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COMPOSIÇÃO E DISTRIBUIÇÃO DE HIFOMICETOS AQUÁTICOS EM RIACHOS SUBTROPICAIS

ERECHIM, NOVEMBRO DE 2020.

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Composição e distribuição de hifomicetos aquáticos em riachos subtropicais

Discente: Mayara Breda

Orientador: Prof. Dr. Luiz Ubiratan Hepp

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26 de novembro de 2020

Resumo – Hifomicetos aquáticos são fungos anamorfos, que se reproduzem assexuadamente com liberação de conídios e são de grande importância no funcionamento dos ecossistemas aquáticos. Entender os padrões de distribuição de hifomicetos entre habitats de diferente qualidade podem revelar importantes características ambientais que afetam suas assembleias, e por consequência, podem alterar o funcionamento aquático. Avaliamos os efeitos de preditores ambientais sobre as assembleias de hifomicetos em um experimento de campo, utilizando substrato padronizado de *Nectandra megapotamica* em 12 riachos subtropicais da Floresta Atlântica. Identificamos 21 espécies de hifomicetos aquáticos, sendo *Lunulospora curvula* espécie dominante, ocorrendo em todos os riachos e correspondendo a 86,3% da produção de conídios, seguido de *Aquanectria submersa* que ocorreu em 11 riachos e *Flagellospora curvula*, ocorrendo em 10 riachos. Já as espécies *Anguillospora* sp., *Campylospora parvula* e *Mycocentrospora acerina* foram encontradas em apenas um riacho. Os riachos não apresentaram padrões claros de ordenação, apresentando variabilidade ambiental entre eles. As variáveis oxigênio dissolvido, condutividade elétrica e pH influenciaram a distribuição das assembleias de hifomicetos. A condutividade elétrica atuou como filtro ambiental para a abundância de conídios, enquanto o oxigênio dissolvido, a condutividade elétrica e fosfato atuaram como filtro ambiental para a riqueza de espécies. Nossos achados ressaltam a importância dos preditores ambientais sobre a assembleia de hifomicetos aquáticos. A influência de preditores ambientais sobre a abundância e riqueza de espécies também pode alterar o funcionamento dos riachos, uma vez que esses organismos apresentam um papel fundamental na decomposição dos detritos. Esse é um trabalho pioneiro no estudo de hifomicetos na perspectiva de distribuição de espécies na região subtropical, sendo que o número de espécies coletadas nesse estudo corresponde a ¼ do total encontrado no Brasil. Uma perspectiva para continuidade do trabalho é a coleta de bancos de folhas e espumas naturais nos riachos já que a ampliação das coletas para diferentes substratos poderemos aumentar a lista de espécies para a região.

Palavras-chave: preditores ambientais, fungos aquáticos, funcionamento de riachos, filtros ambientais, bioindicadores.

Composition and distribution of aquatic hyphomycetes on subtropical streams

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November 26, 2020.

Abstract – Aquatic hyphomycetes are anamorphic fungi, which reproduce asexually with the release of conidia and are important in aquatic ecosystems functioning. Understanding the patterns of distribution of hyphomycetes among habitats of different quality can reveal important environmental characteristics that affect their assemblages, and, consequently, can alter aquatic functioning. We evaluated the effects of environmental predictors on hyphomycete assemblages in a field experiment, using standardized substrate of *Nectandra megapotamica* in 12 subtropical streams of the Atlantic Forest. We identified 21 species of aquatic hyphomycetes, *Lunulospora curvula* being the dominant species, occurring in all streams and corresponding to 86.3% of conidia production, followed by *Aquanectria submersa* which occurred in 11 streams and *Flagellospora curvula*, occurring in 10 streams. The species *Anguillospora* sp., *Campylospora parvula* and *Mycocentrospora acerina* were found in only one stream. The streams did not present clear patterns of ordination, demonstrating environmental variability between them. The variables dissolved oxygen, electrical conductivity and pH influenced the distribution of the hyphomycetes assemblages. Electrical conductivity acted as an environmental filter for conidia abundance, while dissolved oxygen, electrical conductivity and phosphate acted as an environmental filter for species richness. Our findings highlight the importance of environmental predictors on the pool of aquatic hyphomycetes. The influence of environmental predictors on the abundance and richness of species can also change the functioning of streams, since these organisms play a fundamental role in the decomposition of organic matter. This is a pioneering work in the study of hyphomycetes in the perspective of species distribution in the subtropical region, and the number of species collected in this study corresponds to ¼ of the total found in Brazil. A perspective for the continuity of the work is the collection leaf packs and natural foams in the streams since the expansion of collections for different substrates may increase the list of species for the region.

Key-words: environmental predictors, stream functioning, environmental filters, bioindicators.

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1. INTRODUÇÃO GERAL

1.1 FILTROS AMBIENTAIS

Uma das principais questões dentro da teoria ecológica é entender como a diversidade de espécies está distribuída espacial e temporalmente, e quais fatores afetam a coexistência de organismos em determinado local. A compreensão dos processos ecológicos responsáveis pela distribuição de organismos e estruturação das assembleias biológicas é amplamente discutida na ecologia (USSEGLIO-POLATERA et al., 2001; STATZNER e BECHE, 2010). Dentre esses processos, podemos citar as relações competitivas, que foram, por muito tempo estudadas como principal fator na seleção de espécies (CONNEL, 1961; HOLT, 1977). Com o aprimoramento dos estudos, além das relações competitivas, passou-se a considerar que as espécies existentes em um determinado local eram selecionadas por processos filogeográficos, que incluíam os históricos de especiação e migração, assim como processos ecológicos, considerando interações entre fatores bióticos e abióticos (KEDDY, 1992). Dessa forma, os filtros ambientais podem atuar como processo na seleção de espécies e formação das assembleias biológicas.

Filtros ambientais são considerados fatores, os quais selecionam as espécies que não conseguem se desenvolver nas condições existentes em determinado habitat (TONN, 1990; HEINO et al., 2003; TOWNSEND et al., 2003). Dessa forma, a seleção das espécies ocorre quando os fatores bióticos e abióticos limitam o desenvolvimento de algumas espécies (POFF, 1997). Os filtros bióticos são relacionados com as interações ecológicas de competição e predação, que irão influenciar na dispersão das espécies. Por outro lado, os filtros abióticos estão associados às restrições das espécies, que irão se desenvolver ou adaptar sob determinadas condições ambientais (MYERS e HARMS, 2009).

As condições ambientais (fatores bióticos e abióticos) podem ser consideradas filtros, nas quais as espécies precisam passar para que possam sobreviver e reproduzir em determinado local (TONN et al., 1990). Considerando longos períodos de tempo, todas as espécies podem ser capazes de se dispersar para todos os locais em uma região, no entanto, a ausência ou baixa abundância de espécies em um ambiente pode refletir a ação de filtros seletivos (TONN et al., 1990). Dessa forma, para que uma espécie consiga passar um determinado filtro, a mesma deve possuir adaptações funcionais que combinem com a característica do filtro ambiental (POFF, 1997).

Poff (1997) especificou quatro níveis hierárquicos de habitats, que incluem as escalas relevantes para uma ampla gama de espécies em ambientes lóticos, que vão de níveis mais amplos e se estreitam em níveis menores, sendo eles: bacia hidrográfica, segmento do riacho, unidade do canal (corredeira ou poça) e micro-habitat. O uso desses níveis auxilia na identificação dos filtros que podem estar influenciando a distribuição e abundância de espécies na paisagem (POFF, 1997).

Diversos são os filtros que podem estar atuando na seleção de espécies nas diferentes escalas espaciais propostas por Poff (1997). Na escala de bacia hidrográfica, por exemplo, os fatores como: biogeografia da espécie, tipo de vegetação, sazonalidade, temperatura, pH, nutrientes inorgânicos e eutrofização, atuaram como filtros para as comunidades de peixes e macroinvertebrados bentônicos (MOLLES, 1982; POFF, 1997). Avaliando o nível hierárquico de segmento do riacho, fatores como intensidade do fluxo, tipo de recurso alimentar e temperatura atuaram como filtros para as comunidades aquáticas, enquanto que, em níveis menores (unidade do canal e micro-habitat), fatores como tipo e mobilidade de substrato, tipo de matéria orgânica particulada (grossa e fina), teores de oxigênio e temperatura atuaram como filtros para as espécies (POFF, 1997).

Distúrbios antrópicos também podem atuar como filtros ambientais através da exclusão de espécies cuja tolerância fisiológica é excedida, ou cujos requisitos sejam de habitats estáveis. Além disso, também podem possibilitar a entrada de novas espécies de acordo com seus atributos funcionais, como espécies generalistas (CHAPIN et al., 2000). Estudos avaliando a estrutura de comunidades fitoplanctônicas também reportaram a importância de filtros como salinidade, temperatura e precipitação de sais na composição da comunidade (COSTA et al., 2015). Fatores climáticos, pH do solo e precipitação anual atuaram como filtros para as comunidades microbianas terrestres, em pastagens (CAO et al., 2016). Bello et al. (2013), avaliando a comunidade de plantas nos Alpes Franceses, evidenciaram a importância de fatores como a temperatura, topografia e características do solo na estruturação da comunidade.

1.2 HIFOMICETOS AQUÁTICOS

Os hifomicetos aquáticos representados por Basidiomicetos e Ascomicetos, principalmente, apresentam distribuição cosmopolita, com mais de 300 espécies estimadas, sendo a maioria delas observadas em regiões temperadas (SHEARER et al., 2007). No

Brasil, foram registrados um total de 85 espécies, sendo 53 pertencentes ao Bioma Mata Atlântica, 39 a Caatinga, 21 ao Cerrado e 19 a Amazônia (FIUZA et al., 2017). Entretanto, a maioria destes estudos tem avançado os esforços de amostragem para além de folhas em decomposição, aliando amostragem de comunidades em espumas, por exemplo (SCHOENLEIN-CRUSIUS e GRANDI, 2003). Para região subtropical os estudos sobre a diversidade deste grupo ainda são incipientes, porém há indícios de ~25 espécies associadas ao detrito em decomposição (BIASI et al., 2020). É recorrente a indicação de que ambientes tropicais apresentem baixa diversidade de hifomicetos (GRAÇA et al., 2015). Algumas hipóteses para a baixa diversidade deste grupo foram levantadas, como: pouca sazonalidade na entrada de matéria orgânica, condições físicas do riacho, baixa concentração de nutrientes na água, substrato de baixa qualidade, histórias de vida diferentes (que resultam em baixo investimento na reprodução pelos fungos) e competição com outros decompositores, como bactérias (GRAÇA et al., 2016).

Estes hifomicetos apresentam reprodução assexuada com liberação de conídios que permite a identificação das espécies de acordo com a sua morfologia (FIUZA et al., 2013). A atividade reprodutiva (também chamada de esporulação) destes fungos é usualmente utilizada como proxy para a atividade microbiana em detritos em decomposição, e tem se mostrado uma medida eficaz para responder variações na composição química das espécies (GRAÇA et al., 2015), na quantidade de nutrientes dissolvidos na água (BIASI et al., 2017) e também alterações na vegetação ripária (LAITUNG e CHAUVET, 2005).

Diversos são os fatores que regulam a atividade e as assembleias de hifomicetos aquáticos. As mudanças na qualidade da água podem causar redução na biomassa e também na riqueza dos hifomicetos, podendo afetar o papel exercido por esses organismos no funcionamento dos ecossistemas aquáticos (SOLÉ et al., 2008). Dentre essas alterações, Bärlocher et al. (2008) constatou a diminuição na diversidade de fungos aquáticos com o aumento da temperatura e com a presença de poluentes (DUARTE et al., 2008). Além disso, Biasi et al. (2017) observou em um estudo laboratorial o aumento na colonização microbiana e na decomposição foliar ao adicionar nutrientes no microcosmos. No entanto, apesar do aumento na colonização de hifomicetos, essas mudanças na água podem ser responsáveis pela redução observada na diversidade microbiana (ABELHO e GRAÇA, 1996; JUVIGNY-KHENAFOU et al., 2019).

A biodiversidade de fungos aquáticos é controlada por diversos fatores ambientais. A vegetação ripária pode ser considerada um fator que regula a biodiversidade fúngica, dessa

forma, espera-se uma correlação positiva entre a diversidade da vegetação ripária (que fornece substrato para colonização) e diversidade fúngica (FABRE, 1996; KRAUSS et al., 2011). O pH também é um fator amplamente estudado, sendo que o número de hifomicetos aquáticos é normalmente alto em regiões cujo pH varia em 5 a 7, e declina em valores menores ou superiores a esses (BARLOCHER, 1987). No entanto, em casos como a acidificação antropogênica, além da acidificação ocorre o aumento na concentração de íons (e.g. alumínio), que podem reduzir severamente a riqueza e a atividade dos hifomicetos (CHAMIER, 1985; BAUDOIN et al., 2008).

A poluição por metais também afeta negativamente a comunidade fúngica, diminuindo a produção de esporos, biomassa e taxas de decomposição microbiana (LECERF e CHAUVET, 2008). Um importante fator que afeta as funções metabólicas e crescimento e sobrevivência dos microrganismos é a temperatura (MADIGAN et al., 2009). Normalmente os hifomicetos respondem a mudanças sazonais na temperatura principalmente em abundância de conídios e nas taxas de decomposição (BARLOCHER, 1992).

As assembleias fúngicas podem se adaptar às condições locais, no entanto, existem poucos estudos mostrando como as assembleias de hifomicetos aquáticos respondem à mudanças abruptas nas condições da água (PÉREZ et al., 2018). A compreensão e a determinação dos fatores ambientais que afetam a composição das assembleias de hifomicetos aquáticos é fundamental, uma vez que esses organismos apresentam um importante papel no funcionamento dos ecossistemas lóticos (GRAÇA et al., 2015). Dessa forma, o conhecimento desses fatores pode auxiliar em decisões de manejo e conservação

2. OBJETIVOS E ESTRUTURA DA DISSERTAÇÃO

Devido as lacunas no conhecimento da diversidade de hifomicetos aquáticos na região subtropical e sua grande importância no funcionamento do metabolismo aquático esta dissertação teve como objetivo geral avaliar a influência de fatores ambientais sobre a composição das assembleias de hifomicetos aquáticos, em riachos da região subtropical do Brasil.

A dissertação apresenta uma Introdução Geral, a qual contém informações conceituais sobre o tema do trabalho, um Capítulo apresentado na forma de manuscrito e

uma Conclusão Geral apresentando perspectivas de desdobramento do trabalho. O manuscrito intitulado **"Influence of environmental predictors on hyphomycetes assemblages in subtropical streams"** teve como objetivo avaliar a diversidade de hifomicetos aquáticos nos riachos, verificar quais preditores ambientais influenciam a distribuição desses organismos e investigar a existência de filtros ambientais para as assembleias de hifomicetos. Neste trabalho foi observado que a concentração de nutrientes é um importante fator estruturador das assembleias de hifomicetos aquáticos. Este manuscrito foi preparado e submetido ao periódico Acta Oecologica (Qualis/CAPES A3).

3. REFERÊNCIAS BIBLIOGRÁFICAS

ABELHO, M.; GRAÇA M. A. S. Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. **Hydrobiologia**, v. 324, p. 195–204, 1996.

BÄRLOCHER, F. Aquatic hyphomycete spora in 10 streams of New Brunswick and Nova Scotia. **Can J Bot,** v.65, p. 76–79, 1987.

BÄRLOCHER, F.; SEENA, S.; WILSON, K. P.; DUDLEY W. D. Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. **Freshwater Biology,** v. 53, n. 2, p. 368–379, 2008.

BÄRLOCHER, F. Community organization. **The ecology of aquatic hyphomycetes** (Bärlocher F. ed), pp 38–76. Springer - Verlag, Berlin, 1992.

BAUDOIN, J. M.; GUÉROLD, F.; FELTEN, V.; CHAUVET, E.; WAGNER, P.; ROUSSELLE P. Elevated Aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf-litter breakdown. **Microbial Ecology**, v.56, p.260–269, 2008.

BELLO, F. D.; LAVOREL, S.; LAVERGNE, S.; ALBERT, C. H.; BOULANGEAT, I.; MAZEL, F.; THUILLER, W. Hierarchical effects of environmental filters on the functional structure of plant communities: a case study in the French Alps. **Ecography**, v. 36, n. 3, p. 393–402, 2013.

BIASI, C.; FONTANA, L. E.; RESTELLO, R. M.; HEPP, L. U. Effect of invasive *Hovenia dulcis* on microbial decomposition and diversity of hyphomycetes in Atlantic forest streams. **Fungal Ecology***,* v. 44, 2020.

BIASI, C.; GRAÇA, M. A. S.; SANTOS, S.; FERREIRA, V. Nutrient enrichment in water more than in leaves affects aquatic microbial litter processing. **Oecologia**, v. 184, n. 2, p. 555–568, 2017.

CAO, P.; WANG, J. T.; HU, H.W.; ZHENG, Y. M.; GE, Y.; SHEN, J. P.; HE, J. Z. Environmental filtering process has more important roles than dispersal limitation in shaping large-scale prokaryotic beta diversity patterns of grassland soils. **Microbial Ecology**, v. 72, n. 1, p. 221–230, 2016.

CHAMIER, A. Cell-wall-degrading enzymes of aquatic hyphomycetes: a review. **Bot J Linn Soc,** v. 91, p. 67–81, 1985.

CHAPIN III, F. S.; ZAVALETA, E. S.; EVINER, V. T.; NAYLOR, R. L.; VITOUSEK, P. M.; REYNOLDS, H. L.; MACK, M. C. Consequences of changing biodiversity. **Nature**, v. 405, n. 6783, p. 234–242, 2000.

CONNELL, J. H. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. **Ecology**, p. 710–23, 1961.

COSTA, R. S.; MOLOZZI, J.; HEPP, L. U.; COSTA, D. F. D. S.; NUNES DA SILVA, P. R.; ROCHA, R. D. M.; BARBOSA, J. E. D. L. Influence of ecological filters on phytoplankton communities in semi-arid solar saltern environments. **Acta Limnologica Brasiliensia**, v. 27, n. 1, p. 39–50, 2015.

DUARTE, S.; PASCOAL, C.; CÁSSIO, F. High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams. **Microbial Ecology**, v. 56, n. 4, p. 688–695, 2008.

FABRE, E. Relationships between aquatic hyphomycetes communities and riparian vegetation in 3 Pyrenean streams. **CR Acad Sci III Vie** v. 319, p. 107–111, 1996.

FIUZA, P. O.; GUSMÃO, L. F. P. Ingoldian fungi from the semi-arid Caatinga biome of Brazil. **Mycosphere***, v.* 4, p. 1133–1150, 2013.

FIUZA, P. O.; PÉREZ, T.; GULIS, V.; GUSMÃO, L. Ingoldian fungi of Brazil: Some new

records and a review including a checklist and a key. **Phytotaxa**, v. 306, n. 3, p. 171–200, 2017.

GRAÇA, M. A. S. A conceptual model of litter breakdown in low order streams. **International Review of Hydrobiology**, v. 100, n. 1, p. 1–12, 2015.

GRAÇA, M. A. S.; HYDE, K.; CHAUVET, E. Aquatic hyphomycetes and litter decomposition in tropical - subtropical low order streams. **Fungal Ecology**, v. 19, p. 182–189, 2016.

HEINO, J.; MUOTKA, T.; PAAVOLA, R. Determinants of macroinvertebrate diversity in headwater streams: regional and local influences. **Journal of Animal Ecology**, v. 72, n. 3, p. 425–434, 2003.

HOLT, R. D. Predation, apparent competition, and the structure of prey communities. **Theoretical population biology,** v. 12, n. 2, p. 197-229, 1977.

JUVIGNY-KHENAFOU, N. P.; ZHANG, Y.; PIGGOTT, J. J.; ATKINSON, D.; MATTHAEI, C. D.; VAN BAEL, S. A.; WU, N. Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment. **Science of The Total Environment,** v. 716, 2019.

KEDDY, P. A. A pragmatic approach to functional ecology. **Functional Ecology**, v. 6, n. 6, p. 621–626, 1992.

KRAUSS, G. J. Fungi in freshwaters: Ecology, physiology and biochemical potential. **FEMS Microbiology Reviews**, v. 35, n. 4, p. 620–651, 2011.

LAITUNG, B.; CHAUVET, E. Vegetation diversity increases species richness of leaf-decaying fungal communities in woodland streams. **Archiv für Hydrobiologie**, v. 164, n. 2, p. 217–235, 2005.

LECERF, A.; CHAUVET, E. Diversity and functions of leaf- decaying fungi in human-altered streams**. Freshwater Biol,** v. 53, p. 1658–1672, 2008.

MADIGAN, M. T.; MARTINKO, J. M.; DUNLAP, P. V.; CLARK, D. P. **Biology of Microorganisms.** Pearson/Benjamin Cummings, San Francisco, 2009.

MYERS, J. A.; HARMS, K. E. Local immigration, competition from dominant guilds, and the ecological assembly of high-diversity pine savannas. **Ecology**, v. 90, n. 10, p. 2745–2754, 2009.

PÉREZ, J.; MARTÍNEZ, A.; DESCALS, E.; POZO, J. Responses of Aquatic Hyphomycetes to Temperature and Nutrient Availability: a Cross-transplantation Experiment. **Microbial Ecology**, v. 6, p. 328–339, 2018.

POFF, N. L. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. **Journal of the north american Benthological society,** v. 16, n. 2, p. 391–409, 1997.

SCHOELEN-CRUSIUS I. H; GRANDI, R. A. P. The diversity of aquatic Hyphomycetes in South America. **Brazilian Journal Microbiology**, v. 34, n. 3, 2003.

SHEARER, C. A.; DESCALS, E.; KOHLMEYER, B.; KOHLMEYER, J.; MARVANOVÁ, L.; PADGETT, D. Fungal biodiversity in aquatic habitats. **Biodiversity and Conservation**, v. 16, p. 49-67, 2007.

SOLÉ, M. Aquatic hyphomycete communities as potential bioindicators for assessing anthropogenic stress. **Science of the Total Environment**, v. 389, n. 2–3, p. 557–565, 2008.

STATZNER, B.; BECHE, L. A. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems?. **Freshwater Biology**, v. 55, p. 80–119, 2010.

TONN, W. M. Climate change and fish communities: a conceptual framework. **Transactions of the American Fisheries Society**, v. 119, n. 2, p. 337–352, 1990.

TONN, W. M.; MAGNUSON, J. J.; RASK, M.; TOIVONEN, J. Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. **The American Naturalist**, v. 136, n. 3, p. 345–375, 1990.

TOWNSEND, C. R.; DOLÉDEC, S.; NORRIS, R.; PEACOCK, K.; ARBUCKLE, C. The influence of scale and geography on relationships between stream community composition

and landscape variables: description and prediction. **Freshwater Biology**, v. 48, n. 5, p. 768–785, 2003.

USSEGLIO-POLATERA, P.; RICHOUX, P.; BOURNAUD, M.; TACHET, H. A functional classification of benthic macroinvertebrates based on biological and ecological traits: application to river condition assessment and stream management. **Archiv für Hydrobiologie. Supplementband. Monographische Beiträge**, v. 139, n. 1, p. 53–83, 2001.

4. INFLUENCE OF ENVIRONMENTAL PREDICTORS ON HYPHOMYCETES ASSEMBLAGES IN SUBTROPICAL STREAMS[1]

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Abstract

Hyphomycetes are important aquatic organisms to organic matter decomposition, releasing inorganic nutrients to the environment. Understanding distribution patterns of hyphomycetes among habitats of different quality can reveal important environmental characteristics that affect their assemblages, and by consequence, can change stream functioning. We evaluated the effects of environmental predictors over hyphomycetes assemblages in a field experiment in 12 subtropical Atlantic Forest streams. We identified 21 species of aquatic hyphomycetes, being *Lunulospora curvula*, *Flagellospora curvula* and *Aquanectria submersa* dominant species, occurring at 10 streams, while *Anguillospora* sp., *Campylospora parvula* and *Mycocentrospora acerina* were found in only one stream. The variables dissolved oxygen, electrical conductivity and pH influenced hyphomycetes assemblages distribution. The electrical conductivity act as filter to conidia abundance, while dissolved oxygen, electrical conductivity and phosphate act as environmental filter to species richness. Our findings highlight the importance of environmental predictors over aquatic hyphomycetes assemblages. The influence of the environmental predictors over abundance and species richness may also change the stream functioning, since these organisms play an important role in leaf breakdown. Also, since hyphomycetes respond to environmental changes observed in our study, they can be used as bioindicators.

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Keywords: fungal reproduction, bioindicators, aquatic fungi, streams functioning, leaf breakdown, environmental filters.

1. Introduction

Aquatic hyphomycetes are a polyphyletic group of true fungi, also known as freshwater hyphomycetes, “amphibian fungi” or ingoldian fungi (Bärlocher, 1992). These fungi are anamorphs (asexual stages) of ascomycetes or basidiomycetes and have a cyclical life history during litter decomposition, starting from colonization of fresh substrate and followed by mycelial growth and abundant sporulation (Gulis and Bärlocher, 2017). These microorganisms have a conidial shape, that allow its fixation on organic substrates and facilitate its dispersion in the aquatic lotic ecosystems (Graça et al. 2015; Chauvet et al. 2016). More than 600 species of aquatic hyphomycetes have been reported in freshwater ecosystems (Wong et al. 1998; Shearer et al. 2007). However, despite their cosmopolitan distribution, temperate regions had the highest number of described species (Silva and Smits, 2011). In Brazil, 85 taxa have been recorded in the Atlantic Forest biome (53 taxa), Caatinga (39), Cerrado (21) and Amazon (19) (Fiuza et al. 2017). Compared to other countries, the knowledge of aquatic hyphomycetes assemblages is deficient in subtropical regions (Biasi et al. 2020).

Aquatic hyphomycetes preferentially inhabit running, clear, clean and well-oxygenated waters (Krauss et al. 2011). However, these microrganisms can also be found in lentic freshwaters, stream banks, groundwaters, trees and polluted waters (Chauvet et al. 2016; Dasilva et al. 2019). Conidia can be dispersed in water, sticked to the foam, floating on water's surface or being associated with decomposing organic substrates, such as leaves and branches (Schoenlein-Crusius and Grandi, 2003). These fungi play an important role in freshwater ecosystem, since they perform ecosystem services related to organic matter decomposition and the release of inorganic nutrients essentials to environment (Dunck et al. 2015; Graça et al. 2015;).

In small order streams, allochthonous organic matter is the major source of carbon and energy for food webs (Vannote et al. 1980; Neres-Lima et al. 2017). Once in the water, leaf litter is colonized and conditioned, predominantly, by hyphomycetes (Gessner et al. 2007). They are involved in processing carbon in the following ways: carbon mineralization through respiration, maceration of plant tissues using extracellular enzymes and carbon incorporation from organic matter into biomass and spore production, increasing litter palatability for shredders and resulting in mass loss (Gulis and Suberkropp, 2003; Cornut et al. 2010; Graça et al. 2015).

Hyphomycetes are able to obtain nutrients from the colonized substrate or the water column (Suberkropp, 1998). Thus, it is expected that their reproductive activity can be influenced by water quality and also litter type and quality (e.g. leaf physical and chemical defenses), favoring the colonization of some species (Graça et al. 2015). However, several environmental factors can influence hyphomycetes assemblages, such as temperature (Bärlocher et al. 2008; Gonçalves et al. 2013), organic pollutants (Duarte et al. 2008), heavy metal (Solé et al. 2008, Bergmann and Graça, 2020) and dissolved nutrients (Sales et al. 2014, Biasi et al. 2017). Changes in water quality and leaf litter can lead to hyphomycetes biomass and/or richness reduction, affecting their role in ecosystem functioning (Solé et al. 2008). Fungal assemblages can adapt to local conditions, however, little is known about how they respond to abrupt changes in water conditions (Pérez et al. 2018).

It has been reported a reduction in aquatic fungi diversity with the increase of temperature (Bärlocher et al. 2008) and presence of pollutants (Duarte et al. 2008), while higher dissolved nutrients in water enhance microbial colonization and litter decomposition (Biasi et al. 2017). Also, the effect of pH has been reported, where microbial assemblages were structured by alkaline waters, especially because acid waters affect the enzymatic activity of fungi (Duarte et al. 2009). However, despite the increase of hyphomycetes conidia, those changes in water composition can reduce microbial diversity (Abelho and Graça, 1996; Juvigny-Khenafou et al. 2019). Any changes in these factors are normally caused by anthropogenic activities in areas adjacent to freshwater environments. Environmental conditions can be considered as filters affecting the composition of species (Chase, 2007). These filters acts selecting species that are present in a given site due to limiting environmental conditions (Tonn et al. 1990). Thus, only species that have attributes that give them the ability to get through such conditions will be able to remain and proper in a particular site (Poff, 1997). Evaluating hyphomycetes assemblages, environmental filters (e.g. riparian vegetation structure) can act on the spore production and mycelial growth (Biasi et al. 2020).

Comprehending how assemblages distribution patterns occurs is a classic concern to ecologists, and the effect of multiple environmental variables in hyphomycetes assemblages distribution and diversity remains unknown. Determining the factors that affects hyphomycetes composition is a critical for management decisions, since they play an important role in freshwater functioning (Graça et al. 2015). Since little is known about hyphomycetes in subtropical regions, understanding distribution patterns among different

quality habitats can reveal important environmental characteristics that affect their assemblages. In this study, we using a single standardize substrate to fungal colonization in Brazilian subtropical Atlantic Forest streams to assess the effects of environmental predictors over variation in assemblages composition of aquatic hyphomycetes. We expected that aquatic hyphomycetes assemblages would differed between the streams, according to their environmental condition, based on the evidence that aquatic fungi are selected by environmental predictors (Gulis and Bärlocher, 2017; Duarte et al. 2017; Pérez et al. 2018). Thus, we raised the following questions to be answered: (i) which is the diversity of aquatic hyphomycetes in the streams? (ii) Which environmental predictors influence aquatic hyphomycetes distribution? (iii) Is there any environmental filter to hyphomycetes assemblages?

2. Material and Methods

2.1 Study sites

We sampled 12 low order streams (≤3rd order) located in Southern Brazil, in the upper portion of the Uruguay River (Figure 1). The streams had a width 2 m, depth ≤ 0.2 m, and the bottom substrates consisted of stones, leaf packs, silt and sand. The region under study has a temperate subtropical climate (type Cfb according to Köppen), with average annual temperatures of 18ºC and annual mean rainfall of 1.500 mm (Alvares et al. 2013). Most of the region is covered mainly by agricultural and urban activities, and just 20% is covered by arboreal vegetation (Rovani et al. 2019). The vegetation belongs to the Atlantic Forest biome, and comprises a vegetative structure characterized by *Araucaria angustifolia* presence (Oliveira-Filho et al. 2015). Some of the streams studied were surrounded by agricultural practices, receiving influence from this activity. Others are located nearby urban perimeter, receiving influence from domestic and industrial waste, while some of them are considered minimally impacted, with natural riparian vegetation.

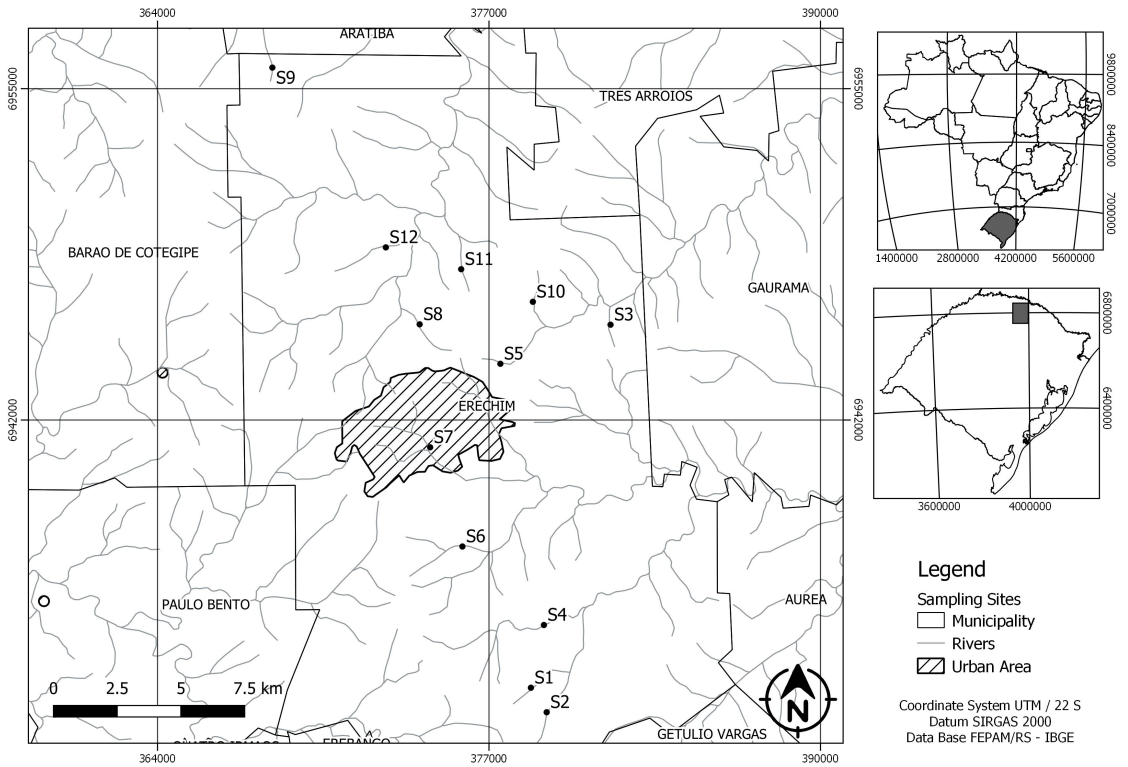

Figure 1. Location of studied subtropical streams (S) in Southern Brazil.

2.2 Environmental predictors

We measured during the study period (June – August, 2019) water temperature, electrical conductivity (EC), pH, dissolved oxygen (DO), turbidity, total dissolved solids (TDS) in loco with an HORIBA® U50 Multiparameter Analyzer. We collected and filtered water (0.45 µm mesh) for total dissolved nitrogen (TDN), total carbon (TC), inorganic carbon (IC) and dissolved organic carbon (DOC) analysis, using a Shimadzu® TOC analyzer. We also collected and froze 100 mL of water from each stream to evaluate anion (fluoride, chloride, nitrite, nitrate, sulfate) and cation (phosphate, sodium, ammonium, potassium, magnesium and calcium) concentrations by High Performance Liquid Chromatography (HPLC) method, using a Chromeleon© Dionex 1996-2008.

2.3 Aquatic hyphomycetes

We collected leaves of *Nectandra megapotamica* (Spreng.) Mez. (Lauraceae), a common specie of riparian vegetation in the region, that occurs in abundance (Oliveira- Filho

et al. 2006) just after abscission and air-dried (~20ºC, 5 days) (see Biasi et al. 2019). We standardize the substrate to single specie, in order to remove the effect of leaf quality on hyphomycetes colonization that allows us to evaluate the effect of the environmental predictors only. We weighted sets of 2.0 ± 0.1 g of leaves and enclosed in litter bags (15 × 20 cm, 0.5 mm mesh). Bags were tied with nylon lines to roots into the streambank (4 bags/stream) and retrieve 28 days after immersion. This period of immersion is appropriate for colonization by fungi on fresh substrates, which is later translated into spore production. The peak of microbial activity in decomposing leaves can vary from a few weeks (Biasi et al. 2019) to more than 30 days (Biasi et al. 2020), depending on the quality of the substrate and the amount of nutrients in the water. The remaining leaf-litter in each bag was rinsed with water. In the laboratory, eight leaf discs (12 mm diameter) were punched out (from eight different leaves) with a cork borer to stimulate aquatic hyphomycetes sporulation rates.

We incubate leaf discs in 125 mL Erlenmeyer flasks containing 35 mL of stream water on an orbital shaker (100 rpm) for 48 h at 18ºC. The resulting conidial suspensions were stored into 50-mL falcon tubes and fixed with 2 mL of 37% formalin (final concentration 10%). The eight leaf discs from each Erlenmeyer were oven-dried (550ºC/5h) and weighed to determine ash-free dry mass (AFDM). In order to guarantee conidia homogenization, we added 100 µL of Triton X-100 0.5% to each sample and kept in a magnetic stirrer. We filtered an aliquot of conidial suspension through a cellulose nitrate membrane filter (25 mm, 5-µm pore size). Filters were dyed with 0.05% Trypan Blue in 60% Lactic acid. We counted the spores (~200 conidia to standardize the sampling effort) and identified under a microscope (400× magnification) using Santos-Flores and Betancourt-Lopez (1997), Fiuza et al. (2017) and Gulis et al. (2020) identification keys. Sporulation rates were expressed as number of conidia released per ash-free dry mass per time.

2.4 Data analyses

Initially, we set up a matrix with environmental predictors for each stream and two matrices with hyphomycete composition data (i.e. presence/absence and conidia abundance) for each stream. Environmental predictors were standardized (range 0 – 1) to avoid data distortions due to the different magnitudes and measurement units of the variables. The conidia abundance values matrix was standardized using the Hellinger method. The environmental predictors were previously evaluated with Pearson correlation analysis to

exclude correlated variables (TDS, TC, IC, TDN, fluoride, nitrite, sodium and magnesium). After this selection, we order the streams according to their limnological characteristics using a Principal Component Analysis (PCA). We compared the species richness and abundance of hyphomycete conidia associated with detritus using a one-way ANOVA with Tukey post hoc test. We applied a Canonical Correspondence Analysis (CCA) between the environmental predictors and the richness of hyphomycetes and a second CCA between the environmental predictors and the abundance of fungi conidia. Finally, we evaluated the best predictive model for each of these biological matrices (i.e. presence/absence and abundance of conidia) using a permutation test (9999 repetitions), following a stepwise model (AIC) (Blanchet et al. 2008). This test allowed to identify the action of possible environmental filters on the hyphomycetes assemblages. All analysis was performed in software R (R Core Team, 2019) using functions of 'vegan' package (Oksanen et al. 2019).

3. Results

3.1 Hyphomycetes assemblages

We identified a total of 21 aquatic hyphomycetes species in the 12 studied streams (Table 1). The hyphomycetes richness varied from 4 species (stream S7) to 12 species (streams S2 and S4) ($F_{(11, 35)} = 6.6$; $p < 0.001$; Figure 2A, Table S1). *Lunulospora curvula* was a dominant specie, occurring in all streams, followed by *Aquanectria submersa,* that appear in 11 streams and *Flagellospora curvula,* in 10 streams (Table 1). The species *Anguillospora* sp., *Campylospora parvula* and *Mycocentrospora acerina* occurred only in one stream (Table 1). *L. curvula* and *F. curvula* together represent 86.3% of the total conidia counted (Figure 2C).

Sporulation rates varied from 8.5 (S7) to 259.6 conidia mg^{-1} DM day^{-1} (S3) ($F_{(11, 35)} = 4.0$; $p < 0.001$; Figure 2B, Table S2). *L. curvula* was the most sporulating specie in streams S3, S9, S10 and S11, followed by *F. curvula*. In the streams S5 and S7 which showed lower sporulation rates had the same pattern, where *F. curvula* was the most reproductive specie.

Table 1. Occurrence of aquatic hyphomycetes on 12 subtropical streams (S) in Southern Brazil.

Species	Species Code	Streams											
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Alatospora acuminata Ingold, 1942	*Ala_acu*		X	X			X		X				
Amniculicola longissima (Sacc. & P. Syd.) Nadeeshan & K.D. Hyde, 2016	*Amn_lon*	X	X		X		X	X	X			X	X
Anguillispora sp.	*Ang_sp*				X								
A. filiformis Greath., 1961	*Ang_fil*					X				X		X	
A. furtiva J. Webster & Descals, 1999	*Ang_fur*		X		X								
Aquanectria submersa (H.J. Huds.) L. Lombard & Crous, 2015	*Aqu_sub*	X	X	X	X	X	X		X	X	X	X	X
Articulospora tetracladia Ingold, 1942	*Art_tet*				X				X			X	
Campylospora chaetocladia Ranzoni, 1953	*Cam_cha*		X	X	X				X	X			
C. parvula Kuzuha, 1973	*Cam_par*				X								
Clavariopsis aquatica De Wild, 1895	*Cla_aqu*	X	X	X	X	X			X	X		X	
Flagellospora curvula Ingold, 1942	*Fla_cur*	X	X	X	X	X			X	X	X	X	X
Fusarium sp.	*Fus_sp*		X			X							
Heliscus tentaculus Umphlett, 1959	*Hel_ten*	X	X		X	X			X	X		X	X
Hydrocina chaetocladia Scheuer, 1991	*Hyd_cha*			X		X	X				X		
Lunulospora curvula Ingold, 1942	*Lun_cur*	X	X	X	X	X	X	X	X	X	X	X	X
Mycocentrospora acerina (Hartig) Deighton, 1972	*Myc_ace*	X											
Tetracladium marchalianum De Wild, 1893	*Tet_mar*									X		X	
Trematosphaeria sp.	*Ter_sp*		X				X						
Tripospermum myrti (Lind) S. Hughes, 1951	*Tri_myr*	X							X			X	
Triscelophorus acuminatus Nawawi, 1975	*Tri_acu*	X				X		X	X		X	X	X
T. monosporus Ingold, 1943	*Tri_mon*		X		X	X		X			X		X
Total species		9	12	7	12	10	6	4	11	8	6	11	7

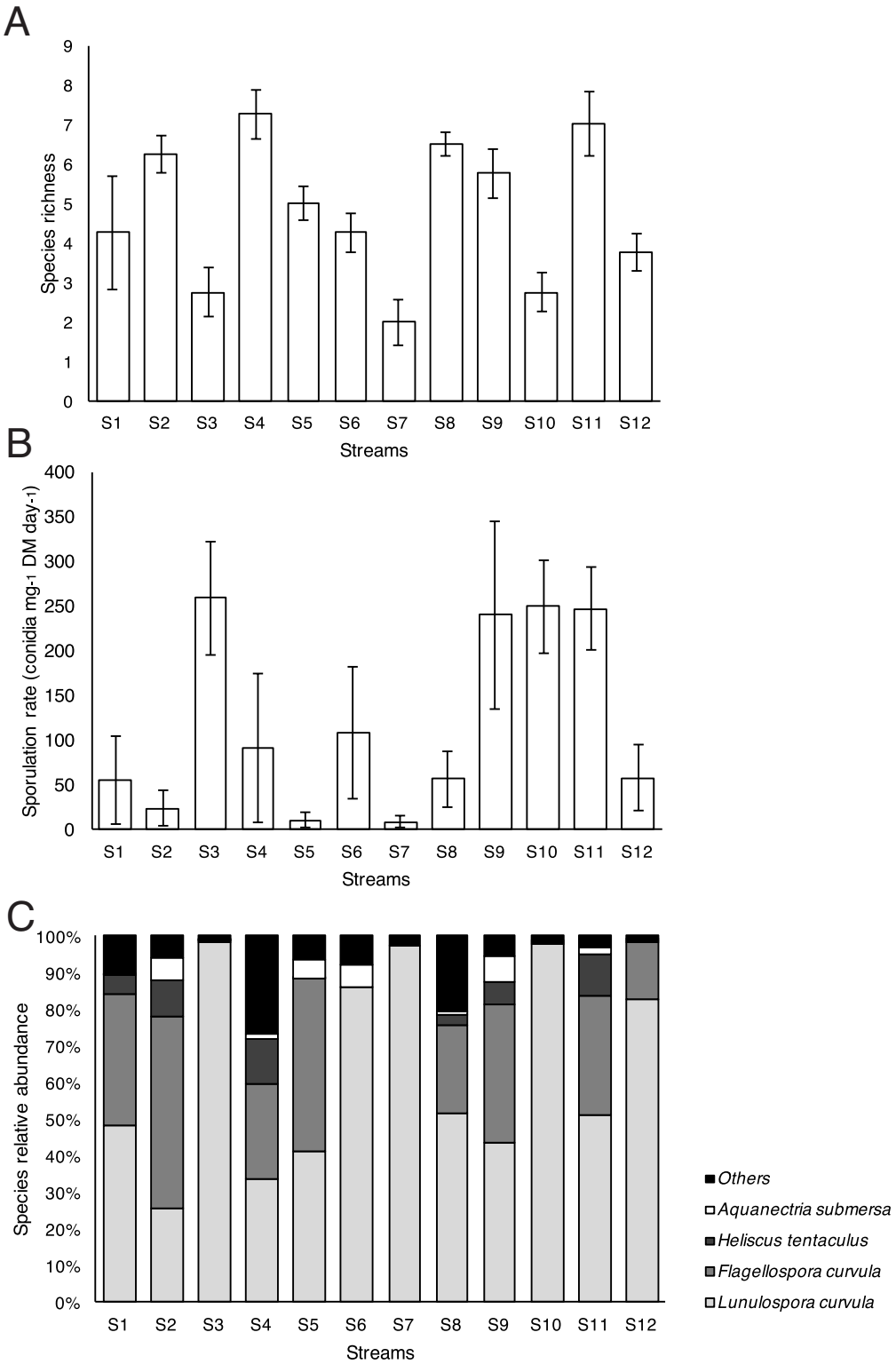

Figure 2. (A) Species richness (mean ± standard error), (B) sporulation rates (mean ± standard error) (B) and (C) species relative abundance of aquatic hyphomycetes on 12 subtropical streams in Southern Brazil.

3.2 Environmental predictors

Streams presented well oxygenated waters (9.21 ± 0.41 mg L^{-1}), circumneutral pH (7.35 ± 0.08) and electrical conductivity mean was 0.111 ± 0.021 mS cm^{-1} (Table 2). Concentrations of DOC ranged from 3.9 to 10.3 mg L^{-1}, TDS varied from 0.039 mg L^{-1} to 0.082 mg L^{-1}, while TDN ranged from 1.197 to 3.781 mg L^{-1} (Table 2). Sulfate content ranged from 0.561 to 9.419 mg L^{-1}, whereas chloride and nitrate ranged from 2.04 to 8.59 and 1.15 to 3.58 mg L^{-1}, respectively (Table 2).

The two first principal components of the PCA of environmental predictors explained 47% of total variation (Figure 3). High concentrations of potassium, dissolved oxygen and ammonia were positively correlated with PC1 (26%), while calcium concentrations were negatively correlated with PC1. The nitrate and phosphate concentrations were positively correlated with PC2 (21%), while chloride and turbidity were negatively correlated with PC2 (Figure 3).

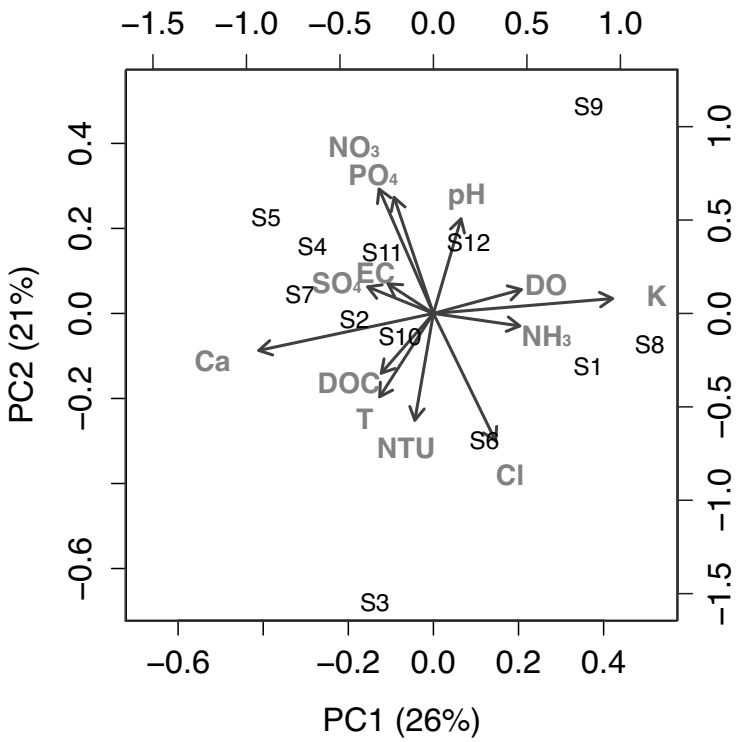

Figure 3. Principal component analysis to streams ordination according to their limnological characteriscs. Temperature (T), electrical conductivity (EC), turbidity

(NTU), pH, dissolved oxygen (DO), dissolved organic carbon (DOC), chloride (Cl), nitrate (NO_3), sulfate (SO_4), phosphate (PO_4), ammonium (NH_3), potassium (K) and calcium (Ca).

Table 2. Environmental variables (mean ± standard error) of 12 streams (S) in Southern Brazil. Electrical conductivity (EC), dissolved oxygen (DO), total carbon (TC), inorganic carbon (IC), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), fluoride (F), chloride (Cl), nitrite (NO2), nitrate (NO3), sulfate (SO4), phosphate (PO4), sodium (Na), ammonium (NH3), potassium (K), magnesium (Mg), calcium (Ca).

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Temperature (ºC)	14.6± 1.5	14.4 ± 1.7	17.8 ± 1.1	13.8 ± 1.4	15.7 ± 1.1	15.9 ± 0.8	18.3 ± 0.5	15.2 ± 0.7	14.2 ± 0.5	13.8 ± 1.4	15.2 ± 0.6	13.6 ± 0.9
EC (mS cm^{-1})	0.114 ± 0.001	0.117 ± 0.010	0.080 ± 0.004	0.343 ± 0.253	0.109 ± 0.009	0.063 ± 0.002	0.125 ± 0.006	0.083 ± 0.002	0.088 ± 0.002	0.060 ± 0.001	0.077 ± 0.002	0.080 ± 0.012
Turbidity (NTU)	10.3 ± 2.9	2.2 ± 1.4	35.4 ± 23.4	7.6 ± 5.1	7.9 ± 3.4	5.6 ± 2.2	0.9 ± 0.5	2.0 ± 1.4	2.5 ± 1.3	2.8 ± 2.8	1.2 ± 0.7	1.3 ± 0.8
TDS (g L^{-1})	0.074 ± 0.001	0.081 ± 0.002	0.044 ± 0.006	0.057 ± 0.001	0.068 ± 0.006	0.044 ± 0.002	0.082 ± 0.004	0.054 ± 0.001	0.056 ± 0.002	0.039 ± 0.001	0.050 ± 0.001	0.053 ± 0.008
pH	7.28 ± 0.34	7.52 ± 0.26	6.83 ± 0.19	7.60 ± 0.12	6.92 ± 0.32	7.28 ± 0.40	7.60 ± 0.03	7.40 ± 0.31	7.77 ± 0.06	7.32 ± 0.11	7.52 ± 0.18	7.22 ± 0.12
DO (mg L^{-}1)	9.64 ± 0.53	8.62 ± 0.09	9.16 ± 0.89	9.48 ± 0.58	6.63 ± 0.27	8.48 ± 0.98	7.97 ± 0.40	10.67 ± 1.93	11.78 ± 2.68	9.46 ± 0.90	10.72 ± 2.07	7.86 ± 0.54
TC (mg L^{-1})	18.00 ± 3.85	19.30 ± 4.58	17.86 ± 4.09	16.97 ± 3.99	18.85 ± 4.73	13.90 ± 3.71	19.06 ± 4.77	16.86 ± 4.24	13.76 ± 3.03	13.48 ± 3.74	16.21 ± 3.87	9.83 ± 1.65
IC (mg L^{-1})	8.38 ± 0.24	9.90 ± 0.80	7.94 ± 0.24	7.49 ± 0.50	8.45 ± 0.59	4.45 ± 0.19	9.12 ± 0.66	7.86 ± 0.46	7.04 ± 0.46	5.27 ± 0.14	7.38 ± 0.40	5.85 ± 0.68
DOC (mg L^{-1})	9.61 ± 3.77	9.39 ± 4.05	)9.92 ± 4.31	9.47 ± 3.74	10.39 ± 4.33	9.45 ± 3.89	9.94 ± 4.46	8.99 ± 3.90	6.72 ± 2.69	8.21 ± 3.60	8.82 ± 3.80	3.97 ± 1.52
TDN (mg L^{-1})	2.61 ± 0.13	2.64 ± 0.28	1.64 ± 0.19	1.73 ± 0.06	3.78 ± 0.54	2.24 ± 0.11	2.61 ± 0.06	1.38 ± 0.04	1.83 ± 0.06	1.36 ± 0.06	1.19 ± 0.10	2.15 ± 0.45
F (mg L^{-1})	0.088 ± 0.006	0.077 ± 0.011	0.105 ± 0.002	0.083 ± 0.017	0.095 ± 0.001	0.133 ± 0.030	0.098 ± 0.006	0.048 ± 0.006	0.084 ± 0.001	0.050 ± 0.001	0.084 ± 0.014	0.091 ± 0.009
Cl (mg L^{-1})	5.72 ± 0.08	6.19 ± 0.97	4.88 ± 0.14	3.75 ± 0.44	8.59 ± 0.43	4.75 ± 0.12	8.47 ± 0.60	1.61 ± 0.00	6.29 ± 0.59	2.04 ± 0.09	2.85 ± 0.39	2.42 ± 0.10
NO_2 (mg L^{-1})	0.02 ± 0.00	<0.01	<0.01	0.02 ± 0.00	0.13 ± 0.00	0.02 ± 0.00	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
NO_3 (mg L^{-1})	2.30 ± 0.03	3.35 ± 0.50	1.79 ± 0.24	2.03 ± 0.32	3.48 ± 0.39	2.44 ± 0.06	3.58 ± 0.10	0.75 ± 0.06	2.85 ± 0.31	1.23 ± 0.02	1.15 ± 0.16	2.84 ± 0.09
SO_4 (mg L^{-1})	1.40 ± 0.03	1.52 ± 0.27	1.26 ± 0.09	1.05 ± 0.09	6.64 ± 1.64	1.41 ± 0.00	9.41 ± 0.39	0.56 ± 0.02	2.69 ± 0.23	1.30 ± 0.01	1.38 ± 0.16	0.69 ± 0.05
PO_4 (mg L^{-1})	<0.1	<0.1	<0.1	<0.1	0.6 ± 0.09	<0.1	<0.1	<0.1	0.5 ± 0.01	0.0 ± 0.0	0.6 ± 0.10	<0.1
Na (mg L^{-1})	1.64 ± 0.31	3.67 ± 0.51	0.98 ± 0.13	3.71 ± 0.51	2.76 ± 0.00	1.79 ± 0.92	0.00 ± 0.00	5.33 ± 1.16	0.00 ± 0.00	0.00 ± 0.00	2.40 ± 0.55	2.90 ± 0.53
NH_3 (mg L^{-1})	0.24 ± 0.06	<0.01	0.19 ± 0.03	<0.01	<0.01	0.25 ± 0.00	0.25 ± 0.00	1.91 ± 0.00	0.17 ± 0.00	0.19 ± 0.00	<0.01	<0.01
K (mg L^{-1})	5.46 ±0.86	0.57 ± 0.00	1.12 ± 0.02	0.56 ± 0.00	1.03 ± 0.00	0.81 ± 0.05	0.93 ± 0.00	5.19 ± 0.93	4.68 ± 0.67	6.29 ± 0.48	2.07 ± 0.29	0.61 ± 0.09
Mg (mg L^{-1})	7.35 ± 0.17	6.00 ± 0.22	5.37 ± 0.04	6.04 ± 0.31	2.05 ± 1.13	3.25 ± 0.16	0.08 ± 0.00	6.30 ± 0.18	6.45 ± 0.41	9.24 ± 0.68	4.52 ± 0.43	6.64 ± 0.29
Ca (mg L^{-1})	6.49 ± 0.29	5.38 ± 0.55	5.70 ± 0.10	5.70 ± 0.28	3.39 ± 0.77	3.51 ± 0.19	5.40 ± 0.10	5.68 ± 0.07	6.01 ± 0.18	5.99 ± 0.32	5.40 ± 0.71	4.25 ± 0.11

3.3 Influence of environmental predictors on aquatic hyphomycetes

The association between environmental predictors with abundance of hyphomycetes explained 51% of total variation (Figure 4A). Highest temperatures and chloride concentrations were positively related to axis 1, while dissolved oxygen and pH were negatively related to this axis. Abundance of *Trematosphaeria* sp. and *Hydrocina chaetocladia* were related to chloride concentrations and high temperature, while *Tripospermum mirty* and *Heliscus tentaculus* showed higher abundance when associated with streams well oxygenated and circumneutral pH. Ammonium concentration were positively related with axis 2, while nitrate and phosphate were negatively related with this axis. Conidia number of *Articulospora tetracladia*, *Alastospora acuminata* and *Campylospora chaetocladia* were related to highest ammonium concentration, numbers of *Tetracladium marchalianum* and *Anguillospora filiformis* were related to highest concentrations of nitrate and phosphate.

In addition, environmental predictors explained 39% of hyphomycetes species richness total variation in the streams (Figure 4B). The variables pH, electrical conductivity and dissolved oxygen were positively related to first axis, while streams with higher temperatures were negatively associated with this axis. The species *Anguillospora* sp., *Articulospora tetracladia* and *Campylospora parvula* were positively related to axis 1, while *Hydrocina chaetocladia*, *Trematosphaeria* sp., and *Fusarium* sp. were negatively related to this axis. Second axis was positively correlated to higher values of electrical conductivity and negatively correlated to higher concentrations of nitrate and phosphate. The species *Trematosphaeria* sp., *Anguillospora furtiva* and *Anguillospora* sp. were positively related to this axis, while *Anguillospora filiformis*, *Fusarium* sp. and *Tetracladium marchalianum* were related to highest nitrate and phosphate concentrations.

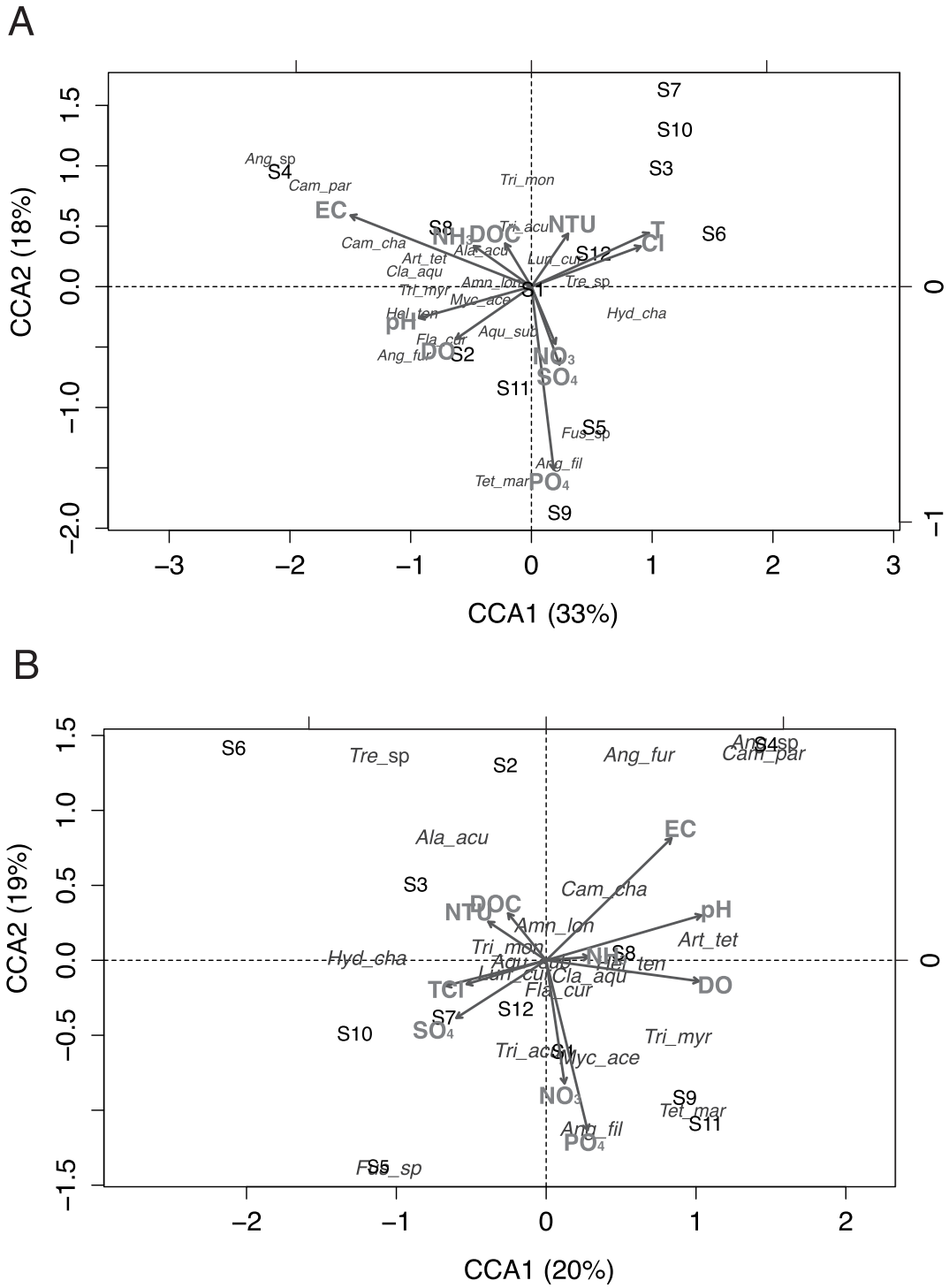

Figure 4. CCA between environmental predictors and (A) conidia abundance of hyphomycetes and (B) hyphomycetes richness. Temperature (T), electrical conductivity (EC), turbidity (NTU), pH, dissolved oxygen (DO), dissolved organic carbon (DOC),

chloride (Cl), nitrate (NO_3), sulfate (SO_4), phosphate (PO_4), ammonium (NH_3). Species code in table 1.

When we tested the models of selection of environmental predictors over hyphomycetes abundance, only electrical conductivity had significant influence over fungi (AIC = 76.9, p = 0.005). However, the best explaining method to hyphomycetes richness was composed by dissolved oxygen concentrations (AIC = 30.8, p = 0.01), phosphate concentrations (AIC = 30.8, p = 0.01) and electrical conductivity (AIC = 31.2, p = 0.005).

4. Discussion

In this study we evaluated the effects of environmental predictors on the structure of aquatic hyphomycete communities. We observed effects of environmental filters on the abundance of conidia and fungi richness. Variables related to water quality (e.g. oxygen, electrical conductivity and phosphorus) were the main environmental filters. In addition, we recorded ~25% of hyphomycetes diversity in Brazil and, considering the conservation status of the region under study (i.e. Subtropical Atlantic Forest), this number is expressive.

4.1 Hyphomycetes assemblages

Our study sampled 21 species of hyphomycetes in 12 streams, where *L. curvula*, *A. submersa* and *F. curvula* were the dominant species. The streams showed high environmental variability, presenting no pattern of ordination. The environmental predictors explained 51% of variation in abundance of species, and electrical conductivity had a significant influence over conidia abundance. However, the environmental predictors explained 39% in hyphomycetes richness, and had significant influence of dissolved oxygen, phosphate concentrations and electrical conductivity.

Previous studies revealed the influence of environmental variables on hyphomycetes assemblages (Duarte et al. 2017; Da Silva et al. 2019). However, there is a lack of studies evaluating those predictors in subtropical regions. Despite their key importance in the functioning of stream ecosystems, aquatic fungi, historically, have received much less attention than macroinvertebrates, algae, fishes, or even bacteria

(Gulis and Bärlocher, 2017). Our study demonstrated that variability in hyphomycetes assemblages in subtropical streams can be explained by environmental predictors.

The diversity of aquatic hyphomycetes found in our study is lower than previous study conducted by Biasi et al. (2020) in three subtropical streams. However, the authors found a total of 23 species on a mixed leaf substrate, and 16 species using a single substrate of *Hovenia dulcis*, while we sampled a total of 21 hyphomycetes species using a single substrate of a native tree specie (i.e. *Nectandra megapotamica*). In comparison to the Atlantic Forest biome, our study comprised a total of 40% of the diversity reported, and 25% of the diversity reported to the Brazil (Fiuza et al. 2017). When we evaluate the diversity of aquatic hyphomycetes worldwide, our study corresponds to 6% of the diversity. However, our study brings a substantial list of species occurring in subtropical streams, even with the use of a single substrate.

The most common species found in our study corroborates with Biasi et al. (2019, 2020), which also found *L. curvula*, *F. curvula* and *A. submersa* as dominant species in Atlantic Forest streams. Sigmoid species like *L. curvula* and *F. curvula* are both common in tropical and subtropical regions (Gessner and Chauvet, 1994; Gomes et al. 2016), being important in intermediate and late stages of foliar decomposition (Gomes et al. 2016). *L. curvula* is represented as dominant specie in tropical and subtropical ecosystems, structuring hyphomycetes assemblages in litter colonization (Sridhar et al. 2009; Rezende et al. 2017; Biasi et al. 2020). Also, *F. curvula* have cosmopolitan distribution, with a wide latitudinal gradient, ranging from artic to tropics (Jabiol et al. 2013). Many species are also reported in both conserved and impacted streams, like *A. longissima*, *A. filiformis* and *F. curvula*, suggesting that some hyphomycetes have a wide distribution and low environmental sensitivity (Fiuza and Gusmão, 2013; Fiuza et al. 2015). The exclusive species that occurred in only one stream (*Anguillospora* sp., *Campylospora parvula* and *M. acerina)* were collected in streams surrounded by agricultural land cover.

The species found in only one stream, like *C. parvula* were the first record in studies in Atlantic Forest. However, studies evaluating Caatinga Biome foams (Fiuza and Gusmão, 2013) and Cerrado submerged litter (Malosso, 1999) also reported the presence of this specie. *M. acerina* was also sampled in only one stream on our study, but was reported in previous studies in Atlantic Forest (Schoenlein-Crusius et al. 2009), Caatinga (Sales et al. 2014) and Cerrado (Malosso, 1999; Rezende et al. 2017). The rarity observed in these species may be due to differences in water chemistry. Some species are more sensitive to a few environmental predictors, and this may limit their occurrence in some

streams. Also, these species may reproduce less, limiting their dispersion and lowering substrate colonization, probably causing changes in streams functioning (Biasi et al. 2020).

4.2 Environmental Predictors

The studied streams did not show a clear pattern of ordination, demonstrating the environmental variability of the streams. However, the streams that presented higher concentration of dissolved oxygen, circumneutral pH and lower electrical conductivity are associated with natural condition sites, while sites located in areas with anthropogenic impact showed a higher nutrient concentration. These factors are important to some hyphomycetes species, since they provide great conditions to their occurrence. Previous studies have also reported the importance of pH and phosphorus concentrations to hyphomycetes assemblages, in addition to electrical conductivity and nutrients (Duarte et al. 2017). However, some species like *L. curvula* and *A. submersa* did not show any relationship with the factors evaluated. Thus, we can suggest that these species tolerate a broader range of ecological conditions and maintain their reproductive activity without any environmental restrictions.

We found that some environmental predictors were related to the species reported, as pH, electrical conductivity, dissolved oxygen and nutrients concentrations. Although aquatic fungi are considered relatively tolerant to anthropogenic changes (Pascoal et al. 2005), few studies showed no differences in fungal diversity and leaf litter processing (Pascoal et al. 2005). In our study, leaf litter incubated in different streams showed distinct conidia abundance, although species richness did not show the same pattern. This may suggest a certain level of functional redundancy in these assemblages (Ferreira and Chauvet, 2012). Some studies showed that the ecosystem functioning is less vulnerable to stress than hyphomycetes diversity (Bärlocher and Graça, 2002; Niyogi et al. 2013). They suggest that the functioning of ecosystem remains stable because of the increase of biomass of tolerant species support the loss of sensitive species. When the assemblages are impacted by several factors, such as those found in our study, the effects of hyphomycetes on ecosystem functioning can be masked (Pérez et al. 2013).

4.3 Environmetal filters to hyphomycetes assemblages

Electrical conductivity was the only environmental predictor measured that acted as filter to species sporulation. High electrical conductivity may be due to the input of organic matter that increases ion concentrations. Streams impacted by anthropogenic activities usually exhibit high values of ion concentrations (Salomoni et al. 2007; Wantzen and Mol, 2013). This factor can reduce the reproductive activity of hyphomycetes in the beginning of leaf decomposition (Sales et al. 2014), which affects their dispersal, since released conidia are carried downstream. Overall, our results show that some species (i.e. *L. curvula* and *A. submersa*) have wide distribution, and present no particular requirements and environmental conditions to spore production.

However, there are environmental predictors acted as filters to species richness. Concentration of dissolved oxygen is an important environmental factor that influences fungal activity, and its depletion can cause changes in structure of hyphomycetes assemblages (Medeiros et al. 2009), although some species tolerate hypoxic conditions, growing in these environments (Field and Webster 1983). Since, in general, hyphomycetes habit preferentially well aerated and clean waters (Krauss et al. 2011), low concentrations of dissolved oxygen may reflect low species richness when compared with high concentrations sites (Sales et al. 2014). A positive correlation of dissolved oxygen and hyphomycetes species richness was found in Indian streams (Rajashekhar and Kaveriappa, 2003).

Electrical conductivity and phosphate concentrations also had influence in hyphomycetes richness. These factors can be related to anthropogenic activities that surround the streams. Electrical conductivity can be easily related to streams with urban impact (Salomoni et al. 2007). Aquatic hyphomycetes assemblages are generally negatively correlated with conductivity (Wood-Eggenschwiler and Bärlocher, 1983; Solé et al. 2008) and soluble phosphorus (Duarte et al., 2017). The concentrations of nutrients like phosphate can reveal the process of eutrophication in streams (Hepp et al. 2013; Duarte et al., 2017), that can also be promoted by urban activities (Milesi et al. 2008; Hepp et al. 2010). Levels of nutrients (e.g. dissolved nitrogen and phosphorus) in water are biologically important to species numbers and spore production of aquatic hyphomycetes (Chamier, 1992), and seems stimulate *A. filiformis*, *Fusarium* sp. and *T. marchalianum*. All of these species (except *Fusarium*) had been found in polluted streams (Solé et al., 2008; Duarte et al. 2017). These factors were reported as influencers on hyphomycetes diversity (Duarte et al. 2017). This way, we can suggest that the environmental predictors selected are acting as selecting filters on the occurrence of

hyphomycetes species in the studied streams.

5. Conclusions

The number of species found in our study corresponds to one quarter of the total found in Brazil. Considering that our list may be underestimated, since we used a standard substrate, the number of species found is relatively high. We can also indicate that environmental variations, as the trophic state of streams, can play an important structuring role in the aquatic hyphomycetes assemblages and demonstrate ecological characteristics preferred by certain species of fungal assemblages. Our study contributes to the knowledge of hyphomycetes occurrence in subtropical streams, helping to understand patterns in the region, since their importance in aquatic ecosystem functioning. Also, we observed that aquatic hyphomycetes have a potential to serve as bioindicators, since they answered to environmental changes. However, there may be a lack in our study, since we performed the sampling using single leaf specie. This way, we suggest the sampling of leafs and natural foam already found in stream-bed that can increase the number of species in the region.

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8. References

Abelho, M., Graça, M.A.S. (1996). Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. *Hydrobiologia*. 324, 195–204. https://doi.org/10.1007/BF00016391

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes Gonçalves, J.L., Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*. 22, 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Bärlocher, F. (1992). Research on aquatic hyphomycetes: historical background and overview. In *The ecology of aquatic hyphomycetes* (pp. 1–15). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-76855-2_1

Bärlocher, F., Graça, M.A.S. (2002). Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. *Freshwater Biology*. 47(6), 1123-1135. https://doi.org/10.1046/j.1365-2427.2002.00836.x

Bärlocher, F., Seena, S., Wilson, K.P., Dudley W.D. (2008). Raised water temperature lowers diversity of hyporheic aquatic hyphomycetes. *Freshwater Biology*. 53(2), 368–379. https://doi.org/10.1111/j.1365-2427.2007.01899.x

Bergmann, M., Graça, M.A.S. (2020). Uranium affects growth, sporulation, biomass and leaf-litter decom-position by aquatic hyphomycetes. *Limnetica*. 39(1), 141–154. https://doi.org/10.23818/limn.39.10

Biasi, C., Cogo, G.B., Hepp, L.U., Santos, S. (2019). Shredders prefer soft and fungal-conditioned leaves, regardless of their initial chemical traits. *Iheringia. Série Zoologia*. 109. http://doi.org/10.1590/1678-4766e2019004

Biasi, C., Fontana, L.E., Restello, R.M., Hepp, L.U. (2020). Effect of invasive *Hovenia dulcis* on microbial decomposition and diversity of hyphomycetes in Atlantic forest streams. *Fungal Ecology*. 44, 100890. https://doi.org/10.1016/j.funeco.2019.100890

Biasi, C., Graça, M.A.S., Santos, S., Ferreira, V. (2017). Nutrient enrichment in water more than in leaves affects aquatic microbial litter processing. *Oecologia*. 184(2), 555–568. https://doi.org/10.1007/s00442-017-3869-5

Blanchet, F.G., Legendre, P., Borcard, D. (2008) Forward selection of explanatory variables. *Ecology.* 89:2623–2632. https://doi.org/10.1890/07-0986.1

Chamier A.C. (1992) Water Chemistry. In: Bärlocher F. (eds) *The Ecology of Aquatic Hyphomycetes. Ecological Studies (Analysis and Synthesis)*. 94. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-76855-2_8

Chase, J.M. (2007). Drought mediates the importance of stochastic community assembly. *Proceedings of the National Academy of Sciences*. 104(44), 17430–17434. https://doi.org/10.1073/pnas.0704350104

Chauvet, E., Cornut, J., Sridhar, K.R., Selosse, M. A.S., Bärlocher, F. (2016). Beyond the water column: aquatic hyphomycetes outside their preferred habitat. *Fungal Ecology*. 19, 112-127. https://doi.org/10.1016/j.funeco.2015.05.014

Da Silva, G.V.R., Castañeda-Ruiz, R.F., Malosso, E. (2019). Comparison of aquatic hyphomycetes communities between lotic and lentic environments in the Atlantic rain forest of Pernambuco, Northeast Brazil. *Fungal Biology*. 123(9), 660–668. https://doi.org/10.1016/j.funbio.2019.05.013

Duarte, S., Cássio, F., Pascoal, C., Bärlocher, F. (2017). Taxa-area relationship of aquatic fungi on deciduous leaves. *PloS one*. 12(7), e0181545. https://doi.org/10.1371/journal.pone.0181545

Duarte, S., Pascoal, C., Cássio, F. (2008). High diversity of fungi may mitigate the impact of pollution on plant litter decomposition in streams. *Microbial Ecology*. 56(4), 688–695. https://doi.org/10.1007/s00248-008-9388-5

Duarte, S., Pascoal, C., Garabétian, F., Cássio, F., Charcosset, J.Y. (2009). Microbial decomposer communities are mainly structured by trophic status in circumneutral and

alkaline streams. *Applied and Environmental Microbiology*. 75(19), 6211–6221. https://doi.org/10.1128/AEM.00971-09

Dunck, B., Lima-Fernandes, E., Cássio, F., Cunha, A., Rodrigues, L., Pascoal, C. (2015). Responses of primary production, leaf litter decomposition and associated communities to stream eutrophication. *Environmental Pollution*. 202, 32–40. https://doi.org/10.1016/j.envpol.2015.03.014

Ferreira, V., Chauvet, E. (2012). Changes in dominance among species in aquatic hyphomycete assemblages do not affect litter decomposition rates. *Aquatic Microbial Ecology*. 66(1), 1–11. https://doi.org/10.3354/ame01556

Field, J.I., Webster, J. (1983). Anaerobic survival of aquatic fungi. *Transactions of the British Mycological Society*. 81(2), 365–369. https://doi.org/10.1016/S00071536(83)80088-6

Fiuza, P.O., de Paiva Ottoni-Boldrini, B. M., Monteiro, J. S., Catena, N. R., Hamada, N., Gusmão, L. F. P. (2015). First records of Ingoldian fungi from the Brazilian Amazon. *Brazilian Journal of Botany*. 38(3), 615–621. https://doi.org/10.1007/s40415015-0157-7

Fiuza, P.O., Gusmão, L.F.P. (2013). Ingoldian fungi from the semi-arid Caatinga biome of Brazil. *Mycosphere*. 4(6), 559–565. https://doi.org/11331150.10.5943/mycosphere/4/6/10

Fiuza, P.O., Pérez, T.C., Gulis, V., Gusmão, L.F.P. (2017). Ingoldian fungi of Brazil: some new records and a review including a checklist and a key. *Phytotaxa*. 306(3), 171–200. https://doi.org/10.11646/phytotaxa.306.3.1

Gessner, M.O., Chauvet, E. (1994). Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*. 75(6), 1807–1817. https://doi.org/10.2307/1939639

Gessner, M.O., Gulis, V., Kuehn, K.A., Chauvet, E., Suberkropp, K. (2007). Fungal

decomposers of plant litter in aquatic ecosystems. *The Mycota: Microbial and Environmental Relationships*. Vol. 4 (Kubicek CP Druzhinina IS, eds), pp. 301–324. Springer, Berlin/Heidelberg, Germany.

Gomes, P., Medeiros, A.O., Gonçalves Jr., J.F. (2016). The replacement of native plants by exotic species may affect the colonization and reproduction of aquatic hyphomycetes. *Limnologica*. 59, 124–130. https://doi.org/10.1016/j.limno.2016.05.005

Gonçalves, A.L., Graça, M.A.S., Canhoto, C. (2013). The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. *Fungal Ecology.* 6(6), 546–553. https://doi.org/10.1016/j.funeco.2013.07.002

Graça, M.A.S., Ferreira, V., Canhoto, C., Encalada, A.C., Guerrero-Bolaño, F., Wantzen, K.M., Boyero, L. (2015). A conceptual model of litter breakdown in low order streams. *International Review of Hydrobiology*. 100(1), 1–12. https://doi.org/10.1002/iroh.201401757

Gulis, V., Bärlocher, F. (2017). Fungi: biomass, production, and community structure. In *Methods in Stream Ecology*. Vol 1, pp. 177–192. Academic Press. https://doi.org/10.1016/B978-0-12-416558-8.00010-X

Gulis, V., Marvanová, L., Descals, E. (2020). An illustrated key to the common temperate species of aquatic hyphomycetes. In *Methods to Study Litter Decomposition.* pp. 223–239, Second Edition. Springer, Dordrecht. https://doi.org/10.1007/978-3-030-30515-4

Gulis, V., Suberkropp, K. (2003). Effects of inorganic nutrients on relative contribu- tions of fungi and bacteria to carbon flow from submerged decomposing leaf litter. *Microbial Ecology*. 45(1), 11–19. https://doi.org/10.1007/s00248-002-1032-1

Hepp, L.U., Milesi, S.V., Biasi, C., Restello, R.M. (2010). Effects of agricultural and urban impacts on macroinvertebrates assemblages in streams (Rio Grande do Sul, Brazil). *Zoologia (Curitiba)*. 27(1), 106–113. http://dx.doi.org/10.1590/S1984-46702010000100016.

Hepp, L.U., Restello, R.M., Milesi, S.V., Biasi, C., Molozzi, J. (2013). Distribution of aquatic insects in urban headwater streams. *Acta Limnologica Brasiliensia*. 25(1), 1–9. https://doi.org/10.1590/S2179-975X2013005000014

Jabiol, J., Bruder, A., Gessner, M.O., Makkonen, M., Mckie, B.G., Peeters, E.T., Vos, V.C.A., Chauvet, E. (2013). Diversity patterns of leaf-associated aquatic hyphomycetes along a broad latitudinal gradient. *Fungal Ecology*. 6(5), 439–448. https://doi.org/10.1016/j.funeco.2013.04.002

Juvigny-Khenafou, N.P., Zhang, Y., Piggott, J.J., Atkinson, D., Matthaei, C.D., Van Bael, S.A., Wu, N. (2019). Anthropogenic stressors affect fungal more than bacterial communities in decaying leaf litter: A stream mesocosm experiment. *Science of The Total Environment*. 716, 135053. https://doi.org/10.1016/j.scitotenv.2019.135053

Krauss, G.J., Sole, M., Krauss, G., Schlosser, D., Wesenberg, D., Bärlocher, F. (2011). Fungi in freshwaters: ecology, physiology and biochemical potential. *FEMS Microbiology Reviews*. 35(4), 620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x

Malosso, E. (1999) *Hyphomycetes em ambientes aquáticos lótico e lêntico – ocorrência e biomassa.* MSc. Thesis, Universidade Federal de São Carlos, São Carlos, 98 pp.

Medeiros, A.O., Pascoal, C., Graça, M.A.S. (2009). Diversity and activity of aquatic fungi under low oxygen conditions. *Freshwater Biology*. 54(1), 142–149. https://doi.org/10.1111/j.1365-2427.2008.02101.x

Milesi, S.V., Biasi, C., Restello, R.M., Hepp, L.U. (2008). Efeito de metais Cobre (Cu) e Zinco (Zn) sobre a comunidade de macroinvertebrados bentônicos em riachos do sul do Brasil. *Acta Scientiarum. Biological Sciences*. 30(3), 283–289. https://doi.org/10.4025/actascibiolsci.v30i3.677

Neres-Lima, V.; Machado-Silva, F.; Baptista, D.F.; Oliveira, R.B.S.; Andrade, P.M.; Oliveira, A.F.; Sasada-Sato, C.Y.; Silva-Junior, E.F.; Feijó-Lima, R.; Angelini, R.; Camargo, P.B.; Moulton, T.P. (2017). Allochthonous and autochthonous carbon flows in

food webs of tropical forest streams. *Freshwater Biology*. 62(6), 1012–1023. https://doi.org/10.1111/fwb.12921

Niyogi, D.K., Harding, J.S., Simon, K.S. (2013). Organic matter breakdown as a measure of stream health in New Zealand streams affected by acid mine drainage. *Ecological Indicators*. 24, 510–517. https://doi.org/10.1016/j.ecolind.2012.08.003

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Stevens, M.H.H. (2019). *vegan: Community Ecology Package*. R package version 2.5. 4. 2019.

Oliveira-Filho, A.T., Budke, J.C., Jarenkow, J.A., Eisenlohr, P.V., Neves, D.R. (2015). Delving into the variations in tree species composition and richness across South American subtropical Atlantic and Pampean forests. *Journal of Plant Ecology*. 8(3), 242–260. https://doi.org/10.1093/jpe/rtt058

Oliveira-Filho, A.T., Jarenkow, J.A., Rodal, M.N. (2006). Floristic relationships of seasonally dry forests of eastern South America based on tree species distribution patterns. *Systematics Association Special Volume*. 69, 159. https://doi.org/10.1201/9781420004496.ch7

Pascoal, C., Marvanová, L., Cássio, F. (2005). Aquatic hyphomycete diversity in streams of Northwest Portugal. *Fungal Diversity*. 19(10).

Pérez, J., Basaguren, A., Descals, E., Larranaga, A., Pozo, J. (2013). Leaf-litter processing in headwater streams of northern Iberian Peninsula: moderate levels of eutrophication do not explain breakdown rates. *Hydrobiologia*. 718(1), 41–57. https://doi.org/10.1007/s10750-013-1610-x

Pérez, J., Martínez, A., Descals, E., Pozo, J. (2018). Responses of aquatic hyphomycetes to temperature and nutrient availability: a cross-transplantation experiment. *Microbial Ecology*. 76(2), 328–339. https://doi.org/10.1007/s00248-018-1148-6

Poff, N. L. (1997). Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. *Journal of the North American*

Benthological Society. 16(2), 391–409. https://doi.org/10.2307/1468026.

R Core Team. (2019). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Vienna, Austria.

Rajashekhar, M., Kaveriappa, K. M. (2003). Diversity of aquatic hyphomycetes in the aquatic ecosystems of the Western Ghats of India. *Hydrobiologia.* 501(1-3), 167–177. https://doi.org/10.1023/A:1026239917232

Rezende, R.S., Sales, M.A., Hurbath, F., Roque, N., Gonçalves Jr, J.F., Medeiros, A.O. (2017). Effect of plant richness on the dynamics of coarse particulate organic matter in a Brazilian Savannah stream. *Limnologica*. 63, 57–64. https://doi.org/10.1016/j.limno.2017.02.002

Rovani, I.L., dos Santos, J.E., Decian, V.S., Zanin, E.M. (2019). Assessing Naturalness Changes Resulting from a Historical Land Use in Brazil South Region: An Analysis of the 1986-2016 Period. *Journal of Environmental Protection*. 10(2), 149–163. https://doi.org/10.4236/jep.2019.102010

Sales, M.A., Gonçalves, J.F., Dahora, J.S., Medeiros, A.O. (2014). Influence of leaf quality in microbial decomposition in a headwater stream in the Brazilian Cerrado: a 1-year study. *Microbial Ecology*. 69, 84–94. https://doi.org/10.1007/s00248-014-0467-5

Salomoni, S.E., Rocha, O., Leite, E.H. (2007). Limnological characterization of Gravataí River, Rio Grande do Sul State, Brazil. *Acta Limnologica Brasiliensia*, 19(1), 1–14.

Santos-Flores, C. J., Betancourt-López, C. (1997). Aquatic and water-borne Hyphomycetes (Deuteromycotina): in streams of Puerto Rico, including records from other Neotropical locations (No. 2). College of Arts and Sciences, University of Puerto Rico.

Schoenlein-Crusius, I.H., Grandi, R.A.P. (2003). The diversity of aquatic hyphomycetes in South America. *Brazilian Journal of Microbiology*. 34(3), 183–193. https://doi.org/10.1590/S1517-83822003000300001

Schoenlein-Crusius, I.H., Moreira, C.G., Bicudo, D.D.C. (2009). Aquatic Hyphomycetes in the Parque Estadual das Fontes do Ipiranga - PEFI, São Paulo, Brazil. *Brazilian Journal of Botany*. 32(3), 411–426. https://doi.org/10.1590/S0100-84042009000300003

Shearer, C.A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H.A., Schmit, J.P., Thorton, H.A., Voglymayr, H. (2007). Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*. 16(1), 49–67. https://doi.org/10.1007/s10531-006-9120-z

Silva, R.F., Smits B.G. (2011). Aquatic hyphomycetes from the headwaters of the Guarico river, state Carabobo, Venezuela. *Interciencia*. 36(11), 831–834.

Solé, M., Fetzer, I., Wennrich, R., Sridhar, K.R., Harms, H., Krauss, G. (2008). Aquatic hyphomycete communities as potential bioindicators for assessing anthropogenic stress. *Science of the Total Environment*. 389(2-3), 557–565. https://doi.org/10.1016/j.scitotenv.2007.09.010

Sridhar, K.R., Duarte, S., Cássio, F., Pascoal, C. (2009). The role of early fungal colonizers in leaf-litter decomposition in Portuguese streams impacted by agricultural runoff. *International Review of Hydrobiology*. 94(4), 399–409. https://doi.org/10.1002/iroh.200811154

Suberkropp, K. (1998). Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. *Mycological Research*. 102(8), 998–1002. https://doi.org/10.1017/S0953756297005807

Tonn, W.M., Magnuson, J.J., Rask, M., Toivonen, J. (1990). Intercontinental comparison of small-lake fish assemblages: the balance between local and regional processes. *The American Naturalist*. 136(3), 345–375. https://doi.org/10.1086/285102

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., Cushing, C.E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*. 37(1), 130–137. https://doi.org/10.1139/f80-017

Wantzen, K.M., Mol, J.H. (2013). Soil erosion from agriculture and mining: a threat to tropical stream ecosystems. *Agriculture*. 3(4), 660–683. https://doi.org/10.3390/agriculture3040660

Wong, M.K., Goh, T.K., Hodgkiss, I. J., Hyde, K.D., Ranghoo, V.M., Tsui, C.K., Ho, W.H, Wong, W.S.W, Yuen, T.K. (1998). Role of fungi in freshwater ecosystems. *Biodiversity & Conservation*. 7(9), 1187–1206. https://doi.org/10.1023/A:1008883716975

Wood-Eggenschwiler, S., Bärlocher, F. (1983). Aquatic hyphomycetes in sixteen streams in France, Germany and Switzerland. *Transactions of the British Mycological Society*. 81(2), 371–379. https://doi.org/10.1016/S0007-1536(83)80089-8.

9. Supplementary material

Table S1. Tukey test summary (p-values) to species richness of aquatic hyphomycetes on 12 subtropical streams in Southern Brazil. Significant results in bold ($p<0.05$).

	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
S1	0.621	0.905	0.108	1.000	1.000	0.563	0.450	0.905	0.905	0.185	1.000
S2	—	**0.031**	0.995	0.971	0.621	**0.009**	1.000	1.000	**0.031**	1.000	0.299
S3	—	—	**0.002**	0.450	0.905	1.000	**0.016**	0.108	1.000	**0.004**	0.995
S4	—	—	—	0.450	0.108	**0.001**	1.000	0.905	**0.002**	1.000	**0.031**
S5	—	—	—	—	1.000	0.175	0.905	1.000	0.450	0.621	0.971
S6	—	—	—	—	—	0.563	0.450	0.905	0.905	0.185	1.000
S7	—	—	—	—	—	—	**0.005**	0.034	1.000	**0.001**	0.853
S8	—	—	—	—	—	—	—	1.000	**0.016**	1.000	0.185
S9	—	—	—	—	—	—	—	—	0.108	0.971	0.621
S10	—	—	—	—	—	—	—	—	—	**0.004**	0.995
S11	—	—	—	—	—	—	—	—	—	—	0.059

Table S2. Tukey test summary (p-values) to sporulation rates of aquatic hyphomycetes on 12 subtropical streams in Southern Brazil. Significant results in bold (p<0.05).

	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
S1	1.000	0.185	1.000	1.000	0.914	1.000	1.000	0.414	0.199	0.197	0.999
S2	—	0.062	0.992	1.000	0.651	1.000	0.991	0.171	0.067	0.067	0.969
S3	—	—	0.497	**0.031**	0.966	0.059	0.505	1.000	1.000	1.000	0.640
S4	—	—	—	0.956	0.998	0.973	1.000	0.792	0.522	0.519	1.000
S5	—	—	—	—	0.463	1.000	0.953	0.093	**0.034**	**0.033**	0.891
S6	—	—	—	—	—	0.572	0.998	0.999	0.972	0.972	1.000
S7	—	—	—	—	—	—	0.972	0.153	0.064	0.063	0.929
S8	—	—	—	—	—	—	—	0.799	0.530	0.527	1.000
S9	—	—	—	—	—	—	—	—	1.000	1.000	0.894
S10	—	—	—	—	—	—	—	—	—	1.000	0.664
S11	—	—	—	—	—	—	—	—	—	—	0.662

5. CONCLUSÃO GERAL E PERSPECTIVAS

Nessa dissertação avaliamos a influência dos fatores ambientais sobre a composição das assembleias de hifomicetos aquáticos em riachos localizados na região subtropical do Brasil. Observamos a influência de variáveis ambientais como condutividade elétrica, oxigênio dissolvido e nutrientes sobre a abundância e riqueza das assembleias, além de verificarmos o potencial uso dos hifomicetos aquáticos como bioindicadores. Pudemos constatar uma substancial diversidade de espécies de hifomicetos aquáticos em riachos subtropicais, assim como observarmos a importância das variações ambientais na estruturação das assembleias de hifomicetos aquáticos, além de demonstrar características ecológicas preferidas por algumas espécies. A influência direta da condutividade elétrica na abundância de hifomicetos e a influência da condutividade, teores de oxigênio dissolvido e fosfato na riqueza das assembleias de hifomicetos aquáticos demonstra a sensibilidade ambiental desses microrganismos às alterações ambientais, dentre elas, degradação de habitats. Dessa forma, ainda, constatamos a possibilidade do uso desses organismos como bioindicadores, uma vez que eles responderam às mudanças ambientais, como teores de oxigênio e concentração de nutrientes.

Estudos na região subtropical do Brasil, avaliando as assembleias de hifomicetos aquáticos ainda são incipientes. Esse é um trabalho pioneiro no estudo de hifomicetos na perspectiva de ocorrência e distribuição de espécies em riachos localizados na região subtropical. O número de espécies identificadas nesse estudo corresponde a ¼ do total encontrado no Brasil. No entanto, podemos considerar esse número relativamente alto, considerando que utilizamos um único substrato para colonização.

Neste sentido, a realização de estudos com a coleta de bancos de folhas e espumas naturais nos riachos pode ser promissor para ampliar a quantidade de espécies identificadas. A diversidade de folhas, com variada qualidade nutricional e tempo dentro do riacho podem favorecer uma assembleia diferenciada de hifomicetos aquáticos. Além disso, esses organismos podem ser encontrados aderidos às espumas naturais dos riachos, em sua forma reprodutiva. Sendo assim, ampliando as coletas para diferentes substratos (bancos de folha e espumas), poderemos aumentar a lista de espécies para a região. Além disso, uma vez que as espécies de hifomicetos aquáticos parecem tolerar determinados fatores bióticos e abióticos, estudos visando a interpretação dos processos de nicho e neutro são importantes para avaliar a dinâmica das espécies nas assembleias.

Devido ao importante papel realizado pelos hifomicetos no funci.namento dos ecossistemas aquáticos, estudos como esse são de suma importância a fim de compreender quais fatores podem estar influenciando na ocorrência e distribuição desses organismos.