importantes para o processo de decomposição foliar (GRAÇA et al., 2016). Estes fungos colonizam os detritos logo após o processo de lixiviação e atuam modificando quimicamente e estruturalmente as folhas através de suas enzimas (GULIS e SUBERKROPP, 2003; GRAÇA et al., 2015). Os invertebrados aquáticos fragmentadores preferem folhas que foram inicialmente colonizados por hifomicetos, por conta da dureza reduzida desses detritos (BIASI et al., 2019). A composição da assembleia de hifomicetos aquáticos nos detritos em decomposição durante condições abióticas e bióticas estressantes, ainda é pouco compreendido (PASCOAL et al., 2021).

1.3.2. Invertebrados aquáticos

Os invertebrados aquáticos desempenham importante papel nos ecossistemas lóticos, pois participam da estrutura trófica, servindo de recurso alimentar para peixes e anfíbios, e também atuam na quebra da matéria orgânica durante o processo da decomposição (CARVALHO e UIEDA, 2004; ALBERTONI e PALMA-SILVA, 2010, RAMÍREZ e GUTIÉRREZ-FONSECA, 2014). São representados por diversos táxons, tais como Annelida, Mollusca e Arthropoda, no entanto, a classe Insecta representa o táxon com maior abundância e riqueza de organismos (RESTELLO e HEPP, 2020).

O estudo destes organismos permite uma melhor compreensão e entendimento acerca da estrutura trófica dos ecossistemas aquáticos, bem como, oferecendo informações a respeito da conservação nestes sistemas (GOULART e CALLISTO, 2003; FERREIRA et al., 2011; OLIVEIRA-JÚNIOR et al., 2013). Neste sentido, são apontados como um complemento fundamental nas análises da água, auxiliando na avaliação ecológica destes ecossistemas (SANTOS e RODRIGUES, 2015).

Os invertebrados aquáticos podem ser classificados em Grupos Tróficos Funcionais (GTF) (TOMANOVA et al., 2006). Este agrupamento é referente as adaptações morfológico-comportamental destes organismos para a aquisição de alimentos (CUMMINS et al., 2005; FIGUEROA et al., 2019). Os coletores se alimentam da matéria orgânica particulada fina (MOPF), podendo ser agrupados em coletores catadores, com aparelhos bucais adaptados para coletar partículas finas, e coletores filtradores, com adaptações especificas para coletar partículas diretamente da coluna da água (RAMÍREZ e GUTIÉRREZ-FONSECA, 2014) Os raspadores que possuem peças bucais adaptadas, removem perifíton e outras partículas ligadas a rochas e outros substratos (RAMÍREZ e GUTIÉRREZ-FONSECA, 2014; ALBERTONI; CARNEIRO; PALMA-SILVA, 2020). Os predadores que capturam e consomem outros invertebrados (CUMMINS et al., 2005). Os fragmentadores possuem aparelho bucal adaptado para macerar e retalhar as partículas grandes dos detritos em decomposição (GRAÇA. 2001; GIMENES et al., 2010). Os fragmentadores são representados, no geral, por insetos pertencentes às Ordens Plecoptera (Família Gripopterygidae), Diptera (Família Tipulidae), Trichoptera (Família Calamoceratidae) e por crustáceos representados por Amphipoda e Isopoda (GIMENES et al., 2010; LOUREIRO et al., 2015; HEPP et al., 2020). Nesse sentido, os fragmentadores desempenham importante papel no processo de decomposição, pois estes organismos participam ativamente da fragmentação dos detritos foliares, transformando a matéria orgânica particulada grossa (MOPG) em matéria orgânica particulada fina (MOPF) (GRAÇA, 2001; BALIBREA et al., 2020).

Os invertebrados aquáticos são comumente utilizados em estudos de decomposição (CLASSEN-RODRÍGUEZ et al., 2019; TONELLO et al., 2021; FONTANA et al., 2022). Muitos destes estudos comparam as taxas de decomposição dos detritos vegetais com a abundância dos invertebrados aquáticos (COMPSON et al., 2013; IÑIGUEZ-ARMIJOS et al., 2016; REZENDE et al., 2018;). Outras abordagens relacionam a redução dos invertebrados aquáticos e da atividade dos fragmentadores, conforme a remoção da vegetação e a implantação de práticas agrícolas nas zonas ripárias (IÑIGUEZ-ARMIJOS et al., 2016; MLAMBO et al., 2019; FONTANA et al., 2020; TONELLO et al., 2021).

2. OBJETIVOS E ESTRUTURA DA DISSERTAÇÃO

As áreas de drenagem de pequenos riachos possuem uma complexidade de usos e cobertura da terra, que podem promover alterações ambientais que afetam direta e indiretamente os padrões e processos ecossistêmicos, entre eles a decomposição dos detritos foliares. Frequentemente as comunidades de hifomicetos e invertebrados são utilizadas em estudos avaliando a influência destes organismos, na decomposição de detritos. Embora saibamos que o ambiente pode influenciar estas comunidades na ação da decomposição, neste estudo utilizamos o detrito apenas como um substrato para a comunidade com um todo, e num segundo plano avaliamos o processo de decomposição. Portanto, esta dissertação teve como objetivo geral avaliar o efeito da variabilidade ambiental sobre a estruturação de comunidades de hifomicetos e invertebrados aquáticos associados a detritos em decomposição.

Esta dissertação é constituída por uma Introdução Geral, contendo informações teóricas e conceituais sobre o tema do trabalho, um Capitulo na forma de manuscrito e uma

Conclusão Geral abrangendo perspectivas de desdobramento do trabalho. O manuscrito intitulado **Does environmental variability in Atlantic Forest streams affect hyphomycetes and invertebrate assemblages associated with leaf litter?** Este manuscrito teve como objetivo compreender como a variabilidade ambiental de riachos afeta a estruturação de comunidades de hifomicetos e invertebrados aquáticos associados a detritos em decomposição. Neste trabalho foi observado que as comunidades não responderam de maneira similar a variabilidade ambiental nos riachos. A comunidade de invertebrados se mostrou mais sensível a variabilidade ambiental do que a comunidade de hifomicetos. Este manuscrito foi preparado e submetido ao periódico Hydrobiologia (Qualis-Biodiversidade/CAPES A1) (Anexo 1), e é apresentado de acordo com as normas do periódico.

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3. CAPITULO 1: DOES ENVIRONMENTAL VARIABILITY IN ATLANTIC FOREST STREAMS AFFECT HYPHOMYCETES AND INVERTEBRATE ASSEMBLAGES ASSOCIATED WITH LEAF LITTER?

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Abstract

We aimed at understanding how the environmental variability produced by limnological variables and by land use and land cover in small streams affect the aquatic hyphomycetes and invertebrate assemblages associated with leaf litter. We quantified land uses and land cover in the draining area of streams located in the subtropical portion of Atlantic Forest, southern Brazil. We incubated *Nectandra megapotamica* leaves in the streams and after 30 days, we collected the decomposing litter to analyze the hyphomycetes and invertebrates associated. We identified 10 species of hyphomycetes associated in the detritus. None of environmental variables was important in the structuring of these organisms. On the other hand, 4068 invertebrates were found, classified in 5 functional feeding groups (FFG). Riparian vegetation and water temperature were the variables responsible for the structuring of invertebrate assemblages associated at detritus. Our study demonstrated that the structure of aquatic assemblages were affected in streams where the landscape presented a complex composition of classes of land uses and land cover and an area of reduced riparian vegetation. Although the hyphomycetes were not affected, we observed that the presence of riparian vegetation in the streams contributed to the environmental integrity of these hydric systems.

Keywords: Ecosystemic processes. Land uses and land cover. Aquatic invertebrates. Functional feeding Groups.

3.1 Introduction

Continental aquatic ecosystems cover only 1% of the surface of the Earth (Häder et al., 2020). Although they are extremely important, these ecosystems are among the most threatened on the planet (Carpenter et al., 2011; Thomsen et al., 2012). Over time, aquatic ecosystems have been impacted by changes in land use and land cover, that have been driven by populational growth (Nagy et al., 2012; Gál et al., 2019). Anthropic activities result in changes in hydrology and, consequently, in the physical and chemical composition of waters in these ecosystems (Giri & Qiu, 2016; Gebremicael et al., 2019). Due to these changes, the main energy source in small streams is affected (Abelho, 2001; Gonçalves-Júnior et al., 2014). This energy source is composed of organic matter which is deposited in the bed of these aquatic environments (Abelho, 2001; Abelho & Descals, 2019).

Once allochthonous organic matter input in the streams, a series of complex ecological processes begin (Loureiro et al., 2015). The process of decomposition of this litter occurs basically in three stages: leaching, conditioning, and fragmentation (Abelho, 2001; Graça et al., 2015). However, the environmental variability produced by land use and land cover and by limnological variables (i.e., water temperature, pH, dissolved oxygen, etc.) may affect the process of leaf decomposition and the assemblages that inhabit the litter (Nessimian et al., 2008; Tonello et al., 2021). Examples of this are the reduction of biodiversity and composition of invertebrate assemblages associated with leaf litter and hyphomycetes structures, which in its turn reduces decomposition indexes (Mlambo et al., 2019; Cornejo et al., 2020; Tonello et al., 2021).

Aquatic hyphomycetes, also known as Ingoldian fungi, fresh water hyphomycetes or amphibian fungi, comprise a heterogeneous group of anamorphic micro fungi that inhabit fresh waters (Pascoal et al., 2005; Barros & Seena, 2022). Hyphomycetes are commonly found in decomposing vegetal litter and they perform sporulation while submerged in water (Schoenlein-Crusius & Grandi, 2003). Aquatic hyphomycetes, along with bacteria, play a part in the conditioning stage during the decomposition process (Graça et al., 2015; Biasi et al., 2019). The presence of hyphomycetes in litter decomposition is important to ensure this ecosystemic process (Seena et al., 2022), since aquatic hyphomycetes are responsible for carrying out chemical and structural changes in litter due to the action of hydrolytic enzymes, hence increasing the palatability and nutritional litter quality (Seena et al., 2022). However, factors such as land use and land cover, eutrophication and climate change may compromise the diversity and the ecological functions performed by aquatic hyphomycetes (Bärlocher, 2016; Breda et al., 2021).

Besides hyphomycetes, aquatic invertebrates also use decomposing leaf litter as shelter or food (Rezende et al., 2015). The invertebrate assemblage is composed of different taxons, among which are Annelida, Gastropoda and Crustacea. However, the most representative group in number of organisms in decomposing leaf litter is the Class Insecta (Lu et al., 2019; Sun et al., 2020). According to the morphological-behavioral adaptations for obtaining food, aquatic invertebrates are classified as Functional Feeding Groups (FFG) (Cummins et al., 2005; Figueroa et al., 2019). Acting directly in the fragmentation stage during the leaf decomposition process, the FFG of shredders is responsible for consuming litter, thus accelerating leaf fragmentation (Graça, 2001; Graça et al., 2015). Shredders are sensitive to changes that occur in streams and the distribution of these organisms is negatively affected by the removal of vegetation in the riparian zone (Lima et al., 2022).

Agricultural activity modifies the natural landscape, especially in the Atlantic Forest, considered one of the most important hotspots in the world (Santana et al., 2020). In the south of Brazil, agricultural activity is predominantly found in the landscape, contributing to vegetation fragmentation, and affecting stream integrity (Rovani et a., 2020; Tonello et al., 2021). Hence, there is a complexity of land use and land cover in the drainage areas of small streams which can contribute to environmental changes that directly and indirectly affect ecosystemic patterns and processes, among which are the decomposition of leaf litter. Hyphomycetes and invertebrate assemblages are often used in studies that evaluate the influence these organisms have on the decomposition of litter. Though we know that the environment can influence these assemblages in their decomposition activities, in the present study we used litter only as a substrate for the assemblage as a whole, while the evaluation of the process of decomposition took on a secondary role. With this in mind, we sought to understand how the environmental variability of streams affects the structure of aquatic hyphomycetes and invertebrate assemblages associated with decomposing litter. In order to do this, our aim was to answer the following questions: i) Do the hyphomycetes and invertebrate assemblages associated with decomposing litter respond to the environmental variability of streams? ii) Is the environmental variability observed in larger scale (i.e., land use and land cover) more significant predictors than local variables (i.e., limnological variables)? iii) Is the environmental variability affecting aquatic hyphomycetes and invertebrate assemblages associated with the indexes of litter decomposition in streams?

3.2 Materials and Methods

3.2.1 Study area

This study was carried out in the southern region of Brazil, in the northern area of the Uruguay River (27°12’59” a 28°00’47” S; 51°49’34” a 52°48’12”W). The approximate area of the region is 591 thousand ha (Rovani et al., 2020); the climate is temperate subtropical humid (Cfb Kôppen-Geiger), with average annual temperatures of 17°C and an average annual rainfall between 1900 to 2200 mm (Alvares et al., 2013). Altitude in the region is between 280m and 900m. The southern portion of the region’s terrain varies from flat to rolling, while the northern terrain varies from rolling to steep (Alvares et al., 2013; Rovani et al., 2020). The area under study is part of the Atlantic Forest biome with vegetation made up of formations of *Araucaria angustifolia* and semi-decidual components that are typical of the Subtropical Atlantic Forest (ROVANI et al., 2019). We selected 10 small order streams in this region (< 3rd order) distributed over a landscape matrix that is predominantly agricultural (Rovani et al., 2020) (Figure 1).

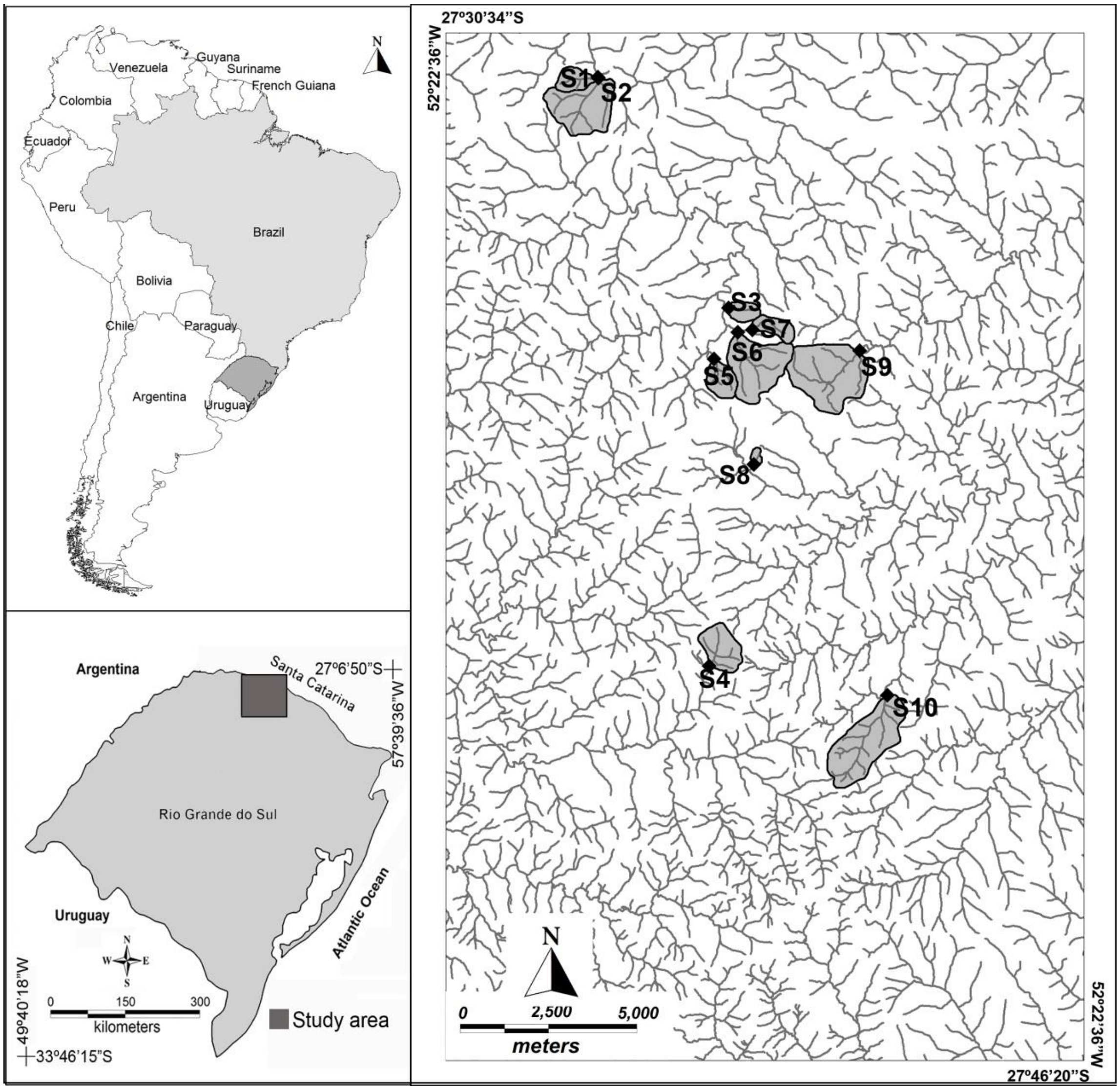


Fig. 1 Geographical location of streams studied in Atlantic Forest, southern Brazil.

3.2.2 Environmental variables

With the use of geoprocessing techniques, drainage areas of streams were defined according to the categories of land use and land cover, agriculture, pastures, native tree vegetation, water depth, forestry, road network, urbanized areas and wetlands. Satellite images taken from INPE (Instituto Nacional de Pesquisas Espaciais – National Institute for Spatial Research) were used for October 2020. Georeferencing of satellite images was done with the collection of coordinates with the UTM SIRGAS/2000 system and time zone 22 S, with a final presentation scale of 1:35.000. Subsequently, the digital classification module was applied by means of the method of

Maximum likelihood (MaxVer) of the IDRISI ANDES app and the Kappa index. Additionally, we carried out the land to define the sample patterns for quantified uses and land cover. Categorization of these land use and land cover classes followed the systematic classification proposed in the Land Use Technical Manual of the Brazilian Institute of Geography and Statistics (IBGE, 2013).

Between the months of October and November of 2021, the *in situ* quantification of the limnological variables electrical conductivity (EC), turbidity, water temperature, dissolved oxygen (DO) and pH was carried out using a HORIBA® U50 multiparameter analyzer. Additionally, we collected approximately 500 mL of water from each stream for total dissolved phosphorus (TDP) quantification by means of the spectrophotometric method and reaction with ascorbic acid (APHA, 2012). We performed three measurements in each stream, distributed throughout the sample segment (~100 m).

3.2.3 Decomposition experiment

Litter bags with senescent leaves of *Nectandra megapotamica* (Spreng.) Mez. (Lauraceae) were incubated in each stream in October 2021 for the colonization of the aquatic organisms (i.e., hyphomycetes and invertebrates) and to determine the indexes of leaf decomposition. We used this vegetal species as a study model because it is commonly found in the riparian areas of the region's streams and is widely used in studies on decomposition (Biasi et al., 2019; Fontana et al., 2020). The senescent leaves of *N. megapotamica* were dried at room temperature (~25°C) for approximately seven days. The leaves were placed in 30 fine meshed litter bags (10 x 20 cm; 0.5 mm mesh) and 30 coarse meshed litter bags (10 x 20 cm; 10 mm mesh). The fine meshed litter bags were used to limit the entrance of aquatic invertebrates. In each litter bag, 3.0 ± 0.1 g of leaves were stored. Three litter bags of each mesh were randomly incubated in each stream, with a total of 60 litter bags (3 duplicates x 10 streams x 2 mesh types).

After approximately 30 days, we collected the litter bags, individually stored them in plastic bags, placed them in insulated boxes and transported them to the laboratory for processing. At the laboratory, we carefully washed the leaves with running water to remove the associated sediment and invertebrates. We removed the retained invertebrates in a granulometric strainer (250 μm mesh) and secured them in 70% alcohol for subsequent identification. After washing, the leaves were dried in an oven at a temperature of 40°C±5ºC for 72 hours and immediately weighed to determine mass loss. We determined the decomposition indexes based on the following equation: k = (DM_0 – DM_f)/days, where k is the decomposition index, DM_0 is the initial weight, Dm_f is the final weight and days is the incubation period.

3.2.4 Aquatic assemblages

We triaged and identified the aquatic invertebrates associated with the leaf litter to the taxonomic level of family, using the taxonomic keys proposed by Fernadez & Domingues (2001), Mugnai et al. (2010). We grouped the invertebrates identified according to their functional characteristics based on the Functional Feeding Groups (FFG) (Cummins 1973; Cummins et al., 2005; Tomanova et al., 2006; Ramírez & Gutiérrez-Fonseca, 2014; Gholizadeh & Heydarzadeh, 2020; Doong et al., 2021).

We identified the aquatic hyphomycetes based on the random selection of five leaves from each fine meshed litter bag. Ten 1 mm leaf disks were removed from this material and stored in 100 mL Erlenmeyer flasks containing 35 mL of stream water. Subsequently, the flasks were placed in agitators, remaining there for 48 hours with 90 r.p.m. and at a temperature of 18±1ºC. After this period, we removed the flasks and transferred the content to falcon tubes. We removed the disks and dried them in an oven for 72 hours at a temperature of 40ºC for subsequent weighing. We then added the dry mass of the disks to the decomposition indexes. The spore suspension was conserved with the addition of 4 mL of formalin solution at 4%. Then, 150 mL of Triton X-100 0.5% were added to each sample to homogenize the conidia. We used a magnetic agitator to ensure the homogenization of the samples and of the conidia. A portion of these samples was filtered in a cellulose nitrate membrane filter (25 mm Ø, pores of 5mm - Unifil®). We also used trypan blue (0.05% trypan blue in 60% lactic acid) to dye the conidia. We observed these under a microscope (enhancement of 400x) and we identified the conidia at the taxonomic level of species with the help of the literature (Fiuza et al., 2017; Gulis et al.,2020). We standardized the sample effort by counting ~200 conidia per sample.

3.2.5 Data analysis

Initially we standardized the matrices related to the environmental variables (land use and land cover and limnological variables) by means of the *deconstand* function of the R *vegan* package software. Due to the low mean percentages below 4%, the categories land use and land cover, water depth, forestry, road network and wetlands were not used in this analysis (Table 2S - Supplementary Information).

Immediately after, we used Principal Component Analysis (PCA) to order the streams according to environmental variables. We applied the Redundancy Analysis (RDA) and in order to do so we used a matrix with environmental variables as an explanatory variable, and a matrix with conidia, invertebrate and FFG abundance as a response variable. Subsequently, a permutation test was applied (Blanchet et al., 2008) through the AIC method (Akaike, 1987) to verify which environmental variables were directly influencing the assemblages under study. Spearman correlations were applied between the scores of the first PCA component and the indexes of decomposition and abundance of conidia, invertebrates and FFG. We also used Spearman correlations between shredder abundance and decomposition indexes mediated by invertebrates and the total. Following that, using the same statistical test, we evaluated the association of hyphomycetes conidia abundance and the decomposition indexes mediated by microorganisms. We used a *t* test (paired) to verify possible differences between the decomposition indexes mediated by microorganisms and invertebrates among the streams. Based on studies by Tonin et al. (2014) and Hepp et al. (2016), we chose to remove the Chironomidae from the analyses to avoid a possible distortion in results in light of the wide dominance of these organisms in the samples (>60%). We considered $p < 0.05$ values significant. We used the R statistical environment (R Core Team, 2022) with the functions of the *vegan* (Oksanen et al., 2022), *ggord* (Beck, 2022) and *ggplot2* (Wickham et al., 2022) packages.

3.3 Results

3.3.1 Environmental variables

The native tree vegetation in the drainage areas of the streams varied from 2.0% to 60.2% (31.3±10.6%) and agricultural use varied from 3.7% to 83.1% (36.7±12.8%). Pastures varied from 0.5% to 35.2% (11.4±6.1%). The area of urbanization presented the largest amplitude of variation, from 0% to 94.3% (16.5±17.2%) (Table 1). The stream waters were shown to be well oxygenated (>9.0 mg L^{-1}); pH was slightly acid to neutral (6.7 ±0.4); and temperatures varied from 14.7ºC to 19.5ºC (18.0±0.9ºC). Electrical conductivity varied from 0.05 mS cm^{-1} to 0.15

mS cm^{-1} (0.10±0.01 mS cm^{-1}); TDP presented a variation of 32.4 to 102.4 μg L^{-1} (57.0±20.9 μg L^{-1}) (Table 1).

We used a PCA to order the streams based on environmental variables (land use and land cover and limnological). The two first axes explained 48% of total variation (Figure 2). We observed that the PC1 explained 26% of total variation, and that the high concentrations of TDP and urbanization were positively correlated to this component, while tree vegetation was negatively correlated with this axis. PC2 explained 22% of total variation, and turbidity and pH ordered this axis.

Table 1 Land use and land cover of drainage areas and limnological variables of streams in the south of Brazil. (EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved oxygen)

Streams	Pasture (%)	Agriculture (%)	Vegetation (%)	Urbanization (%)	Water Temperature (°C)	pH	EC (mS cm^{-1})	Turbidity (NTU)	DO (mg L^{-1})	TDP (µg L$^{-1)}$
S1	18.9	35.7	36.1	0.0	19.5±0.5	7.1±0.1	0.06±0.02	19.1±0.4	9.7±0.2	102.4±8.1
S2	35.2	38.1	22.1	0.0	19.5±0.1	7.3±0.1	0.10±0.00	12.9±2.2	8.8±0.0	52.4±9.7
S3	0.5	34.8	60.2	0.0	17.9±0.1	7.3±0.0	0.09±9.81	9.3±3.1	8.2±0.1	32.4±2.1
S4	9.7	64.9	23.0	0.0	17.5±0.0	5.4±0.4	0.05±0.00	18.6±0.4	11.2±2.2	32.8±0.8
S5	15.0	26.5	39.3	18.1	18.8±0.0	6.8±0.0	0.15±0.00	10.7±1.8	7.6±0.1	54.0±22.0
S6	17.0	24.9	38.6	16.4	14.7±0.1	5.5±0.3	0.11±0.00	5.3±0.8	8.9±0.1	51.1±15.5
S7	7.5	27.3	55.8	0.0	16.3±0.1	7.2±0.1	0.08±0.00	6.4±0.1	9.4±0.3	10.7±3.0
S8	0.0	3.7	2.0	94.3	19.1±0.0	6.3±0.2	0.08±0.01	12.4±6.1	8.8±0.4	136.1±24.1
S9	5.2	28.2	26.0	36.2	18.5±0.0	3.8±0.2	0.11±0.00	5.5±0.4	11.5±1.6	47.0±9.7
S10	5.0	83.1	9.9	0.0	19.3±0.4	6.3±0.1	0.12±0.00	4.4±0.2	7.6±0.1	51.5±6.7

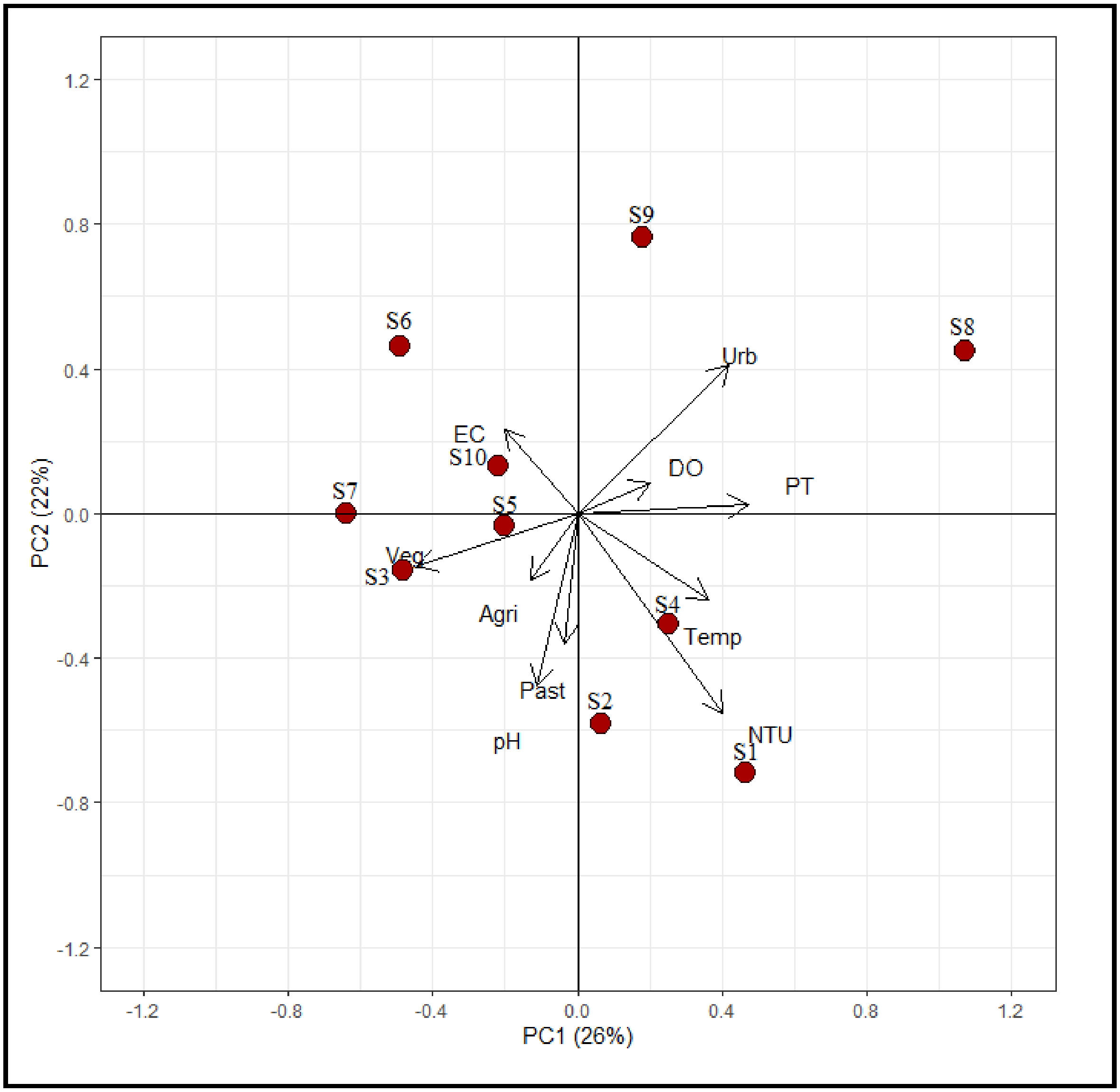


Fig. 2 Principal component analysis (PCA) for stream ordination based on environmental variables. (Veg = vegetation; Urb = urbanization; Agri = agriculture; Past = pastures; Temp = water temperature; NTU = turbidity; EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved oxygen).

3.3.2 Aquatic hyphomycetes and associated invertebrate

We identified 10 species of aquatic hyphomycetes in the sampled streams (Figure 4A; Table 3S - Supplementary Information). *Campylospora chaetocladia* and *L. curvula* represent 71% of the total conidia counted. *Lunulospora curvula*, *Anguillospora filiformis* and *Flagellospora curvula* were found in all the streams, while *Heliscus tentaculus* was found in only one stream (S7).

We identified a total of 4068 aquatic invertebrates associated with the decomposing leaf litter of *Nectandra megapotamica* distributed in 41 taxons (Figure 4b; Table 4S – Supplementary Information). Chironomidae represented 67% (2734 larvae) of all the fauna collected, followed by Caenidae with 9% (368 larvae) and Calamoceratidade with 4% (155 larvae). Of the total of organisms collected, we observed five functional feeding groups (Figure 4c; Table 4S - Supplementary Information), and collectors were the most representative with 82%. The shredders correspond to 6% (243 larvae) of the total of organisms collected. The family Calamoceratidae represented 64% (155 larvae) of the fauna of shredders, followed by Gripopterygidae with 31% (75 larvae).

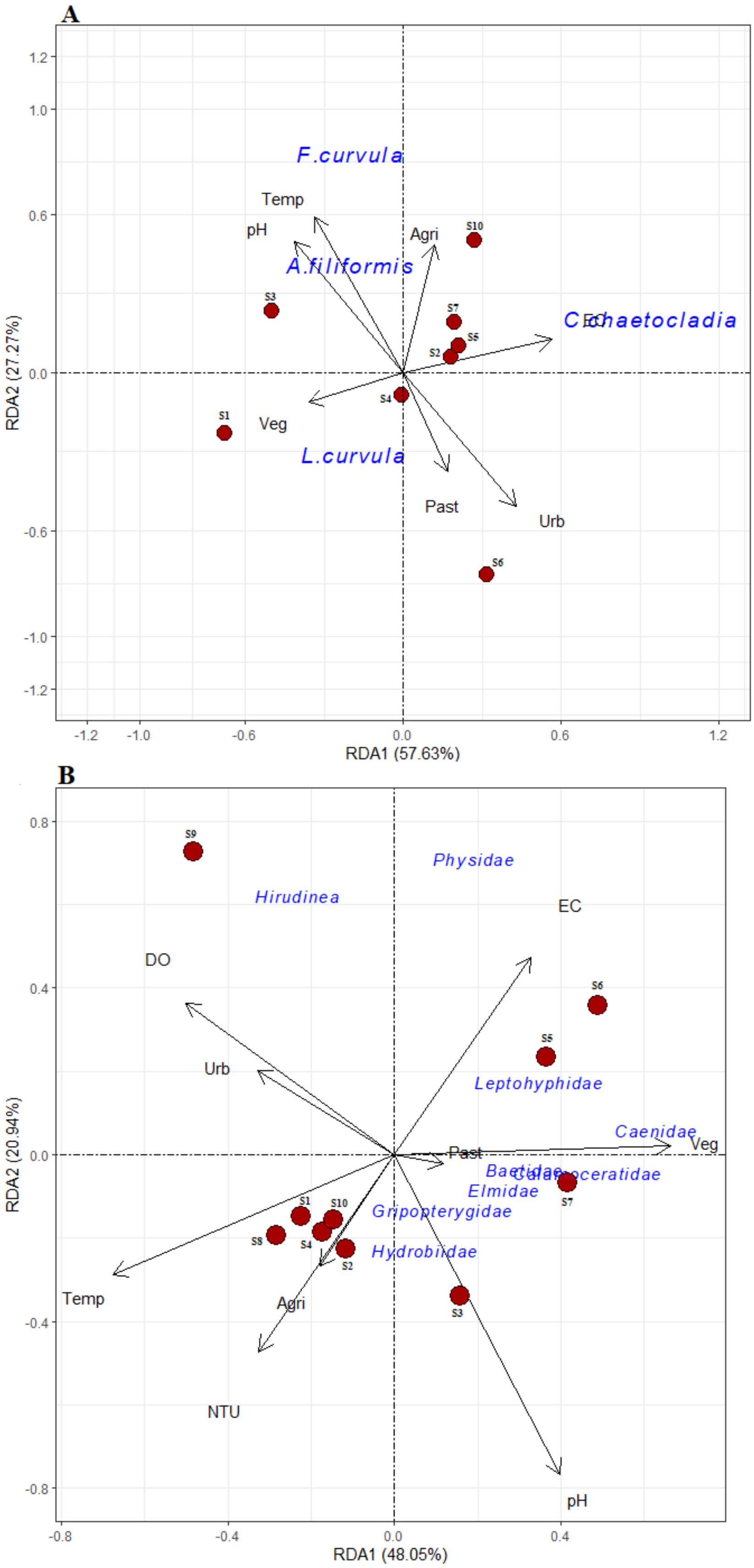
246

A
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10
Others
Lunulospora curvula
Flagellospora curvula
Anguillospora filiformis
Campylospora chaetocladia
B
Others
Hydrobiidae
Physidae
Hirudinida
Elmidae
Calamoceratidae
Gripopterygidae
Leptohyphidae
Caenidae
Baetidae
C
Predator
Shredder
Scraper
Filters
Collector

Fig. 3 Proportions of hyphomycetes (A), invertebrate (B) and FFG (C) distribution associated with decomposing leaf litter in subtropical streams. *Stream S8 did not present ant species and in stream S9 the fine mesh litter bags were lost.

3.3.3 Environmental variables and aquatic assemblage relationships

The RDA between the environmental variables and the aquatic hyphomycetes composition explained 84.9% of data variation (RDA1= 57.6%; RDA2= 27.3%) (Figure 5a). However, none of the environmental variables significantly influenced the hyphomycetes assemblage in the streams based on the AIC model (Table 5S – Supplementary Information). For the associated invertebrates, we observed that the RDA explained 69% of data variation (RDA1= 48.0%; RDA2= 20.9%) (Figure 5b). Vegetation in the drainage area (AIC= 29.1; p=0.04) and water temperature (AIC= 29.1; p=0.03) were environmental variables that had an effect on the associated invertebrate assemblage (Table 6S – Supplementary Information). The variation shown by the RDA for FFG abundance explained 70.8% of the total variation (RDA1= 46.6%; RDA2= 33.2%) (Figure 5c). Vegetation in the drainage area (AIC= 21.9; p=0.02) was the most important environmental variable in FFG structuring (Table 7S – Supplementary Information).


A
F.curvula
Temp
pH
Agri
A.filiformis
EC
C.chaetocladia
Veg
L.curvula
Past
Urb
S1
S2
S3
S4
S5
S6
S7
S10
RDA1 (57.63%)
RDA2 (27.27%)
B
Physidae
Hirudinea
EC
DO
Urb
Leptohyphidae
Caenidae
Veg
Past
Baetidae
Calamoceratidae
Elmidae
Gripopterygidae
Hydrobiidae
Temp
Agri
NTU
pH
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
RDA1 (48.05%)
RDA2 (20.94%)

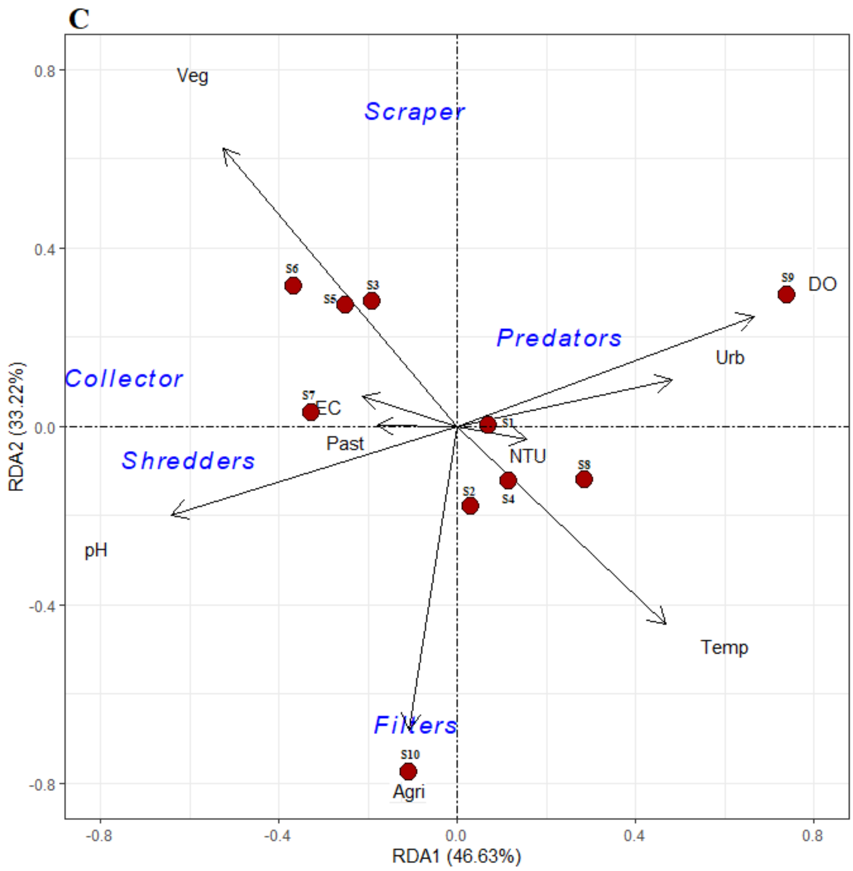


Fig. 4. Redundancy analysis (RDA) between environmental variables and hyphomycetes (A), invertebrates (B) and FFG (C). (Veg = vegetation; Urb = urbanization; Agri = agriculture; Past = pastures; Temp = temperature; NTU = turbidity; EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved oxygen).

3.3.4 Decomposition rates

After 33±4 days, the *Nectandra megapotamica* leaves lost 74±5 % of leaf mass in the fine meshed litter bags and 59±9 % in the coarse meshed litter bags. The decomposition index of litter mediated by microorganisms was k_{micro} = -0.0085±0.0010 day $^{1.}$ On the other hand, the index mediated by invertebrates was k_{inv} = -0.0047±0.0025 day^{-1} and the total index was k_{total} = -0.0124±0.0026 day^{-1} (Figure 5). There was no difference between the index of decomposition mediated by microorganisms and invertebrates among the streams k_{total} = -0.0124±0.0026 day^{-1} (Figure 5).

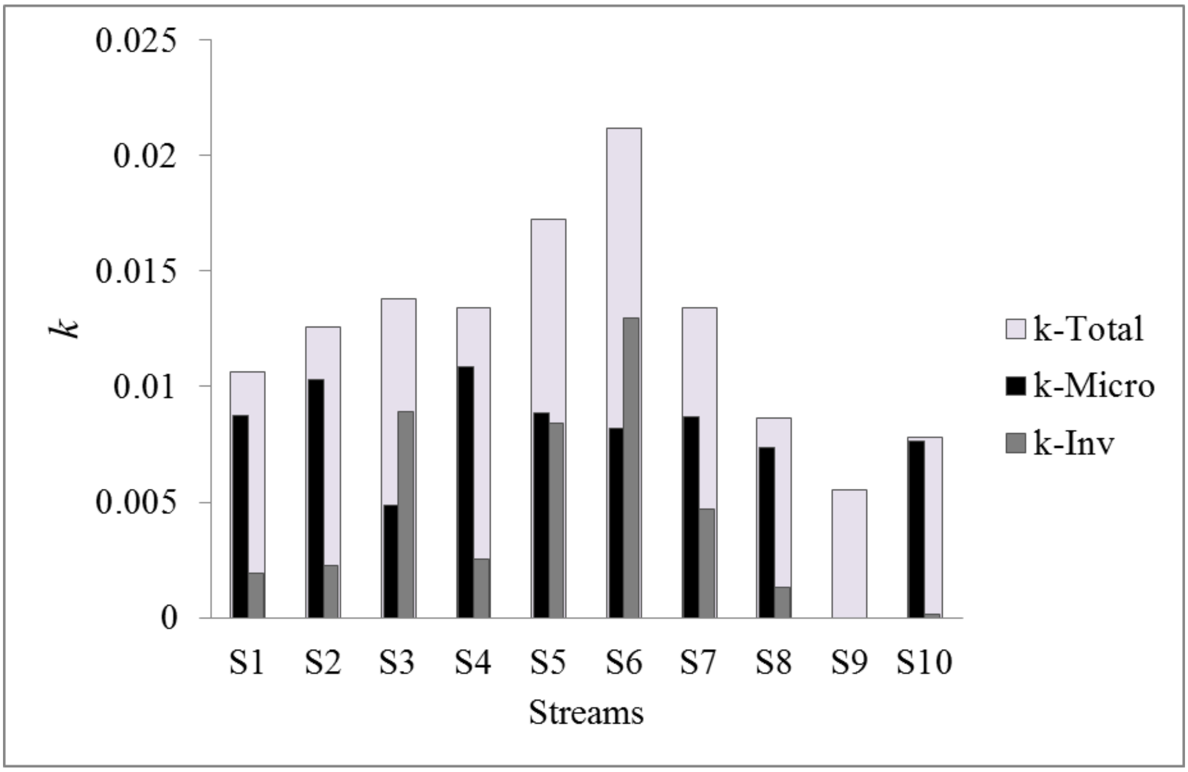


Fig. 5. Decomposition indexes mediated by microorganisms (k_{micro}), by invertebrates (k_{inv}) and total (k_{total}). Stream S9 did not present values for k_{micro} and k_{inv} due to the loss of fine mesh litter bags.

We did not observe any association of PCI scores with conidia abundance (r=-0.33; p=0.41), filtrators (r= -0.24; p= 0.49), predators (r= -0.12; p= 0.72) and scrapers (r= -0.50; p= 0.14). On the other hand, we did observe an association for total abundance of invertebrates (r=-0.84; p=0.004), shredders (r=-0.85; p=0.001) and collectors (r= -0.67; p= 0.03). There was no association between environmental variables (PCI scores) and the decomposition indexes mediated by microorganisms (k_{micro}) (r=0.47; p=0.24), invertebrates (k_{inv}) (r=-0.61; p=0.08) and taxa (k_{total}) (r=-0.57; p=0.08). In addition, when we directly evaluated the abundance of shredders with decomposition indexes mediated by invertebrates and the total, we did not observe a significant relation (r= 0.61; p=0.07 e r= 0.56; p=0.10, respectively). Finally, we did not observe a direct association between conidia abundance of hyphomycetes and the decomposition indexes mediated by microorganisms (r= 0.47; p= 0.24).

3.4 Discussion

In the present study, we aimed at understanding how the environmental variability produced by limnological variables and land use and land cover in drainage areas of small streams affect the structure of aquatic hyphomycetes and invertebrate assemblages associated with decomposing litter. In a general sense, we noticed that the landscape of the studied area is very fragmented, which is reflected in streams with a higher percentage of anthropic uses (i.e., agriculture and urbanization) in drainage areas. As a consequence, there is a considerable replacement of tree vegetation for monocultures that will result in changes in the limnological conditions of streams. This contributes to the increase in chemical sediments, nutrients, and contaminants; some variables suffer the direct influence from temperatures, turbidity, DO and TDP (Barakat et al., 2016; Sánchez-Morales et al., 2018; Wu et al., 2018; Gál et al., 2019; Puerto et al., 2022). Moreover, the environmental variability produced by these changes in land use and land cover, associated with limnological alterations, constitute important predictors for the structuring of aquatic assemblages, as was observed in this study.

Aquatic hyphomycetes and invertebrate assemblages did not respond in a similar way to the environmental variability observed in the streams. We did not observe a direct influence of environmental variables on the hyphomycetes assemblage. The absence of a direct relation between environmental variables and the occurrence and distribution of hyphomycetes has been reported in the literature (Breda et al., 2021). In this case, we suggest that other substances dissolved in the stream waters (i.e., heavy metals, pesticides) can explain possible effects on this assemblage (Cornejo et al., 2021; Bertol et al., 2022). Environmental conditions are important for the colonization of hyphomycetes in vegetal litter in streams (Graça et al., 2015). In this sense, the limnological variability found in aquatic environments set in a complex landscape matrix may demonstrate the importance of the action of environmental filters on fungi (Breda et al., 2021) at a smaller scale (i.e., streams).

In this model analysis, we did not observe any environmental variable significantly influencing the hyphomycetes assemblage. Although the RDA demonstrated a high explanatory value in the first two axes, the ordering of the species of fungi in the streams occurred in a similar way and the environmental variables measured were not enough to explain possible predictors. Among the environmental variables measured, temperature, pH, DO, and nutrients are important for hyphomycetes distribution (Biasi et al., 2017; Duarte et al., 2017; Breda et al., 2021). In our study, the environmental variability of the streams was not sufficient to establish a pattern of distribution among fungi.

On the other hand, the aquatic invertebrate assemblage demonstrated a higher sensitivity to the environmental variables measured, especially riparian vegetation and water temperature. We observed that the streams with the highest percentages of vegetation in the drainage area and the lowest temperatures presented the greatest richness of invertebrates associated with litter. Riparian vegetation is directly related to the amount of allochthonous organic matter in streams and is an important source of energy for these systems (Fontana et al., 2022). Furthermore, primary vegetation provides shade for streams thus reducing the incidence of light and, consequently, water temperatures (Tonello et al., 2021). In addition, the presence of riparian vegetation in streams can curb possible negative effects, such as those affecting the aquatic invertebrate assemblage by human activity (Chellaiah & Yule, 2018; Espinoza-Toledo et al., 2021; Guimarães-Souto et al., 2021; Sargac et al., 2021). These features account for the important function riparian vegetation has in the taxonomic and functional structuring of invertebrate assemblies, acting as an environmental filter (Firmiano et al., 2021).

As expected, the effect of the presence of riparian vegetation was important for the FFG composition of invertebrates. The FFG are influenced by environmental conditions due to the availability of food resources (Fu et al., 2016; Abdul & Rawi, 2019). Hence, tree vegetation in drainage is an important environmental variable to understand FFG occurrence (Ono et al., 2020; Lima et al., 2022). Landscape structure and habitat complexity act at different spatial scales contributing to FFG occurrence and distribution, though it is shaped by the availability of organic material (König et al., 2014; Ferreira et al., 2017; Guimarães-Souto et al., 2021; Lima et al., 2022). In our study, this pattern was clear since shredder abundance was positively associated with the streams presenting higher percentages of tree vegetation on the margins.

Environmental variability was related to invertebrate abundance as well as with shredders and collectors. However, this result did not reflect on a significant association with decomposition indexes. The negative correlation between the PC1s and the shredders demonstrates that the increase in farm area results in a decrease in the abundance of these organisms. Shredders are susceptible to the removal of riparian vegetation (Fu et al. 2016; Solis et al. 2019; Silva-Araújo et al. 2020; Tonello et al., 2021). Since these are the main organisms responsible for transforming coarse organic matter into fine matter, there is a strong dependence on the amount of allochthonous material in streams (Mangadze et al., 2019; Mlambo et al., 2019; Silva-Araújo et al., 2020). Tonello et al. (2016) state that a reduced abundance of shredders in streams can decrease decomposition indexes by 23%. However, our results do not demonstrate this relation (p=0.07 e p=0.10). Probably this result is explained by the sample size used in the correlation analysis (n = 8). In this sense, considering the statistical result, we could speculate that, with an increase in the sample mesh, we could demonstrate the relation between environmental variability, shredder abundance and decomposition indexes more clearly.

The collectors presented a significant correlation with environmental variability and, in the same way as with the shredders, their abundance depends on an increase in the vegetation on stream margins. However, this relation is not directly associated to the process of stream litter decomposition. Collectors consume fine particles of organic matter, not the coarse matter (Miserendino & Masi, 2010; Chellaiah & Yule, 2018). Hence, collectors may have used the litter banks as shelter (Biasi et al., 2013; Barrios et al., 2022).

The absence of a significant relation between hyphomycetes and decomposition indexes (p=0.24) is a reflex of the lack of a pattern in the distribution of the organisms in the streams under study. The pressures of the anthropic activities *agriculture and urbanization* on the aquatic ecosystems produce alterations in the physical and chemical composition of these environments and, as consequence, end up affecting the composition and distribution of the assemblages that inhabit these locations (Borgwardt et al., 2019; Breda et al., 2021). In addition, the hyphomycetes assemblage may be taking on a secondary role in the decomposition process in the streams we studied. Even if there are records indicating there is high density of hyphomycetes in colder water streams, the presence of shredders may decrease the importance of these organisms in the decomposition process.

3.5 Conclusion

In this study we sought to understand how the environmental variability of streams affects the structure of aquatic hyphomycetes and invertebrate assemblages associated with decomposing litter. We observed that only the aquatic invertebrate assemblage was structured based on environmental variables. In addition, we observed that both environmental variability at a wider scale (i.e., land use and occupation) and local variables (i.e., limnological) were important predictors for the aquatic invertebrate assemblage. However, this influence did not reflect directly on the decomposition indexes, only on the shredders.

Our study demonstrated that regions with a fragmented landscape, in other words, made up of a complex composition of classes of land use and a reduced area of native vegetation, decisively affect the structure of aquatic assemblages. Though the hyphomycetes were not affected, we observed that the presence of riparian vegetation in streams provide better conditions for ecological integrity to these streams. These conditions are connected to food supply (amount of organic material), shelter (leaf banks on streambed), and the interaction with limnological variables (i.e., water temperature and nutrient concentration).

Therefore, it is crucial to conserve the riparian vegetation and the residual vegetation in the drainage area of these streams, especially in a scenario where urbanization and agricultural practices are advancing. Our results can help in decision-making regarding administrative and management actions for hydric resources. In this sense, we suggest the creation of programs to encourage the restauration of vegetation in the riparian area, which would be essential to solve the pressing issue of the removal of this vegetal land cover. It would also act towards the conservation of hydric resources. Even with the protection of riparian vegetation recommended by national laws, there is a need for constant monitoring, inspection, and actions for the recovery of vegetation.

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Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author contributions: LAE field data collection, identification of organisms, data analysis and article writing. BLP field data collection and identification of organisms. CB Sampling and identification of hyphomycetes. LUH experimental design, data analysis and article writing. RMR field data collection, identification of organisms and writing of the article.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest

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Supplementary Information

Does environmental variability in Atlantic Forest streams affect hyphomycetes and invertebrate assemblages associated with leaf litter?

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Table 1S. Physical characteristics of sample streams in the south of Brazil

Streams	**Latitude (GMS)**	**Longitude (GMS)**	**Altitude (m)**	**Largura (m)**	**Profundidade (m)**
S1	27°30'53.75"S	52°19'53.04"W	645	3.9	0.12
S2	27°30'53.03"S	52°19'54.16"W	633	1.8	0.14
S3	27°35'43.19"S	52°16'47.17"W	557	1.6	0.08
S4	27°43'15.35"S	52°17'13.60"W	753	1.7	0.14
S5	27°36'48.02"S	52°17'7.80"W	590	1.8	0.07
S6	27°36'14.30"S	52°16'34.70"W	654	3.7	0.10
S7	27°36'8.80"S	52°16'15.10"W	650	2.8	0.10
S8	27°38'58.7"S	52°16'13.5"W	699	1.7	0.06
S9	27°36'38.80"S	52°13'40.30"W	696	3.1	0.11
S10	27°43'51.82"S	52°13'1.78"W	635	4.4	0.16

Table 2S. Land use and land cover in the drainage areas of streams in the south of Brazil

Streams	Drainage Area (ha)	Pasture (%)	Agriculture (%)	Vegetation (%)	Urbanization (%)	Wetlands (%)	Silviculture (%)	Road (%)
S1	418.10	18.9	35.7	36.1	0.0	0.2	7.6	1.1
S2	107.08	35.2	38.1	22.1	0.0	0.0	4.6	0.0
S3	79.08	0.5	34.8	60.2	0.0	0.0	4.5	0.0
S4	218.01	9.7	64.9	23.0	0.0	0.3	0.5	1.6
S5	132.23	15.0	26.5	39.3	18.1	0.0	0.0	1.2
S6	442.76	17.0	24.9	38.6	16.4	0.1	1.8	1.2
S7	104.97	7.5	27.3	55.8	0.0	0.0	8.9	0.5
S8	22.39	0.0	3.7	2.0	94.3	0.0	0.0	0.0
S9	628.28	5.2	28.2	26.0	36.2	0.4	1.0	1.4
S10	546.93	5.0	83.1	9.9	0.0	0.0	0.3	0.6

Table 3S. Hyphomycetes species and quantity of conidia (conidia/mg) associated with decomposing leaf litter in streams in the south of Brazil. Stream S8 did not present any species and litter bags were lost in stream S9.

Species	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S10**
Campylospora chaetocladia	0	250	4	65	290	167	274	274
Anguillospora filiformis	41	79	85	59	74	2	59	11
Flagellospora curvula	48	33	222	26	38	2	64	294
Lunospora curvula	325	244	299	244	202	213	174	18
Tricladium chaetocladium	0	2	0	2	0	1	4	8
Clavariopsis aquatica	0	0	2	0	0	0	6	0
Heliscus tentaculus	0	0	0	0	0	0	3	0
Heliscus submersus	4	2	5	14	8	38	6	1
Lemonniera aquatica	0	1	0	0	0	0	16	1
Triscelophorus acuminatus	0	1	0	0	0	0	3	9

Table 4S. Distribution of aquatic invertebrates and respective Functional Food Groups (FFG) associated with decomposing leaf litter in streams in the south of Brazil.

Taxa	FFG	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
ANNELIDA											
Oligochaeta	Collector	10	0	0	0	0	0	2	1	0	1
Hirudinea	Predator	0	0	0	0	0	1	0	0	124	1
ENTOGNATHA											
Collembola	Collector	16	0	3	0	5	0	1	1	0	1

GASTROPODA											
Physidae	Scrapers	0	0	0	0	19	36	1	0	20	0
Planorbidae	Scraper	1	1	0	0	0	0	2	0	1	0
Hydrobiidae	Scraper	0	0	55	0	0	0	0	0	0	0
Ancilidae	Scraper	2	0	0	1	0	0	1	1	1	0
INSECTA											
Diptera											
Chironomidae	Collector	123	143	132	177	305	189	410	233	436	586
Simulidae	Filter	0	2	0	0	0	0	0	0	0	77
Tabanidae	Predator	0	0	2	0	0	0	1	0	0	5
Tipulidae	Shredder	2	0	0	0	2	4	0	0	0	0
Culicidae	Collector	0	0	1	0	0	0	0	0	0	0
Psychodidae	Collector	0	0	1	0	0	0	0	0	0	5
Ceratopogonidae	Predator	4	2	6	1	8	0	0	8	0	2
Stratiomidae	Collector	0	0	1	0	0	0	0	0	0	0
Empididae	Predator	0	0	0	4	6	0	0	3	0	0
Ephemeroptera											
Baetidae	Collector	1	1	4	9	5	20	20	0	0	5
Caenidae	Collector	3	6	25	1	123	104	102	0	0	4
Leptophlebiidae	Collector	3	2	0	4	2	2	6	0	0	3
Leptohyphidae	Collector	0	0	0	2	5	20	18	0	0	1
Plecoptera											
Gripopterygidae	Shredder	0	7	0	4	0	10	13	1	0	40
Lepidoptera											
Pyralidae	Scraper	0	0	0	0	1	0	0	0	0	0
Trichoptera											
Calamoceratidae	Shredder	3	1	52	0	18	43	33	5	0	0
Hydropsychidae	Filter	0	0	0	0	0	0	0	0	0	9

Odontoceridae	Shredder	0	0	0	1	0	0	0	0	0	1
Hydrobiosidae	Predator	0	0	0	0	0	1	3	0	0	0
Philopotamidae	Filter	0	0	1	0	0	0	0	0	0	0
Hydroptilidae	Scraper	0	2	0	0	0	0	0	0	1	0
Coleoptera											
Elmidae	Collector	0	14	5	5	26	10	6	2	0	3
Hydrophilidae	Predator	4	1	12	0	0	0	1	0	0	0
Dryopidae	Shredder	0	0	3	0	0	0	0	0	0	0
Lampyridae	Predator	0	0	0	0	2	0	0	0	0	0
Staphylinidae	Predator	0	0	0	0	0	0	0	0	0	5
Coleoptera											
Dytiscidae	Predator	1	1	0	0	1	0	0	0	0	2
Odonata											
Calopterygidae	Predator	0	0	0	0	2	9	3	0	0	0
Aeshnidae	Predator	0	0	1	0	0	0	0	1	0	0
Coenagrionidae	Predator	0	0	0	0	0	1	0	0	0	0
Hemiptera											
Belostomatidae	Predator	0	0	2	0	0	0	0	0	0	0
Veliidae	Predator	0	0	1	0	0	0	0	0	0	0
MALACOSTRACA											
Isopoda	Collector	0	0	0	0	0	0	0	1	0	0
MAXILLOPODA											
Copepoda	Collector	0	0	0	0	2	0	0	0	0	0

Table 5S. Explanatory environmental variables based on permutation tests for the composition of associated aquatic hyphomycetes (EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved phosphorus).

	Gl	AIC	F	p
Turbidity (NTU)	1	18.310	1.876	0.160
EC (mS cm^{-1})	1	18.791	1.416	0.260
pH	1	18.921	1.297	0.255
Urbanization (%)	1	18.935	1.283	0.365
TDP (µg/L)	1	19.071	1.161	0.380
Temperature (ºC)	1	19.075	1.158	0.355
Agriculture (%)	1	19.558	0.738	0.585
Vegetation	1	19.676	0.639	0.660
DO (mg L^{-1})	1	19.736	0.590	0.616
Pasture (%)	1	19.852	0.495	0.620

Table 6S. Explanatory environmental variables based on permutation tests for the composition of associated aquatic invertebrates. (EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved phosphorus). (*= significant).

	Gl	AIC	F	p
Vegetation (%)	1	29.057	2.7755	0.045*
Temperature (ºC)	1	29.115	2.7133	0.030*
pH	1	29.693	2.1116	0.070
DO (mg.L^{-1})	1	30.315	1.5021	0.180
TDP (µg/L)	1	30.602	1.2331	0.295
Turbidity (NTU)	1	30.711	1.1330	0.380
EC (mS cm^{-1})	1	30.823	1.0312	0.410
Agriculture (%)	1	31.021	0.8538	0.510
Urbanization (%)	1	31.165	0.7273	0.565
Pasture (%)	1	31.648	0.3157	0.930

Table 7S. Explanatory environmental variables based on permutation tests for GTF abundance. (EC = electrical conductivity; DO = dissolved oxygen; TDP = total dissolved phosphorus). (*= significant).

	Gl	AIC	F	p
Vegetation (%)	1	21.937	2.8201	0.020*
DO (mg L^{-1})	1	22.107	2.6376	0.050
pH	1	22.565	2.1618	0.145
Temperature (ºC)	1	23.102	1.6301	0.245
Agriculture (%)	1	23.086	1.6463	0.255
Urbanization (%)	1	23.686	1.0839	0.305
Turbidity (NTU)	1	23.826	0.9578	0.470
EC (mS cm^{-1})	1	24.107	0.7101	0.565
TDP (µg/L)	1	24.133	0.6871	0.600
Pasture (%)	1	24.478	0.3926	0.760

4. CONCLUSÃO GERAL

Nesta dissertação buscamos compreender como a variabilidade ambiental de riachos, afeta a estruturação de comunidades de hifomicetos e invertebrados aquáticos associados a detritos em decomposição. Observamos que apenas a comunidade de invertebrados aquáticos associados aos detritos, foi estruturada com base nas variáveis ambientais. A variabilidade ambiental em escala mais ampla, (vegetação na área de drenagem), quanto em escala local (temperatura da água), foram importantes preditoras para a comunidade de invertebrados aquáticos e para a composição dos GTF. Porém, esta influência não refletiu diretamente nas taxas de decomposição, apenas nos organismos fragmentadores.

Observamos que locais onde a paisagem estava fragmentada, com reduzida área de vegetação nativa, afetou a estrutura da comunidade de invertebrados aquáticos, como por exemplo, favoreceu a dominância de alguns grupos (*e.g.* Chirononimidae). Embora os hifomicetos não tenham sido afetados, observamos que a presença de vegetação ripária nos riachos, proporciona condições melhores de integridade ecológica aos riachos. Estas condições se referem à oferta de alimento (aporte de material orgânico), refúgio (banco de folhas no leito riacho), além de interagir com variáveis limnológicas (e.g. temperatura da água e concentração de nutrientes).

Com isso, se torna imprescindível a conservação da vegetação ripária na área de drenagem destes riachos, principalmente em um cenário de avanço da urbanização e das práticas agrícolas. Nossos resultados podem auxiliar na tomada de decisão para ações de gestão e manejo dos recursos hídricos. Neste sentido, sugerimos a criação de programas para o incentivo a restauração da vegetação na zona ripária, o que seria fundamental para a solução ante a problemática da remoção dessa cobertura vegetal, e ainda, atuaria na conservação dos recursos hídricos. Mesmo com a proteção da vegetação ripária recomendada pelas leis nacionais, há a necessidade de constante monitoramento, fiscalização e ações de recuperação da vegetação. Iniciativas que incentivem a conservação da vegetação na zona ripária, podem atuar significativamente na proteção dos recursos hídricos. Com isso, iniciativas semelhantes ao Programa de Recuperação de Recursos Hídricos e Pagamento por Serviços Ambientais (PSA), devem ser implementadas. O PSA foi desenvolvido, e está sendo implementado pela Secretaria Municipal de Meio Ambiente de Erechim, tendo como um dos objetivos recompensar os agricultores que atuam conservando a vegetação ripária em torno dos riachos nas suas propriedades.

Como perspectiva de continuidade, tendo em vista o crescimento populacional e o avanço da urbanização, sugerimos estudos envolvendo a decomposição de detritos foliares em riachos inseridos no meio urbano, e, analisar o efeito deste uso, sobre as comunidades associadas. Estudos com esta temática são escassos na região subtropical. Ainda sugerimos a inclusão de análise de metais pesados na água e como estes afetam as comunidades associadas a detritos em decomposição, principalmente os hifomicetos aquáticos.

ANEXOS

Anexo 1. Comprovante de submissão

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Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Methods

Methods should be described with enough details to allow the reproduction of the work Thus, we request full disclosure in all papers. Disclosure "refers to the process of describing in full the study design and data collected that underlie the results reported, rather than a curated version of the design, and/or a subset of the data collected." (Munafò et al., 2017). Methods must present details on experimental designs, on experimental units or observational units (in case of field research), local of investigation, time and duration of the experiment, description of statistical tests used, statistical programs and their versions, number of replicates (or sampling units in the case of observational studies), transformations employed, data deleted with reasons for deletion, codes used, and any other information necessary to get the work fully reproducible. Finally, **nucleotide sequence data** must have been submitted to databases and informed accordingly in the paper.

Munafò, M. C. et al. 2017. A manifesto for reproducible science. Nature Human Behavior 1, 0021. DOI: 10.1038/s41562-016-0021

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References

References in the text will use the name and year system: Adam & Eve (1983) or (Adam & Eve, 1983). For more than two authors, use Adam et al. (1982). References to a particular page, table or figure in any published work is made as follows: Brown (1966: 182) or Brown (1966: 182, fig. 2). Cite only published items; grey literature (abstracts, theses, reports, etc.) should be avoided as much as possible. Papers which are unpublished or in press should be cited only if formally accepted for publication.

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- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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Anexo 3. Riachos estudados.

Figura 1. Riacho S1 (Barra 1)

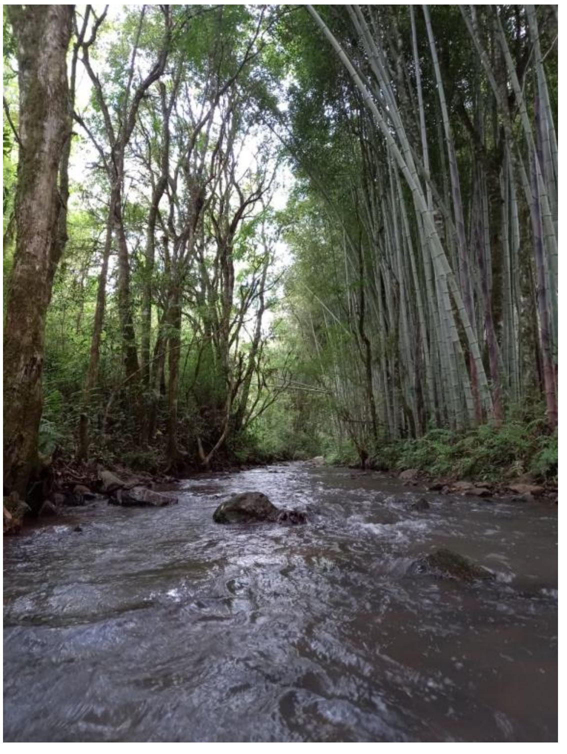

Figura 2. Riacho S2 (Barra 2)

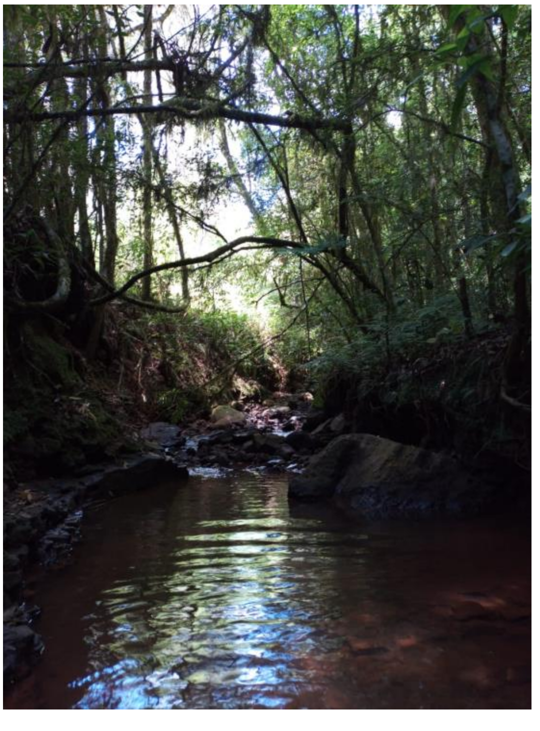

Figura 3. Riacho S3 (Lucas)

Figura 4. Riacho S4 (Cravo)

Figura 5. Riacho S5 (Capela)

Figura 6. Riacho S6 (Sonia)

Figura 7. Riacho S7 (Dourado)

Figura 8. Riacho S8 (URI 1)

Figura 9. Riacho S9 (URI 2)

Figura 10. Riacho S10 (Capo 2)