STREAM INTEGRITY IN OCEANIC ISLANDS – A FUNCTIONAL APPROACH USING ORGANIC MATTER DECOMPOSITION

Tese de Doutoramento

Ana Balibrea Escobar

Doutoramento em Biologia

SICUT AURORA SCIENTIA LUCET

UNIVERSIDADE DOS AÇORES

Ponta Delgada
2025

STREAM INTEGRITY IN OCEANIC ISLANDS – A FUNCTIONAL APPROACH USING ORGANIC MATTER DECOMPOSITION

Doctoral thesis submitted as a partial requirement for obtaining the degree of Doctor in Biology

University of Azores, Department of Biology

Ponta Delgada

2025

Ana Balibrea Escobar

Supervisors:

Professor Vítor Gonçalves

Doctor Pedro Miguel Raposeiro

Doctor Verónica Ferreira

INTEGRIDADE DOS RIBEIROS EM ILHAS OCEÂNICAS – UMA ABORDAGEM FUNCIONAL UTILIZANDO A DECOMPOSIÇÃO DE MATÉRIA ORGÂNICA

Tese de Doutoramento submetida como requisito parcial para obtenção do grau de Doutor em Biologia

Universidade dos Açores, Departamento de Biologia

Ponta Delgada

2025

Ana Balibrea Escobar

Orientadores:

Professor Doutor Vítor Gonçalves

Doutor Pedro Miguel Raposeiro

Doutora Verónica Ferreira

INSTITUTIONS AND FINANTIAL SUPPORT

Funded by:

Doctoral Grant awarded by FRCT-Fundo Regional da Ciência e Tecnologia (SRMCT-Secretaria Regional do Mar, Ciência e Tecnologia, Governo Regional dos Açores; Refª M3.1. a/F/040/2020.

Developed at:

Faculty of Sciences and Technology, University of the Azores and CIBIO, Research Center in Biodiversity and Genetic Resources, InBIO Associate Laboratory, BIOPOLIS Program in Genomics, Biodiversity and Land Planning – UNESCO Chair – Land Within Sea: Biodiversity & Sustainability in Atlantic Islands, University of the Azores, Rua da Mãe de Deus, 9500-321 Ponta Delgada, Portugal.

With the support of:

This work was supported by FCT, I.P., the Portuguese national funding agency for science, research, and technology, under the Projects UIDB/50027/2020 & UIDP50027/2020 (CIBIO/InBIO), UIDP/04292/2020 & UIDB/04292/2020 (MARE) and LA/P/0069/2020 (ARNET).

Projeto de investigação financiado pelo Fundo Regional da Ciência e Tecnologia (FRCT), Governo dos Açores, Bolsa de Doutoramento, M3.1.a/F/040/2020 (Programa PRO-SCIENTIA).

DECLARATION OF AUTHORSHIP

I, Ana Balibrea Escobar, hereby declare that this thesis and work presented here it was developed by me as student of the Doctoral Program in Biology at the University of Azores.

I further declare that data and results from Chapter 3 were published over the course of this thesis project. The published paper has the following reference.

Balibrea, A., Ferreira, V., Gonçalves, V., & Raposeiro, P. M. (2023). Effects of leaf litter naturally enriched with metals on consumption, growth, and survival of an endemic Azorean shredder. Aquatic Sciences, 85(2), 65.

Headwaters of Guilherme Stream, Tronqueira, São Miguel, Azores

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisors, Vítor Gonçalves, Pedro M. Raposeiro and Verónica Ferreira. Thank you, Vítor, for your support and the insight you brought to every aspect of this work. Thank you, Pedro, for trusting me and for the years of guidance that have been as challenging as they have been rewarding. Thank you, Verónica, for your expert advice and constructive feedback throughout the entire thesis process, your support was invaluable, especially during moments when deadlines were pressing and creative ideas for continuing the writing process were scarce.

My thanks extend to my colleagues from FRESCO as well. I am particularly grateful to Martin and Bea for the enjoyable and entertaining fieldwork excursions that made our collective efforts not only productive but also filled with laughs and light-hearted moments. A heartfelt thanks goes to Mª Angeles Vila Perea for her continuous dedication and invaluable help during the fieldwork for Chapter 2 and to Núria Plana for her persistent support and assistance during the fieldwork of Chapter 3.

I also owe a great debt of gratitude to my family, father, mother, and sister, for their unwavering support through every up and down. Your love and encouragement have been the steady beacon that guided me through the most challenging days during this period and the pillar on which I commemorated every joy and success. A special thanks also goes to my Tia Carmen for her continuous words of motivation to lifted me up and to la Gordita, my beloved grandmother, whose soul now shines down on me like a brilliant star, inspiring me to keep moving forward.

Each one of you has contributed in a unique way to the realization of this thesis. Thank you all for being part of this unforgettable journey!

TABLE OF CONTENTS

ABSTRACT

This thesis presents an integrative evaluation of stream ecosystem functioning in the Azores, focusing on how land-use changes and natural metal enrichment derived from volcanic activity affect organic matter decomposition, an essential process that determines freshwater ecosystem integrity. Through field experiments and laboratory trials, this work demonstrates that both anthropogenic and natural stressors interact to alter the physical environment and biotic community structure of island streams, leading to variations in decomposition dynamics.

To evaluate the impact of riparian forest modifications (cryptomeria plantations and pastures) on organic matter decomposition in streams, a field experiment was conducted to compare these altered environments with non-impacted streams dominated by native laurel vegetation. The study used *Ochroma pyramidale* wood and *Clethra arborea* leaves as substrates, enclosed in fine and coarse mesh bags, to differentiate between microbial-driven decomposition and decomposition mediated by both microbes and macroinvertebrates, respectively. Land-use changes, particularly the conversion of native laurel forests to pastures and cryptomeria plantations, significantly influenced decomposition rates. While microbial-driven decomposition remained stable across native and commercial plantation streams, suggesting potential functional redundancy of decomposer communities, pasture streams exhibited enhanced decomposition related to higher water temperatures and nutrient content. Shredder contributions, particularly by endemic taxa such as *Limnephilus atlanticus*, were stream-specific and strongly shaped by habitat features and elevation.

Metal enrichment of streams due to volcanic activity constitutes a specific environmental pressure from natural origin that influences the organic matter decomposition process. To evaluate the impact of natural metal enrichment on shredders and microbial decomposition, two microcosm experiments were conducted. The first experiment assessed the feeding preferences, consumption rates, growth, and survival of the endemic Azorean shredder caddisfly *Limnephilus atlanticus*. We used three types of leaf litter: the high-quality exotic *Alnus glutinosa* (as control) and the more recalcitrant native species *Ilex perado* and *Laurus azorica*. These were submerged in a metal-enriched

stream and a reference stream with low metal concentrations. The second experiment focused on microbial decomposer responses. Leaf litter from *Clethra arborea* was initially submerged in two streams with contrasting metal concentrations (low and high). Subsequently, the litter was incubated in six water treatments, representing a gradient of metal concentrations: 100% (only metal-enriched stream water), 75%, 50%, 25%, 10%, and 0% (only reference stream water). Microbial decomposition was assessed through leaf litter mass loss and aquatic hyphomycete responses, including sporulation rate, taxa richness, and community structure. Metal deposition on leaf litter surfaces reduced palatability for shredders and altered microbial colonization, leading to diminished fungal sporulation and decomposition efficiency. These effects were more pronounced under high metal concentrations and were influenced by leaf litter traits, such as toughness and secondary compounds content. Evidence of compensatory feeding and hormesis responses in decomposers underscored the complexity of biotic responses to metal stress.

Together, these findings reinforced the value of organic matter decomposition as a functional indicator of ecosystem integrity. However, they also highlighted the necessity of context-specific interpretation, especially in metal-enriched environments. While this work contributes to a deeper understanding of ecosystem processes on oceanic islands, further in situ studies are needed to calibrate these tools under natural environmental variability. Ultimately, this thesis emphasizes the importance of incorporating functional indicators alongside structural metrics in freshwater bioassessment frameworks to enhance conservation strategies in island ecosystems.

RESUMO

Esta tese apresenta uma avaliação integrada do funcionamento dos ecossistemas ribeirinhos nos Açores, focando-se no efeito das alterações no uso do solo e enriquecimento natural em metais na decomposição da matéria orgânica, um processo essencial determinante para a integridade dos ecossistemas de água doce. Através de experiências de campo e ensaios em laboratório, este trabalho demonstra que os fatores de stress, tanto antrópicos quanto naturais, interagem para alterar o ambiente físico e a estrutura das comunidades bióticas dos ribeiros insulares, resultando em alterações nas dinâmicas de decomposição.

Para avaliar o impacto das modificações na vegetação ripária (plantações de criptoméria e pastagens) na decomposição da matéria orgânica nos ribeiros, realizou-se uma experiência de campo para comparar estes ambientes alterados com ribeiros rodeados por vegetação nativa. Neste estudo usou-se madeira de *Ochromona pyramidale* e folhas de *Clethra arborea* dentro de sacos de malha fina e grossa para diferenciar a decomposição exclusivamente microbiana da realizada conjuntamente por microrganismos e macroinvertebrados, respetivamente. As mudanças no uso do solo, especialmente a conversão de florestas nativas de Laurissilva em pastagens e plantações de criptoméria, influenciaram significativamente a taxa de decomposição. Enquanto a decomposição mediada por microrganismos manteve-se semelhante em ribeiros em floresta nativa e plantações comerciais, sugerindo uma possível redundância funcional das comunidades decompositoras, os ribeiros em áreas de pastagem apresentaram uma decomposição mais acelerada, relacionada com temperatura da água e concentração de nutrientes mais elevadas. A contribuição dos detritívoros, especialmente da espécie endémica *Limnephilus atlanticus*, foi específica de cada ribeiro e fortemente influenciada pelas características do habitat e altitude.

O enriquecimento dos ribeiros em metais, resultante de manifestações de vulcanismo, constitui uma pressão ambiental de origem natural que influencia o processo de decomposição da matéria orgânica. Para avaliar o impacto desse enriquecimento natural em metais sobre a decomposição mediada por microrganismos e detritívoros foram realizadas duas experiências em microcosmos. Na

primeira experiência avaliou-se a preferência alimentar, taxa de consumo, crescimento e sobrevivência do detritívoro endémico *Limnephilus atlanticus* alimentado com três tipos de folhada, *Alnus glutinosa,* espécie exótica de elevada qualidade (controlo) e *Ilex perado* e *Laurus azorica,* duas espécies nativas mais recalcitrantes - previamente submersa num ribeiro naturalmente enriquecido em metais e num ribeiro com baixa concentração de metais. Numa segunda experiência, focada na resposta da decomposição microbiana, folhada de *Clethra arborea* foi submersa em dois ribeiros com concentrações de metais contrastantes (baixa e elevada) e depois encubada em seis tratamentos representativos de um gradiente de concentração de metais: 100% (água do ribeiro naturalmente enriquecido em metais), 75%, 50%, 25%, 10% e 0% (água do ribeiro de referência). A decomposição microbiana foi avaliada através da perda de massa da folhada, e taxa de esporulação, riqueza específica e estrutura das comunidades de hifomicetes aquáticos. A deposição de metais sobre a superfície foliar reduziu a sua palatabilidade para os detritívoros e alterou a colonização microbiana, levando à diminuição da esporulação dos fungos aquáticos e da eficiência do processo de decomposição. Esses efeitos foram mais acentuados sob altas concentrações de metais e foram influenciados por características intrínsecas dos substratos, como dureza e teor de compostos secundários. Evidências de compensação nas taxas de alimentação e respostas do tipo hormese nos decompositores demostraram a complexidade das respostas bióticas ao stress por metais.

Conjuntamente, estes resultados reforçam o valor da decomposição da matéria orgânica como um indicador funcional da integridade dos ecossistemas. No entanto, também evidenciam a necessidade de interpretações contextualizadas, especialmente em ambientes naturalmente enriquecidos em metais. Embora este trabalho contribua para um entendimento mais profundo dos processos ecológicos em ilhas oceânicas, são necessários mais estudos in situ para calibrar essas ferramentas em cenários ambientais naturais. Finalmente, esta tese ressalta a importância de incorporar indicadores funcionais juntamente com medidas estruturais na monitorização dos ecossistemas aquáticos insulares a fim de melhorar as estratégias para a sua conservação.

<u>Chapter 1</u>

General introduction.

1.1. CONTEMPORARY LAND-USE TRANSFORMATIONS.

During early 20th century, more than half of the Earth's land had transitioned from wild landscapes to areas dominated by human influence (Ellis et al., 2010), a reflection of humanity's need to sustain a rapidly growing population that has now exceeded 8 billion people (Alexandratos & De Haen, 1995; Bahadur et al., 2018; Aznar-Sánchez et al., 2019). Despite urbanization being possibly the most dramatic land use change as it converts green into grey infrastructure, the global coverage of urban areas was estimated to be 0.63% of total land area in 2010 (Gong et al., 2012; Liu et al., 2018). The demand for food, biofuels and other agricultural products has also driven remarkable changes in land uses (de Fraiture et al., 2008; Gopalakrishnan et al., 2009; Tubiello et al., 2015). Currently, agricultural lands account for more than one-third of the total land area (Food and Agriculture Organization (FAO), 2024), of which croplands occupy around 11%, while pastures for livestock grazing cover 33% of the total land area (Jose et al., 2017; Motta-Delgado et al., 2019). The expansion of agricultural activities has been accompanied by significant forest loss, with an estimated 10 million hectares of forest being cleared annually between 2015 and 2020 (Food and Agriculture Organization (FAO), 2020). In addition to agriculture, commercial forestry plantations, dominated by fast-growing conifer species such as pines and spruces, cover more than 140 million hectares, representing over 50% of the total area of forest plantations (Food and Agriculture Organization (FAO), 2020). Nowadays, these changes in land use are particularly pronounced in tropical regions, where large-scale deforestation is often driven by the need for cropland and pastures (Lambin et al., 2003; Don et al., 2011; Velastegui-Montoya et al., 2022). However, landscape alterations are also widespread across temperate and boreal zones due to commercial forestry, infrastructure expansion and urbanization (Newbold et al., 2014; Curtis et al., 2018). The intensification of land use has profound implications for ecosystems, leading to habitat fragmentation, biodiversity loss, soil degradation and the disruption of critical ecosystem services, such as carbon sequestration, water filtration and nutrient cycling (Houghton, 1994; Novotny, 1999; Newbold et al., 2015). These global scale shifts highlight the pressing challenge of balancing human needs with environmental sustainability.

1.2. DEGRADATION OF RIPARIAN AREAS: CONSEQUENCES OF LAND-USE CHANGES ON FRESHWATER ECOSYSTEMS.

Riparian areas, which act as transitional zones between terrestrial and aquatic ecosystems, are among the ecosystems most affected by widespread changes in land uses (Giller et al., 2004; Clerici et al., 2014; Fernandes et al., 2016; Park et al., 2021). These areas provide vital ecosystem services, including sediment retention, water filtration, habitat provision and nutrient cycling, while also playing a key role in maintaining the ecological integrity of freshwater ecosystems (Richardson & Moore, 2010; Ferreira et al., 2023a; Nabout et al., 2023). Moreover, riparian vegetation regulates stream microclimates by providing shade that reduces solar radiation and moderates water temperatures (Knight & Bottorff, 1984; Johnson & Almlöf, 2016). It also supplies organic matter, such as leaves and woody debris, that serves as the primary energy source for heterotrophic aquatic food webs (Gregory et al., 1991; Pozo et al., 1997; Wallace et al., 1997). However, land-use changes, such as the conversion of forests into agricultural fields, pastures or commercial plantations, often result in the removal or degradation of riparian vegetation, with cascading impacts on stream ecosystems (Niyogi et al., 2003; Hladyz et al., 2011a; Chauvet et al., 2016). Deforestation, for instance, as consequence of land conversion into pastures or agricultural lands, exposes stream channels to direct sunlight, increasing water temperatures and stimulating primary production to the detriment of heterotrophic processes (Moore et al., 2005; Hladyz et al., 2011c). Agricultural runoff contributes excessive nutrients and sediments to water channels, leading to eutrophication, sedimentation and reduced water quality (Hessen et al., 1997; Smith et al., 1999; Withers et al., 2014). Similarly, the establishment of monoculture tree plantations, such as conifer forests, alters the timing and quality of organic matter inputs to streams, with conifer needles providing lower-quality resources compared to native broadleaf vegetation (Martínez et al., 2016; Larrañaga et al., 2021). Pastures and grazing lands also have significant impacts, as livestock activity often compacts soil, reduces vegetation cover and accelerates erosion, leading to higher sediment loads in streams (Bojsen & Jacobsen, 2003; Hladyz et al., 2010). Together, these land-use changes compromise the ability of riparian areas to act as effective buffers, undermining their capacity to regulate nutrient inputs, provide habitat and maintain biodiversity (Turner & Rabalais, 2003; Riis et al., 2020; Ferreira et al., 2023b).

The degradation of riparian zones has profound ecological consequences for freshwater ecosystems, as these areas play a central role in regulating the exchange of materials and energy between terrestrial and aquatic environments (Naiman & Décamps, 1997; Richardson & Moore, 2010). The loss of riparian vegetation disrupts organic matter inputs, which are critical for sustaining detrital food webs in streams (Ferreira et al., 2016c; Rugenski et al., 2017). In particular, the replacement of native forests with pastures or commercial plantations often reduces the diversity, quantity and quality of leaf litter entering streams, with significant implications for key ecosystem processes such as primary production, nutrient cycling and organic matter decomposition (Casas et al., 2013; Martínez et al., 2013). These changes can alter aquatic community composition and reduce the overall efficiency of nutrient cycling in streams (Lake et al., 2000; Frainer & McKie, 2015). Additionally, land-use changes often cause changes in environmental characteristics, such as sedimentation, increased water temperature and nutrient enrichment, all contributing to habitat degradation and biodiversity loss (Clapcott & Barmuta, 2010; Studinski et al., 2012; Ferreira et al., 2016c). In regions with intensive agricultural practices, nutrient runoff from fertilizers can lead to algal blooms and hypoxic conditions, further compromising aquatic habitats (Willis & McDowell, 1982; Wurtsbaugh et al., 2019; Blanco & Lal, 2023). Moreover, riparian zones are also highly susceptible to pollution from heavy metals, pesticides and herbicides, which accumulate in stream sediments and disrupt microbial and invertebrate communities responsible for key ecological processes (Angier et al., 2002; Quinton & Catt, 2007; Aguiar et al., 2015). The combined effects of these stressors highlight the importance of preserving riparian buffers as a fundamental strategy for mitigating the impacts of human activities on freshwater ecosystems.

1.3. FRAGILITY OF ISLAND FRESHWATER ECOSYSTEMS.

While most research on the effects of changes in land uses has been focused on continental streams, the challenges faced by riparian areas are often amplified on islands due to their unique ecological, geographical and climatic characteristics. Islands typically have limited land area, which concentrates and intensifies human pressures on natural ecosystems (Keppel et al., 2014; Calado et al., 2016; Ferreira et al., 2017). This limited space often results in high levels of habitat fragmentation, bringing natural areas into close proximity with urban, agricultural and industrial land uses, increasing their vulnerability (Jupiter et al., 2014; McMillen et al., 2014). Island ecosystems are characterized by lower species richness, but higher levels of endemism compared to continental regions, making them particularly susceptible to habitat degradation and biodiversity loss (Hughes & Malmqvist, 2005; Raposeiro et al., 2012; Sánchez-Ortiz et al., 2020). The endemic species that inhabit islands are often highly specialized and less adaptable to environmental changes, leaving them at greater risk of extinction when riparian and freshwater habitats are disturbed (Whittaker & Fernández-Palacios, 2007; Triantis et al., 2010). Land-use changes, such as deforestation for agriculture, invasion by exotic species or the establishment of large areas of commercial tree plantations, can disproportionately affect island riparian areas due to their inherent ecological fragility (Lussier et al., 2008; Ferreira et al., 2017; Rull et al., 2017). These ecosystems are often constrained by steep topographies, small watersheds and limited water availability, further amplify the effects of deforestation and vegetation loss (Smith et al., 2003; Raposeiro et al., 2011; Calado et al., 2016; Chi et al., 2020). The removal of native vegetation destabilizes soil, increasing erosion and sedimentation, which not only degrades water quality but also alters the physical structure of stream habitats (Louvat et al., 1998; Ferreira et al., 2017). This can lead to the loss of microhabitats that support aquatic species, further reducing biodiversity and threating island endemic species (Clapcott & Barmuta, 2010; Studinski et al., 2012). Additionally, the shallow soils and steep gradients typical of many islands may increase the rate and magnitude of runoffs, resulting in flashier hydrological regimes and greater vulnerability to extreme weather events that are becoming more frequent due to climate change (Alpert et al., 2002; Toreti et al., 2013). Moreover, the widespread introduction of exotic plant species, which is common on islands, can displace native vegetation and further reduce the functional capacity of riparian zones to buffer against environmental stressors (Russell et al., 2017; Costa et al., 2021).

Freshwater ecosystems on islands may also be particularly sensitive to pollution (including heavy metals) from agricultural runoff and urban effluents due to their limited capacity to dilute contaminants. This sensitivity becomes greater when watersheds are small or altered, stream channels are diminished and soils are undeveloped, reducing their ability to filter pollutants (Mieth & Bork, 2005; Golabi et al., 2014; Buttermore et al., 2018). As a consequence, the excessive nutrient inputs can lead to eutrophication, algal blooms and oxygen depletion among other effects compromising aquatic biodiversity and ecosystem services (Hessen et al., 1997; Withers et al., 2014). Additionally, increased sedimentation due to deforestation and agricultural activities can bury benthic habitats and reduce the availability of suitable refuge and feeding sites for aquatic organisms (Löfgren et al., 2009; Valente-Neto et al., 2015; Mathers et al., 2017). Thus, the combined effects of these stressors, including the replacement of native vegetation with pastures, urban areas or commercial plantations, compromise the resilience of island freshwater ecosystems, creating cascading effects that make them highly susceptible to both natural and anthropogenic disturbances (Delgado et al., 2017; Steibl & Laforsch, 2019; Fernández-Palacios et al., 2021; Khan et al., 2022).

1.4. ECOSYSTEM BIOASSESSMENT: THE IMPORTANCE OF INTEGRATING FUNCTIONAL INDICATORS.

Traditionally, assessments of stream ecological integrity have relied heavily on structural parameters, as exemplified by the Water Framework Directive (WFD, Directive 2000/60/EC), a key policy framework for aquatic ecosystems assessment in Europe (Directive Water Framework, 2003; Hödl, 2018; Feio et al., 2021). This approach primarily focus on the composition and diversity of key biological groups, including benthic invertebrates, diatoms, macrophytes and fish under the assumption that shifts in community structure reflect underlying environmental changes (Directive Water Framework, 2003; Resh, 2008; Moog et al., 2018). As a result, a wide range of biotic indices based on the taxonomic composition of aquatic organisms have been developed to evaluate ecological status, often emphasizing the presence or absence of indicator species that signal environmental stressors such as pollution, habitat degradation or hydrological alterations (Metcalfe, 1989; Dolédec & Statzner, 2010; Abbasi & Abbasi, 2011; Birk et al., 2012). While structural indicators provide valuable insights into species composition and biodiversity, they do not always capture the full context of ecosystem functioning (Sandin & Solimini, 2009; Hering et al., 2010; Santos et al., 2021; Vadas et al., 2022). Ecosystem integrity is inherently a combination of both structural and functional components, as recognized by the WFD, however there is increasing evidence that structure and function are not always tightly correlated (Furse et al., 2009; Sandin & Solimini, 2009; Vadas et al., 2022). For instance, several studies have demonstrated that alterations in riparian vegetation, such as deforestation or replacement with monoculture plantations, lead to pronounced effects on stream ecosystem processes, including organic matter decomposition and nutrient cycling, without necessarily inducing significant changes in benthic community structure (Nyberg & Eriksson, 2001; Fuchs et al., 2003; Haggerty et al., 2004; Goodman et al., 2006; McKie & Malmqvist, 2009; Riipinen et al., 2009). This discrepancy highlights a fundamental limitation of assessments based on structural indicators, as streams may experience substantial functional impairments even when taxonomic indices indicate minimal ecological disturbance.

Recognizing these limitations, several studies have emphasized the need to incorporate functional indicators into freshwater bioassessment (Lecerf & Richardson, 2010; Ferreira et al., 2020; Vadas et al., 2022). Ecosystem functioning is closely tied to ecosystem services such as nutrient retention, organic matter processing and water purification, which are critical for supporting human well-being (Wilson & Carpenter, 1999; Feld et al., 2010; Harrison et al., 2014; Ferreira et al., 2023a). Thus, functional parameters, including organic matter decomposition rates, nutrient cycling and metabolic processes, provide valuable insights into how ecosystems respond to environmental stressors and may serve as early-warning indicators of ecological degradation (Xu et al., 2001; Gessner & Chauvet, 2002; Young et al., 2008; Young & Collier, 2009; Kandziora et al., 2013; Von Schiller et al., 2018). More recently, some systematic reviews of freshwater monitoring programs analysed multiple bioassessment frameworks, revealing that although structural indicators remain dominant, there is a growing trend toward incorporating such functional measures (Ferreira et al., 2020; Harrison et al., 2021; Cheung & Burrows, 2024). This trend reflects the recognition that a comprehensive evaluation of ecological integrity must account for both biodiversity patterns and the underlying processes that sustain freshwater ecosystems.

1.5. ORGANIC MATTER DECOMPOSITION AS A BIOASSESSMENT TOOL OF STREAM FUNCTIONAL INTEGRITY.

One widely used functional indicator is organic matter decomposition, which offers a direct measure of ecosystem functioning providing insights into stream health and resilience (Elosegi et al., 2006; Young & Collier, 2009; Chauvet et al., 2016; Benfield et al., 2017; Ferreira et al., 2020). Organic matter decomposition is shaped by complex interactions among biotic and abiotic factors, with both anthropogenic and natural stressors altering decomposition rates by enhancing or inhibiting microbial and invertebrate activity (Gessner & Chauvet, 2002; Young et al., 2008). It is widely known that land-use changes and riparian vegetation loss have profound effects on organic matter decomposition dynamics (Allan, 2004; McKie & Malmqvist, 2009; Ferreira et al., 2016c; Silva-Junior, 2016). The removal of native vegetation for agriculture, urbanization or commercial forestry plantations modifies the quantity and quality of organic matter entering streams (Hladyz et al., 2011c; Martínez et al., 2016; Larrañaga et al., 2021). For instance, in land conversion for commercial forestry uses, normally deciduous leaf litter from native forest, which decomposes relatively quickly due to its high nutrient concentration and lower structural complexity, is often replaced by tough and nutrient-poor coniferous litter (Casas et al., 2013; Martínez et al., 2013; Ferreira et al., 2017). This shift in the amount and quality of litter can slow decomposition by reducing food for microbial decomposers and invertebrate shredders (Martínez et al., 2013; Sakai et al., 2013). However, studies have shown that increased solar radiation in deforested streams, such as those in pastures, can enhance invertebrate communities by boosting their richness and density under higher solar exposure (Quinn et al., 1997), thereby accelerating organic decomposition rates. Moreover, higher solar exposure can elevate water temperature, stimulating microbial activity and primary production (Young & Huryn, 1996; Quinn et al., 1997; Neill et al., 2001), further altering the organic matter decomposition process (Niyogi et al., 2003; Hladyz et al., 2011c).

Nutrient enrichment due to eutrophication is another major driver of changes in organic matter decomposition (Gulis et al., 2006; Woodward et al., 2012; Ferreira et al., 2015; Pereira et al., 2016). The introduction of excessive nitrogen and phosphorus from agricultural runoff, wastewater discharges or urbanization can accelerate microbial decomposition, as aquatic fungi and bacteria benefit from higher nutrient availability (Gulis & Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006). Moreover, invertebrate activity may amplify the stimulatory effects of nutrient enrichment on microbial activity, making overall litter decomposition more responsive to nutrient inputs than decomposition driven solely by microbes (Pascoal et al., 2003; Gulis et al., 2006; Woodward et al., 2012; Tant et al., 2015). However, prolonged nutrient enrichment can lead to shifts in microbial and invertebrate decomposers favouring fast growing opportunistic species that are tolerant to these nutrient enrichment condition, further impacting on nutrient cycling efficiency (Pascoal & Cássio, 2004; Young et al., 2008).

Heavy metal contamination and pesticide exposure also impair organic matter decomposition (Neumann et al., 2002; Quinton & Catt, 2007; Batty & Hallberg, 2010; Aguiar et al., 2015). Streams affected by mining runoff, volcanic activity or industrial pollution often have elevated concentrations of iron, copper, zinc and aluminium, which are toxic to decomposer communities (Gonçalves et al., 2011; Ferreira et al., 2016b; Balibrea et al., 2023). Heavy metals can inhibit fungal sporulation and microbial respiration, leading to significantly reduced decomposition rates (Sridhar & Bärlocher, 2011; Hogsden & Harding, 2012; Funck et al., 2013a). Moreover, metal precipitates coating substrate surfaces may constrain invertebrate feeding activity (Duarte et al., 2008; Roussel et al., 2008; Batista et al., 2012; Campos et al., 2014). Similarly, pesticides and herbicides introduced through agricultural runoff negatively affect both microbial decomposers and shredding invertebrates, slowing organic matter breakdown and reducing the efficiency of nutrient recycling (Lecerf et al., 2006; Rasmussen et al., 2012).

Hydrological alterations, such as flow regulation, dam construction and sedimentation, further disrupt organic matter decomposition (Lehner et al., 2006; Sabater & Tockner, 2010; Dos Santos Fonseca et al., 2013; Ferreira et al., 2020). Changes in streamflow patterns affect the transport, retention and breakdown of organic matter, influencing microbial colonization and invertebrate feeding activities (Flores et al., 2013; Singh et al., 2014; Li et al., 2020). Reduced flow conditions may lead to organic matter accumulation, altering oxygen availability and shifting microbial and invertebrate community composition (Inoue et al., 2012; Flores et al., 2013; Li et al., 2020). Conversely, highly variable flow regimes or sediment deposition from deforestation and agriculture can bury organic matter, making it physically inaccessible to decomposers translating into slower decomposition rates (Wantzen et al., 2005, 2008; Rueda-Delgado et al., 2006).

Climate change may also have severe influences in decomposition dynamics, where rising temperatures directly affect microbial metabolism and often accelerate decomposition rates (Dang et al., 2009; Nickus et al., 2010; Gonçalves et al., 2013; Griffiths & Tiegs, 2016; Xenopoulos et al., 2021). However, higher water temperatures may also reduce oxygen levels, potentially impairing invertebrate activity and microbial efficiency (Carpenter et al., 2011; Follstad Shah et al., 2017; Galic et al., 2019). Additionally, extreme climate events, such as droughts and floods, alter hydrological conditions and disrupt decomposer communities, leading to unpredictable shifts in decomposition rates (Carpenter et al., 1992; Bello et al., 2017; Zittis et al., 2022).

Despite its sensitivity to environmental changes, the use of organic matter decomposition in freshwater bioassessment has both strengths and limitations. One of its greatest advantages is that it is highly integrative, capturing the cumulative effects of multiple stressors on stream function (Gessner & Chauvet, 2002; Young et al., 2008; Truchy et al., 2022). It is also directly linked to key ecosystem services, such as nutrient retention, carbon processing and water purification, making it a scientifically relevant and ecologically meaningful measure of ecosystem health (Feld et al., 2010; Holland et al., 2011; Ferreira et al., 2020, 2023a). Furthermore, decomposition experiments using leaf litter bags are low cost effective, relatively simple to implement and applicable across diverse environments and geographic regions, allowing for broad comparability across different freshwater systems (Lecerf & Richardson, 2010; Chauvet et al., 2016; Zhang et al., 2019; Ferreira et al., 2020). However, there are challenges to its widespread application in bioassessment programs. One of the primary limitations is its high variability due to abiotic influences, including natural fluctuations in temperature, hydrology and organic matter quality, which can make it difficult to distinguish anthropogenic impacts from natural environmental variability (Langhans et al., 2008; Martínez et al., 2016; Bastias et al., 2020). Additionally, the lack of standardized methodologies for measuring decomposition constitutes challenges the comparison across studies, as variations in leaf species, mesh size and exposure time can lead to inconsistent results (Young et al., 2008; Ferreira et al., 2021). Another potential weakness is that faster decomposition rates do not always indicate improved ecological conditions; for instance, increased microbial activity in polluted waters may reflect opportunistic microbial dominance rather than a healthy ecosystem (Gulis & Suberkropp, 2003). Finally, while decomposition effectively detects nutrient enrichment, contamination and hydrological changes, it may be less responsive to stressors affecting higher trophic levels, such as invasive species or overfishing (Hladyz et al., 2011b). Despite these limitations, organic matter decomposition remains a powerful tool for assessing freshwater ecosystem health, particularly when integrated with complementary structural and functional indicators. Expanding its application in bioassessment frameworks will improve our understanding of ecosystem responses to environmental stressors, ultimately supporting more effective conservation and management strategies.

1.6. AZORES ARQUIPELAGO AS A CASE STUDY OF OCEANIC ISLANDS.

The Azores archipelago, an autonomous region of Portugal, comprises nine volcanic islands and several islets (Morton & de Frias Martins, 2019). The Azores are situated in the Macaronesia subregion along with Madeira, the Canaries and Cape Verde (Vanderpoorten et al., 2007; Fernández-Palacios, 2010). Azores islands represent the most remote archipelago in the North Atlantic, located approximately 1300 km from mainland Europe and 1900 km from the American continent (Santos et al., 2004). The region is characterised by oceanic temperate climate with mean annual temperatures ranging from 14°C to 18°C and precipitation levels between 740 mm and 2400 mm (Bettencourt, 1979; Climate Atlas, 2012).

In the Azores archipelago, freshwater ecosystems are abundant, comprising 736 stream basins (DROTRH-INAG, 2001; Cruz & Soares, 2018). Lotic systems are characterized by small, short and steep watersheds with short streams with maximum length of 29 km (Smith et al., 2003; Hughes & Malmqvist, 2005; Raposeiro et al., 2013). Permanent streams exist only in Santa Maria, São Miguel, São Jorge, Faial and Flores islands where they are fed by lakes or spring waters (DROTRH-INAG, 2001). Additionally, the Azores feature over 88 lentic systems (Porteiro, 2000), including crater lakes and maars (Azevedo, 1998; Nunes, 1999), occupying 0.4% of the regional territory, mainly on São Miguel, Terceira, Pico, Flores, and Corvo islands (Porteiro, 2000). As a typical island biota, the freshwater fauna of the Azores is depauperated in comparison to continental systems, resulting in biotic assemblages that have low diversity and high percentage of endemism (11%) (Raposeiro et al., 2012; Balibrea et al., 2020b). Furthermore, some taxonomical groups common in continental freshwaters, such as Plecoptera, are absent in Azorean streams. The isolation, recent age of the islands, numerous geological events and volcanic eruptions have acted as biogeographical filters (Borges & Brown, 1999; Hughes, 2005; Raposeiro et al., 2009) limiting the range of freshwater organisms able to colonize island streams (Malmqvist, 2002; Hughes & Malmqvist, 2005). The Azorean freshwater fauna is dominated by insects, in particular the Diptera order (Borges et al., 2010), where Chironomidae is the most diverse family (Raposeiro et al., 2009). Diptera has the highest number of endemic taxa, representing 6.69% of the total species richness in this order (Raposeiro et al., 2012). Other taxonomical groups such Trichoptera and Coleoptera also have relatively high degree of endemism (Gonçalves et al., 2008; Raposeiro & Costa, 2009). However, some orders of aquatic insects are represented by a single species, such as the Ephemeroptera represented by *Cloeon dipterum* (Linnaeus, 1761), and other orders are even absent in the region such as the Plecoptera (Raposeiro et al., 2009).

Despite the importance of the Azores colonization by the Portuguese in the 15th century, human activities have historically constituted significant threats in the Azorean archipelago. Since the establishment of the first human settlements (official Portuguese settlement in 1432 CE), pressure on the ecosystems has increased exponentially, mainly associated with landscape disturbance due to changes in land uses (Triantis et al., 2010; Connor et al., 2012; Rull et al., 2017; Raposeiro et al., 2021). Deforestation began in coastal river basins for village establishment, extending to higher elevations by the 20th century due to agricultural expansion and infrastructure development (Silva & Smith, 2004; Raposeiro et al., 2011). Current pressures include intensive agriculture, agrochemical use, roads and urban construction, livestock effluent discharge and the expansion of commercial wood plantations as an important economic activity (Constância, 1963; Calado et al., 2015; Castanho et al., 2021) which degrade water quality and streams integrity (Raposeiro et al., 2014a; Ferreira et al., 2017).

In 2003, the Regional Government of the Azores adopted the European Water Framework Directive (WFD, Directive 2000/60/EC), establishing continuous bioassessment programs that evaluate water quality and ecological integrity (Directive Water Framework, 2003; Cruz et al., 2012). This monitoring plan uses physicochemical parameters (such as pH, temperature, dissolved oxygen, nutrient and contaminant concentrations) and structural indicators such macroinvertebrate, phytoplankton, macrophytes and diatom communities (DROTRH-INAG, 2001; Cruz et al., 2017). Despite functional indicators are still not being used as a tool for bioassessment in the archipelago, studies over the last decade have focused on functional processes in Azorean streams. Those studies

(Raposeiro et al., 2014b; Ferreira et al., 2016d; Balibrea et al., 2017; Ferreira et al., 2017; Raposeiro et al., 2018; Balibrea et al., 2020a, 2023), have focused on organic matter decomposition and the role of decomposer communities, offering an initial step towards further exploration of functional indicators in ecosystems quality monitoring. Thus, these studies provide a valuable starting point for future research that could lead to a clearer and more comprehensive application of functional indicators in freshwater bioassessment in the Azores.

However, the volcanic nature of the Azores adds complexity to stream bioassessment due to the presence of streams naturally enriched with metals derived from effluents and springs linked to active volcanism (Cruz, 2003; Quintela et al., 2013) and runoffs from lava flow aquifers discharges (Freire et al., 2013; Cabral Pinto & Ferreira da Silva, 2019). High concentrations of metals such as iron, aluminium, manganese, copper and zinc often coat stream habitats with metal hydroxide and metal oxide precipitates. Moreover, these conditions are often accompanied by low pH and elevated temperatures (Terroso et al., 2006; Gonçalves et al., 2016; Balibrea et al., 2023). It is well known that elevated metal levels may diminish species richness and disrupt essential ecosystem processes such as organic matter decomposition (Hogsden & Harding, 2012; Peters et al., 2013; Ferreira et al., 2016b). Decomposition is particularly sensitive to metal toxicity, affecting microbial decomposers and macroinvertebrate shredders, which impair energy flow in stream food webs (Maltby et al., 1995; Carlisle & Clements, 2005). Elevated metal concentrations in stream water may contaminate leaf litter, leading to reduced consumption, slower growth rates and increased mortality in shredders (Abel & Barlocher, 1988; Gonçalves et al., 2011; Batista et al., 2012; Campos et al., 2014; Ferreira et al., 2016b). The adsorption of metals onto leaf litter surfaces can also inhibit microbial colonization, thereby limiting microbial conditioning (Roussel et al. 2008; Duarte et al. 2008; Sridhar & Bärlocher 2011; Funck et al. 2013) and reducing palatability, further restricting shredder feeding (Schlief & Mutz 2006; Gonçalves et al. 2011; Batista et al. 2012; Niyogi et al. 2013; Funck et al. 2013). Moreover, metal contamination can inhibit growth, reproduction and diversity of aquatic hyphomycetes (Sridhar & Bärlocher, 2011; Batista et al., 2012; Ferreira et al., 2016b). Additionally, metals can negatively affect microbial activity indirectly by disrupting enzymatic pathways essential for decomposition, interfering with enzymatic activity by inducing oxidative stress and disrupting cell membranes (Sridhar et al., 2001; Krauss et al., 2005; Azevedo et al., 2007; Duarte et al., 2008). Consequently, assessing biological communities and functional processes in metal-enriched streams, particularly those influenced by active volcanism, is crucial for establishing accurate reference conditions.

1.7. AIMS, HYPHOTESES AND THESIS STRUCTURE.

Given the unique environmental challenges presented by the volcanic nature of the Azores and the increasing anthropogenic pressures, the main aim of this thesis is to assess the potential for organic matter decomposition to be used as a functional indicator of stream ecosystem health in the Azores archipelago. The thesis aims to shed some light on Azorean stream functioning under various common land use changes.

The main hypothesis of this thesis is that organic matter decomposition process will be sensitive to both natural and human disturbances, including land-use changes and natural metal enrichment from volcanic activity, making it a valuable tool for bioassessment. The thesis contains three main chapters, each addressing specific objectives, with a final chapter dedicated to a general discussion and final conclusions:

- **Organic matter decomposition as an indicator of island stream functioning under riparian forest modification (Chapter 2)** that evaluates the effects of riparian forest modifications (pastures and commercial plantations) on stream ecosystem functioning, particularly focusing on organic matter decomposition mediated by both microbial communities and macroinvertebrate shredders.
- **Effects of leaf litter naturally enriched with metals on invertebrate decomposers (Chapter 3)** that assesses the impact of natural metal enrichment on macroinvertebrate

decomposers, particularly endemic shredders, assessing how metal-enriched leaf litter influences their feeding rates, growth and survival.

- **Effects of leaf litter naturally enriched with metals on microbial decomposers activity and community structure (Chapter 4)** that examines the effects of natural metal enrichment on microbial decomposers, exploring changes in microbial activity, aquatic hyphomycetes reproductive output and community structure under varying metal concentrations in stream water.
- **General discussion and conclusions (Chapter 5)** presents a general discussion synthesizing the findings from previous chapters and highlights the key insights gained from the thesis. It also presents the conclusions of the work, emphasizing the importance of functional indicators for improving bioassessment and conserving stream ecosystems in the Azores.

Chapter 2

Organic matter decomposition as an indicator of island stream functioning under riparian forest modification.

2.1 ABSTRACT

Changes in land uses in Azorean islands are characterised especially by native forest clearance, establishment of commercial plantations with exotic species and intensification of agricultural and livestock activity. The replacement and removal of native vegetation may have strong effects on streams communities and processes. Aquatic decomposers and organic matter decomposition may be particularly sensitive to land uses changes due to their dependence on terrestrial litter supply. Here we assessed organic matter decomposition in streams under riparian forest modifications (cryptomeria plantations and pastures) in comparison with non-impacted streams (native laurel vegetation). *Ochroma pyramidale* wood and *Clethra arborea* leaves were used as substrates enclosed in fine and coarse mesh bags to assess microbial-driven organic matter decomposition and decomposition carried out by both microbes and macroinvertebrates, respectively. We found that organic matter decomposition was faster in streams surrounded by pastures, while streams flowing through native riparian vegetation and cryptomeria plantations showed similar organic matter decomposition. Decomposition was higher in coarse mesh bags compared to fine mesh bags in streams adjacent to cryptomeria plantations due to increased shredder abundance. Moreover, leaves decomposed faster than wood because their more labile characteristics. Despite sporulation rate of aquatic hyphomycetes showed to be higher in pastures streams, no significant differences were found between stream types. Reduced riparian vegetation was translated into lower of aquatic hyphomycetes richness in pastures streams, however, this effect did not influence benthic invertebrate richness and abundance among stream types. These findings highlight the complex interactions between land use transformation and organic matter decomposition processes, emphasizing the importance of riparian management for maintaining ecosystem functioning.

2.2 INTRODUCTION

Riparian areas serve as crucial interfaces between terrestrial and aquatic ecosystems, playing a pivotal role in the exchange of energy and materials between these ecosystems (Richardson & Moore, 2010). Riparian areas are not only vital for maintaining ecological connectivity between terrestrial and aquatic ecosystems but also provide essential ecosystem services, including water supply, biodiversity conservation and places for recreation (Ferreira et al., 2023a). The riparian vegetation also contributes to shape the physical, chemical and biological characteristics of streams (Tolkkinen et al., 2020). In forested streams, the riparian vegetation acts as a natural filter, reducing solar radiation input and supplying organic matter that constitutes the primary source of energy and carbon for aquatic food webs (Wallace et al., 1997). Heterotrophic microbes, particularly aquatic hyphomycetes, and macroinvertebrate shredders are the main decomposers of allochthonous organic matter, incorporating litter carbon and nutrients into secondary production in streams (Cummins et al., 1973; Hieber & Gessner, 2002; Cornut et al., 2010). The rate at which organic matter is incorporated into aquatic food webs greatly depends on its characteristics, with organic matter that has low carbon-to-nutrient ratios being decomposed faster than more recalcitrant organic matter (Ostrofsky, 1997; Jabiol et al., 2019; Ramos et al., 2021). The strong dependence of streams on their riparian vegetation makes them highly vulnerable to changes in land use (Allan, 2004; Silva-Junior, 2016; Hladyz et al., 2011).

Changing land use into forest plantations is a widespread forestry practice, with conifer plantations for commercial purposes occupying about 52% of the total forest plantation area worldwide (FAO, 2005). The replacement of deciduous native vegetation by evergreen conifer plantations changes the timing and quantity of organic matter inputs to streams (Inoue et al., 2012; Sakai et al., 2013; Martínez et al., 2016; Larrañaga et al., 2021). Also, conifer needles, characterized by their tough structure, low nitrogen concentration and high concentrations of lignin and polyphenols, are generally of poorer quality compared to deciduous leaf litter (Casas et al., 2013; Martínez et al., 2013). Changes in the timing, quantity and quality of organic matter inputs to streams flowing through conifer plantations may alter the community structure and activity of microbial decomposers and invertebrate shredders (Sedell et al., 1975; Bärlocher & Oertli, 1978; Girisha et al., 2003; Hisabae et al., 2011; Sakai et al., 2013). These changes may potentially impact organic matter decomposition in streams, but the few available studies show contrasting results (Ferreira et al., 2017; Hisabae et al., 2011; Martínez et al., 2013; Riipinen et al., 2009; Whiles & Wallace, 1997). Some studies found faster organic matter decomposition in streams flowing through pine and mixed spruce and larch plantations than through native broadleaf forests, due to greater shredder abundance able to exploit conifer needle inputs (Whiles & Wallace, 1997; Riipinen et al., 2009). On the contrary, other studies found slower organic matter decomposition in streams flowing through cryptomeria and pine plantations than through native broadleaf deciduous forests, due to reduced shredder densities constrained by low-quality food resources (Hisabae et al., 2011; Martínez et al., 2013). A few studies also found no major differences in organic matter decomposition between streams flowing through different conifer plantations and broadleaf deciduous forests, despite differences in macroinvertebrate shredders and aquatic hyphomycete communities between stream types, suggesting that structure and function are not always closely linked (Ferreira et al., 2017; Riipinen et al., 2010). The heterogeneity in conifer plantation effects on aquatic decomposers and organic matter decomposition may be due to numerous factors, such as the identity of the conifer species, type of native forest, identity and type of decomposing organic matter, abundance and diversity of decomposer community, and abiotic factors.

Forest conversion into pasture for livestock grazing represents another significant land use change affecting riparian habitats (Hladyz et al., 2011), covering about 33 % of the global land area (Jose et al., 2017; Motta-Delgado et al., 2019). This land use change often results in clear-cutting and deforestation of the riparian area, which in turn produces strong changes in the physical habitat and water chemistry (Akselsson et al., 2007; McKie & Malmqvist, 2009). For instance, sedimentation may increase due to soil erosion and concentrations of dissolved organic carbon, nitrate and phosphate may increase with runoff from fertilized fields (Löfgren et al., 2009). Consequently, microbial activity is promoted while the abundance and diversity of invertebrate consumers with low nutrient tolerance are constrained (Bojsen & Jacobsen, 2003; Lorion & Kennedy, 2009; Davies et al., 2010). Also, reduced vegetation cover increases solar radiation and water temperature which may stimulate primary production (Young & Huryn, 1996; Quinn et al., 1997; Neill et al., 2001) and organic matter decomposition due to the stimulation of microbial activity (Niyogi et al., 2003; Hladyz et al., 2010). However, a decrease in organic matter inputs to streams may reduce the habitat heterogeneity and limit food availability to aquatic organisms (Clapcott & Barmuta, 2010; Studinski et al., 2012; Wild et al., 2019). In pasture streams, grass species may constitute the main terrestrial organic matter inputs to streams (Menninger & Palmer, 2007; Leberfinger & Bohman, 2010; Hladyz et al., 2011c), but inputs are generally limited and the diversity and activity of the decomposer community are reduced (Niyogi et al., 2003; Hladyz et al., 2010; Dangles et al., 2011). Few studies done on pasture streams showed that grass inputs were mainly decomposed by microbes rather than invertebrate consumers due to the scarcity or absence of shredders (Young et al., 1994; Niyogi et al., 2003; Menninger & Palmer, 2007). Moreover, other studies found that organic matter decomposition mediated by microbes was faster in streams flowing through pastures than through native forests, while the opposite pattern occurred for decomposition mediated by invertebrates (Bird & Kaushik, 1992; Danger & Robson, 2004; Encalada et al., 2010; Hladyz et al., 2010).

Most studies addressing the effects of riparian forest changes on streams have predominantly focused on continental streams, with limited attention being given to island streams. However, evidence for continental streams may not be generalizable to island streams, because these generally have naturally lower diversity and high species endemicity (Hughes, 2005; Malmqvist et al., 1995; Raposeiro et al., 2011). Also, island streams are highly susceptible to human pressure due to space and resources limitation (Calado et al., 2016; Chi et al., 2020; Ferreira et al., 2017; Keppel et al., 2014; Raposeiro et al., 2013). The Azores archipelago exemplifies this scenario, where human settlement has increased pressure on natural ecosystems through land use changes (Triantis et al., 2010; Connor et al., 2012; Rull et al., 2017; Raposeiro et al., 2021). Land uses in the Azores archipelago are strongly linked to the elevational gradient, being shaped by topography and climatic characteristics (Quaternaire-Portugal, 2008). In some islands, streams are surrounded by small patches of native laurel forest, which are minimally impacted by human activities. More frequently, however, the landscape at lower elevations is shaped by urban and agricultural areas and streams often drain areas of pasture with riparian vegetation partially or totally removed (Raposeiro et al., 2011; Silva & Smith, 2004), while streams at medium and high elevations are more frequently surrounded by commercial tree plantations, mainly conifers (e.g. *Cryptomeria japonica* (L.f.) D.Don), or by forests dominated by exotic species (Borges et al., 2009; DRRF, 2014; Gonçalves et al., 2015; Raposeiro et al., 2011).

In this study, we assessed the effects of riparian forest modifications on the communities and functioning of Azorean streams by comparing streams flowing through cryptomeria plantations and pastures with non-impacted streams (with native laurel vegetation). We focused on benthic macroinvertebrates, and macroinvertebrates and aquatic hyphomycetes associated with decomposing leaf substrate. Organic matter decomposition was used as a surrogate of stream functioning, for which wood and leaves were enclosed in coarse- and fine-mesh bags to allow or prevent access by macroinvertebrates, respectively. We hypothesize that organic matter decomposition would respond in different ways when comparing altered streams with native streams depending on substrate quality, abiotic factors (temperature and nutrient concentration) and consumer abundance and diversity in streams. Thus, (1) we expected faster organic matter decomposition in pasture than in native streams due to higher water temperature and nutrient concentrations in the former streams, and no differences in decomposition between cryptomeria and native streams (following similar results of previous studies in the archipelago; Ferreira et al. 2017). Moreover, (2) in altered streams, organic matter decomposition would be faster in pasture streams than in cryptomeria streams due to higher temperature and nutrient concentration in the former streams. We also hypothesize that (3) differences in organic matter decomposition among stream types would be higher for leaves than wood due to their higher susceptibility to macroinvertebrate activities and that (4) these differences would be higher in coarse- than in fine-mesh bags due to shredder access. Regarding aquatic communities, we hypothesize that (5) fungal activity would be higher in stream types with higher temperature and nutrient concentrations (pasture streams) and that (6) taxa richness of benthic macroinvertebrates and macroinvertebrates and aquatic hyphomycetes associated with leaf litter would be lower in stream types with reduced riparian vegetation diversity (pasture < conifer streams) than in streams with higher vegetation diversity (native streams).

2.3 MATERIALS AND METHODS

Study Region

This study was conducted on São Miguel, the largest island in the Azores archipelago. The archipelago, comprising nine islands with a total land area of 2325 km^2, is located in the North Atlantic Ocean at the junction of the Eurasian, African, and North American plates, approximately 1500 km from mainland Portugal (Santos et al., 2004). The climate is temperate oceanic, characterized by mean annual temperatures ranging from 14 to 18 ºC (Machado & Gonçalves, 2004) and total annual precipitation between 1500 and 3000 mm (Silva & Smith, 2004), varying with elevation.

The landscape, already significantly altered by the beginning of the 19th century due to forest resource exploitation and clearance for agriculture and urbanization, is now characterized by diverse land uses, including extensive livestock activities, commercial tree plantations, agriculture, urbanization, and exotic and native forests (Constância, 1963; Castanho et al., 2021). Livestock activities are one of the most relevant economic activities in the Azores, with cattle fields and pastures covering around 42% of the land area (Calado et al., 2015). Intensively managed pastures are covered mainly by *Trifolium repens* L., *Lolium perenne* L. and *Lolium multiflorum* Lam., while semi-natural pastures are covered mainly by *Holcus lanatus* L. (Melo et al., 2022).

Furthermore, *Cryptomeria japonica* D. Don. (cryptomeria), a conifer tree native to Japan and southern China, was introduced in the archipelago as an ornamental tree in the mid-19th century and progressively became an important commercial tree species (Albergaria, 2000; Dias et al., 2007). Currently, cryptomeria covers 26% of the forest area, which represents ca. 22% of the land area and 60% of the forest plantation area in the Azores (SRAM/DROTRH, 2007; DRRF, 2014). Plantations occasionally include exotic invasive species such as *Pittosporum undulatum* Vent., *Hedychium gardneranum* Sheppard ex Ker Gawl. and *Clethra arborea* Aiton.

The native forest in the Azores is commonly known as Laurissilva, due to the presence of laurel species (Lauraceae family), and it is characterized by an association of native evergreen tree and shrub species (Triantis et al., 2010), such as *Juniperus brevifolia* (Seub.) Antoine, *Laurus azorica* (Seub.) Franco, *Ilex azorica* Loes., *Frangula azorica* Grubov, *Erica azorica* Hochst. ex Seub, *Myrsine retusa* Aiton, *Vaccinium cylindraceum* J. E. Sm., *Calluna vulgaris* (L.) Hull. and *Culcita macrocarpa* C. Presl. Nowadays, native forest cover is strongly reduced, and it is mainly restricted to high elevations (>800 m a.s.l) and represents less than 10% of the overall surface area of the archipelago (Borges et al., 2010; DRRF, 2014; Gaspar et al., 2008).

Stream Selection

Nine permanent streams were selected based on comprehensive hydrographic basin surveys in São Miguel Island. Streams were categorized into three types according to their riparian vegetation: native (n=3), cryptomeria (n=3), and pasture (n=3) (Table 2.1 and Figure 2.8). Native streams, flowing through riparian forests of native species with little direct human influence, were used as reference sites. In contrast, cryptomeria and pasture streams, representing impacted sites, were characterized by the replacement of native riparian forest with cryptomeria plantations and pasture for livestock, respectively (Table 2.1 and Figure 2.8).

Land use cover at each study stream site was determined for a 300-m radius area upstream of the sampling site. The land uses were categorized into five categories: native vegetation, cryptomeria plantations, exotic vegetation, pastures, and artificial land uses (urbanization and roads) (Table 2.1 and Figure 2.9). Images were acquired from Google Earth (accessed in May 2022) and colored polygons were used to calculate the percentage of each land use in relation to the total area (excluding adjacent basins areas within the 300-m radius area) (Figure 2.9).

Water Variables

Water temperature was recorded every 4 hours for the duration of the experiment (from 9th May to 12th July 2022) using submerged data loggers (Hobo Pendant MX, Onset Computer Corp., MA, USA). Concurrently, a suite of additional water parameters, encompassing pH, electrical conductivity and total dissolved solids, were measured on five occasions using a multiparametric field probe (Horiba model U-52G, Horiba Instruments, UK). Water samples were also collected at the same time, transported to the laboratory, filtered using fiber glass filters (47-mm diameter, 1.2-µm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK), and frozen until analysis. Nutrient concentrations were determined using a Continuous Flow Analyser Skalar San++ (Skalar Analytical B.V., Breda, The Netherlands) with segmented flow analysis (SFA) according to Skalar methods M461-318 (EPA 353.2) for total nitrogen and nitrate, M155-008R (EPA 350.1) for ammonium and M503-555R (Standard Method 450-P I) for phosphate (Skalar, 2004). Water analyses were done at MARINNOVA—Marine and Environmental Innovation, Technology and Services, Oporto.

Litter Species

Two substrates were selected for this study, *C. arborea* (clethra) leaves and *Ochroma pyramidale* (Cav. Ex Lam.) Urb. (balsa) commercial wood. Clethra leaves were collected directly from trees, in Autumn 2021, in Planalto dos Graminhais (37°48'52.34" N, 25°14'16.44" W; 740 m a.s.l), transported to the laboratory, air-dried at ambient conditions and stored in the dark until used. This species is an exotic broadleaf perennial tree commonly present in the riparian vegetation of Azorean streams. Previous studies in the region have shown that clethra leaf litter decomposition can capture differences in the abundance of invertebrate shredders between streams (Balibrea et al., 2020; Raposeiro et al., 2018). Commercial wood substrates offer practical advantages due to their easy acquisition and high standardization in terms of chemical composition. In addition, wood decomposition is highly sensitive to differences in dissolved nutrient concentrations among incubation sites (Ferreira et al., 2006)(Figure 2.1).

Experimental Setup

Air-dried clethra leaves were weighed in 3.0–3.5 g (0.1 mg precision) batches, sprayed with distilled water for softening and enclosed in fine-mesh bags (0.5-mm mesh size) and coarse-mesh bags (5-mm mesh size with 10-mm holes). Fine-mesh bags were used to assess microbial-driven decomposition and coarse-mesh bags allow estimation of decomposition carried out by both microbes and macroinvertebrates. Individual balsa wood veneers (10 × 8 cm, 1-mm thick) were weighed (0.1 mg precision) and enclosed in fine-mesh bags (0.5-mm mesh size) (Figure 2.1). Since wood decomposition is mostly microbial-driven (Sinsabaugh et al., 1992; Tank & Webster, 1998; Arroita et al., 2012) and considering the potentially high flow rates in certain Azorean streams (Raposeiro et al., 2013, 2014), fine-mesh bags were used to protect this substrate from excessive physical fragmentation.

On 9th and 10th May 2022, 12 litter bags for each substrate and mesh size were incubated in each of the nine streams (clethra: 3 stream types × 3 streams × 2 mesh sizes × 4 sampling times × 3 replicates, n=216; balsa wood: 3 stream types × 3 streams × 1 mesh size × 4 sampling times × 3 replicates, n=108). These bags were fixed to the streambed with iron nails and secured submerged with stones. Additionally, an extra group of 9 bags (3 bags per substrate and mesh size) were submerged at one stream for 10 minutes and transported back to the laboratory. This was conducted to determine a conversion factor between initial air-dry mass and initial ash-free dry mass (AFDM), considering mass loss due to handling.

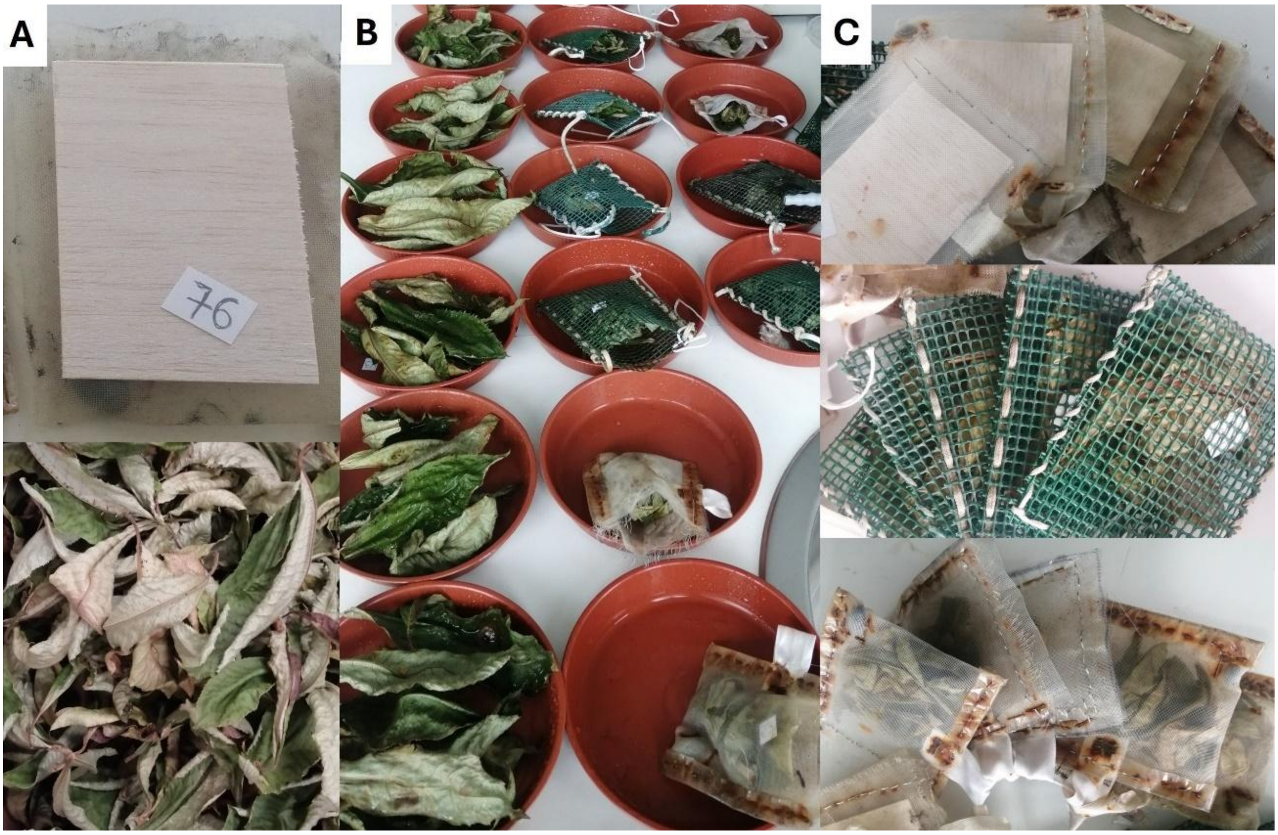

Figure 2.1. Selected substrates, clethra leaves and commercial balsa wood (A); clethra leaves getting moistened for being enclosed in mesh bags (B); commercial balsa wood enclosed in fine mesh bags and clethra leaves enclosed in coarse and fine mesh bags (C).

Litter Mass Remaining

After 15, 30, 45, and 63 days, three randomly selected bags for each substrate and mesh size were collected from each stream (3 bags × 3 substrates and mesh size × 9 streams; n=81 bags per sampling date). Collected bags were individually enclosed in ziplock bags, transported cool to the laboratory (Figure 2.2) and rinsed with distilled water on top of a sieve (0.5-mm mesh size) to remove fine sediments while retaining small litter fragments (Figure 2.3). Balsa wood veneers were processed for determination of AFDM remaining, while clethra leaves were processed for determination of AFDM remaining, spore production by aquatic hyphomycetes (fine- and coarse-mesh bags) and macroinvertebrate colonization (coarse-mesh bags) (see below).

Figure 2.2. At each sampling date bags from each substrate and mesh size were randomly selected (A); enclosed in zip lock bags and transported to the laboratory (B).

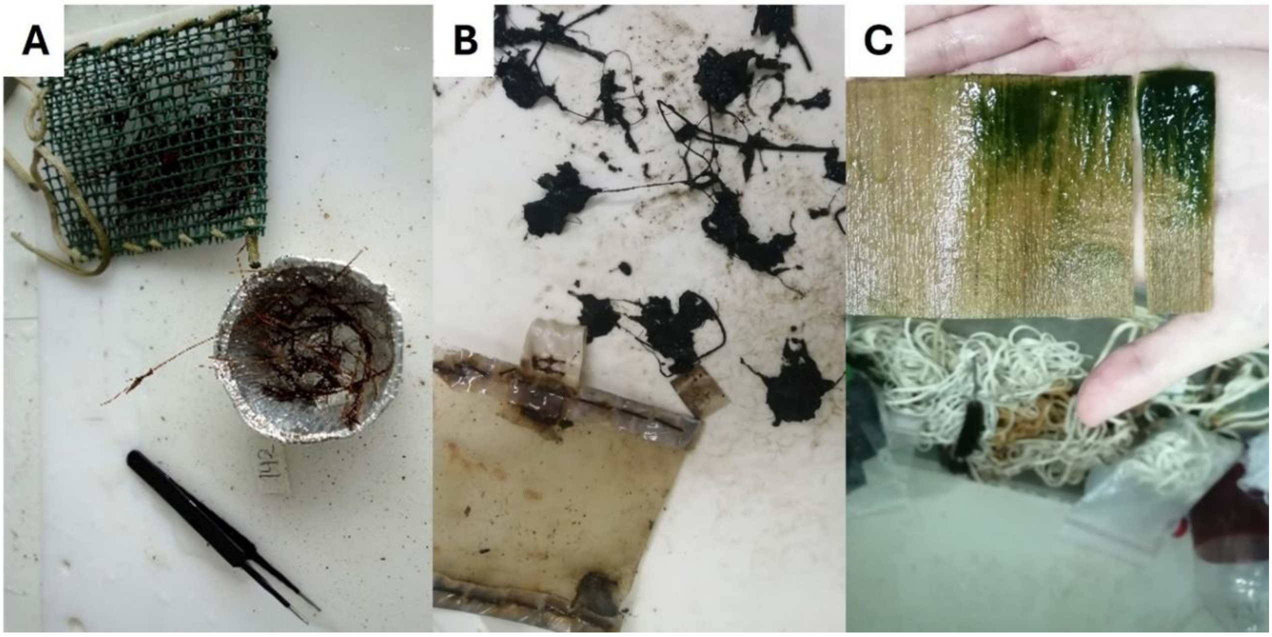

Figure 2.3. Clethra coarse-mesh bags (A), clethra fine-mesh bags (B) and balsa wood substrate (C) remaining rinsed gently with distilled water at laboratory to remove fine sediments and transferred to pre-weighed aluminum pans.

Balsa wood veneers were carefully transferred into pre-weighed aluminum pans, oven-dried (60 ºC for 48 h) (Memmert GmbH + Co, Schwabach, Germany) and weighed (0.1 mg precision) to determine final dry mass (DM). Dry substrate samples were ignited (500 ºC for 4 h) (Lenton EF 11/8B, Hope Valley, UK) and ashes were weighed (0.1 mg precision). The AFDM remaining was determined as DM–ash mass and results were expressed as the percentage of initial AFDM. Clethra leaves were processed as balsa wood, except that five leaf discs were extracted from each sample to induce sporulation (see below); the discs AFDM were later added to the bulk leaf AFDM (Figure 2.4).

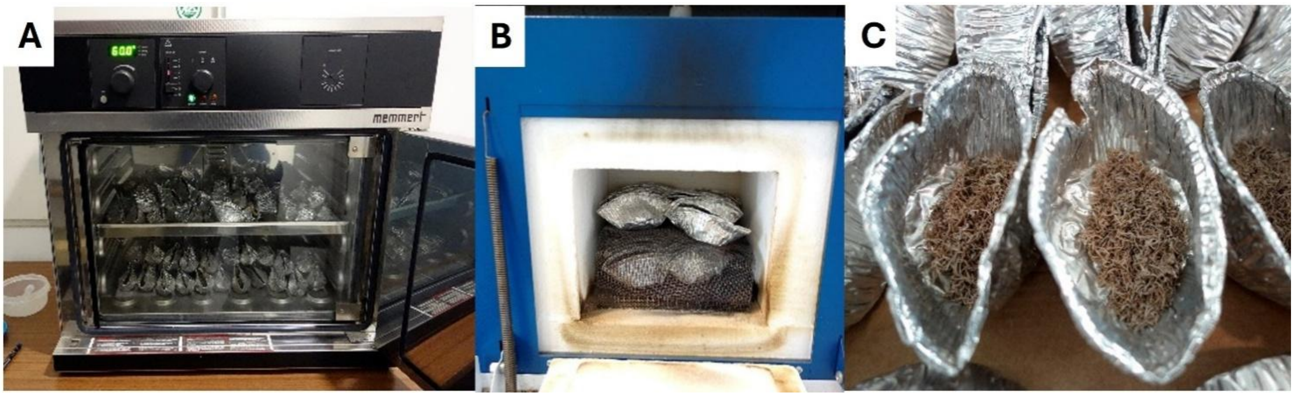

Figure 2.4. Remaining substrate in pre-weighed aluminum pans for oven-dried inside oven (A), for ignition inside muffle (B) and ash remaining after ignition (C).

Aquatic Hyphomycetes

The community structure and sporulation rates of aquatic hyphomycetes associated with clethra leaf litter in coarse- and fine-mesh bags were assessed by inducing sporulation under controlled laboratory conditions (Bärlocher, 2020). Leaves from each bag were gently rinsed with distilled water and five leaf discs (each one selected from different leaves) were cut with a corkborer (12-mm diameter) and placed in 100-mL Erlenmeyer flasks with 25 mL of filtered stream water (47-mm diameter, 1.2-µm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK) (Figure 2.5). Flasks were incubated on an orbital shaker (at 100 r.p.m.) (VWR Standard 3500 Orbital Shaker, VWR, USA) and placed inside an environmental test chamber (Economic Lux Chamber EC01-094, Snijders Scientific B.V., Tilburg, Holland) for 48 h at 10 ºC and with 10 h light:14 h dark photoperiod to simulate environmental light conditions (Figure 2.6). Spore suspensions were then gently shaken to dislodge spores attached to the flask walls, transferred into 50-mL graduated tubes, and fixed with 2 mL of formalin 37%. The sample final volume was adjusted to 35 mL with distilled water. Spore suspensions were stored in the dark until used. Leaf discs were transferred to preweighted aluminum cups, oven-dried at 60 ºC for 48 h and weighed (0.1 mg precision) for DM determination. Dry leaf discs were ignited at 500 °C for 4 h to determine ash mass and AFDM.

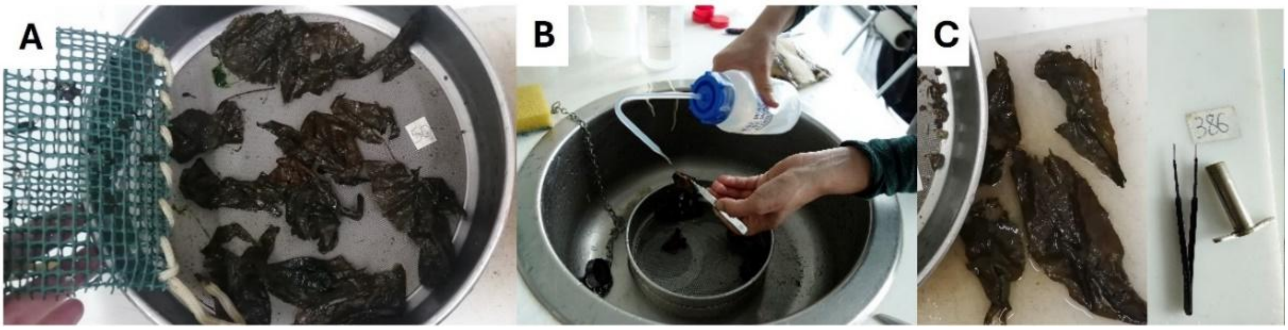

Figure 2.5. Remaining clethra substrate from coarse-mesh bags transferred to sieve (A), rinsed gently with distilled water to remove sediments (B) and cut leaf disc with corkborer (C).

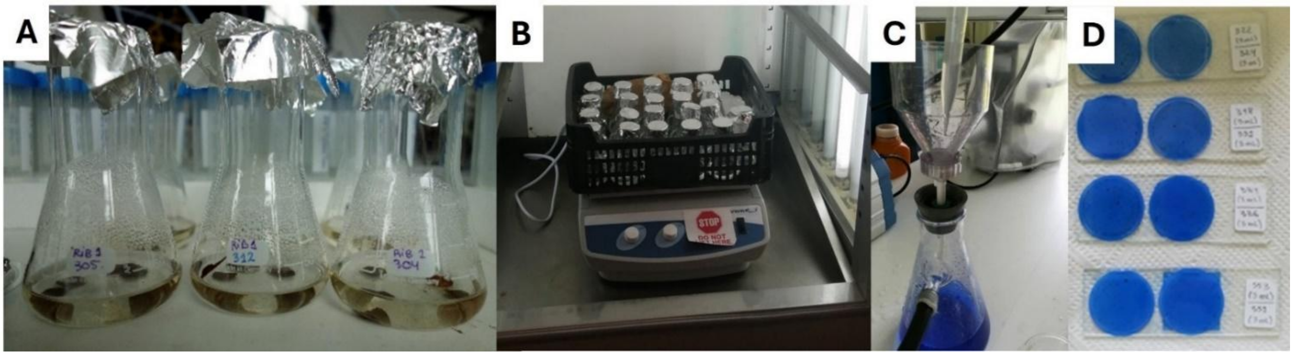

Figure 2.6. Erlenmeyer flask with leaf discs and filtered stream water (A); deployed in an orbital shaker placed inside an environmental test chamber (B); after sporulation aliquots of suspension filtered through cellulose nitrate filters and stained with cotton blue in lactic acid (C); stained filter mounted on a microscope slide for spores counting and identification under microscope (D).

For microscope slides preparation, spore suspensions were transferred into a beaker with 100 µL Triton X-100 solution (0.5%) and homogenized with a magnetic stirrer to ensure a uniform distribution of spores. Aliquots of the suspension were filtered through cellulose nitrate filters (25-mm diameter, 5-µm pore size; SMWP, Merk Millipore Ltd. Cork, Ireland). Filters were stained with cotton blue in 60% lactic acid (0.05%) and mounted on a microscope slide (Figure 2.6). Spores were identified and counted under a microscope (Leica DM2500, Leica Microsystems CMS GmbH, Wetzlar, Germany) at 200× magnification. Sporulation rates were expressed as the number of spores released per mg AFDM per day and aquatic hyphomycete taxa richness as the number of taxa per sample.

Benthic and Litter Macroinvertebrates

Benthic macroinvertebrate samples were obtained at the beginning (day 0) and end (day 63) of the experiment from each stream following standard protocols (INAG, 2008). Samples were collected with a kicknet (0.33-m wide, 0.5-mm mesh) along a 50-m reach. Each sample comprised six subsamples (1-m long) distributed proportionally among the existing microhabitats. Samples were preserved in 70% ethanol, and macroinvertebrates were counted and identified under a stereo microscope (Leica Stereozoom S9i, Leica Microsystems CMS GmbH, Wetzlar, Germany). Identification keys (Borges et al., 2010; Kriska, 2013; Tachet et al., 2000) were used for identification of individuals to the lowest possible taxonomic level. Total abundance was expressed as the total number of individuals per sample and relative abundance was expressed as the percentage of each taxon relative to the total number of individuals within each sample. Taxa richness was expressed as the number of taxa per sample. Organisms were assigned to functional feeding groups (Schmidt-Kloiber & Hering, 2015) and shredder density was expressed as the number of individuals per m^2.

Macroinvertebrates associated with clethra leaves enclosed in coarse-mesh bags were collected from the sieve and preserved in 70% ethanol for later identification and counting as described for benthic macroinvertebrates (Figure 2.7). Shredder density was expressed as the number of shredders per gram of leaf litter AFDMr.

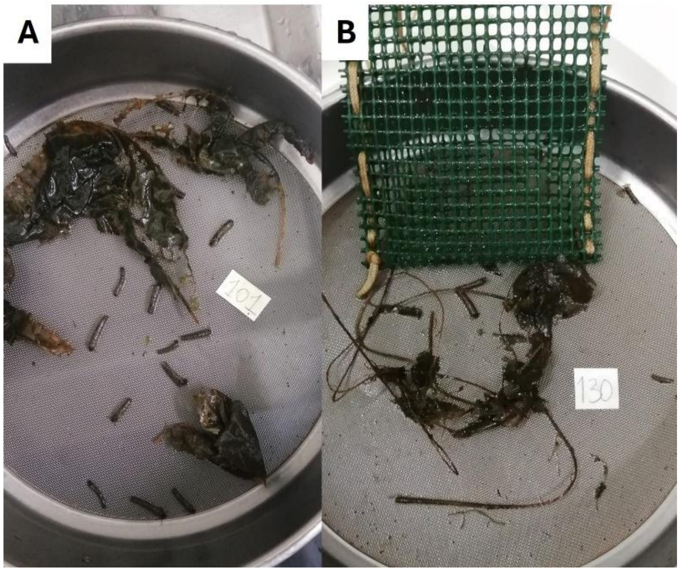

Figure 2.7. Remaining clethra substrate of coarse-mesh bags transferred into a sieve to collect macroinvertebrates associated to litter in cryptomeria streams at day 30 (A); and at day 63 (B).

Data Analysis

Water parameters were compared among stream types and streams (nested within stream types) using two-level nested ANOVA, followed by Tukey's honest significant difference (HSD) test when significant effects were detected.

Litter decomposition across the incubation period is often expressed as decomposition rate; however, neither the negative linear model nor the negative exponential model (with either fixed or free intercept) successfully fitted our data across all treatments, in particular for wood incubated in native and cryptomeria streams, for which decomposition was very slow for the first 45 days, increasing thereafter (Figure 1). Thus, comparisons of the proportion of AFDM remaining among stream types, streams (nested within stream types), substrates (clethra leaves enclosed in coarse- and fine-mesh bags and balsa veneers enclosed in fine-mesh bags) and time were done using four-level nested ANOVA. Subsequently, Tukey's HSD test was used to identify pairwise differences when significant effects were detected. Moreover, to overcome possible bias in the interpretation of our results due to differences in temperature among stream types, decomposition rate on a per day (k, /d) and on a per degree-day (k, /dd) basis were calculated using samples from day 63 only as –ln(proportion AFDM remaining)/t, with t being time (days) or the cumulative mean daily temperature (ºC) by day 63, respectively. Three-level nested ANOVA was used to compare decomposition rates on a per day and on a per degree-day basis among stream types, streams (nested within stream types) and substrates, followed by Tukey's HSD test.

Aquatic hyphomycete communities (based on spore production; log (x + 1) transformed) associated with clethra in coarse- and fine-mesh bags were compared among stream types, streams (nested within stream types), mesh size and time by PERMANOVA based on Bray-Curtis similarity matrix followed by pairwise test. Aquatic hyphomycete sporulation rates and taxa richness were compared among stream types, streams (nested within stream type), mesh and time by four-level nested ANOVA followed by Tukey's HSD test.

Benthic macroinvertebrate communities (log (x + 1) transformed) were compared among stream types, streams (nested within stream types) and time by PERMANOVA based on Bray-Curtis similarity matrix followed by pairwise test. Dispersion of principal coordinates analysis (PCO) was done to identify the predominant feeding group responsible for dissimilarities between stream types. Shredder densities in the benthos were compared among stream types using one-way ANOVA followed by Tukey's HSD test. Moreover, *Limnephilus atlanticus* Nybom (1948) (Trichoptera, Limnephilidae) density was also compared among stream types because previous studies have shown that it plays an important role in organic matter decomposition when present (Balibrea et al., 2020; Raposeiro et al., 2018). Macroinvertebrates abundance (log (x + 1) transformed) associated with clethra in coarse-mesh bags were compared among stream types, streams (nested within stream types) and time by PERMANOVA based on Bray-Curtis similarity matrix followed by pairwise test. Shredder density (log (x + 1) transformed) associated with decomposing litter was compared among stream types and time by two-way ANOVA followed by Tukey's HSD test.

Data were checked for homoscedasticity (Bartlett's test) and normality (Shapiro-Wilk's test) before analyses and transformed when needed. Univariate analyses were performed using STATISTICA 7 (StatSoft, Tulsa, OK, USA) and IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA). Community analyses were performed using PRIMER 6 v6.1.11 & PERMANOVA v1.0.1 (Primer-E Ltd, Plymouth, UK).

2.4 RESULTS

Streams

The predominant land use in native stream hydrographic basins was native vegetation, which covered 100% of the total area (Table 2.1 and Figure 2.9). The main land use in cryptomeria stream was cryptomeria plantations, which occupied 67% to 95% of the total area (Table 2.1 and Figure 2.9). The primary land use in the pasture stream hydrographic basins was extensive pasture for livestock, which covered 52% to 88% of the area (Table 2.1 and Figure 2.9). Pasture streams were also surrounded by a thin strip (i.e., 2–10 meters wide) of exotic species, mainly *P. undulatum*, *Acacia* *melanoxylon* R.Br., *Arundo donax* L., *H. gardneranum*, *Rubus ulmifolius* Schott and *Solanum mauritianum* Scop., which represented 9% to 45% of the land use in the total area (Table 2.1 and Figure 2.9).

Table 2.1. Location, elevation, and land use cover of the nine study streams. Land use cover was determined for a 300-m radius area upstream of the sampling sites (excluding basin area from other streams). Land uses were categorized into five types: native vegetation, cryptomeria plantations, exotic forest, pastures, and artificial land uses (urbanization and roads).

Stream	**Code**	**Latitude (N)**	**Longitude (W)**	**Elevation (m a.s.l.)**	**Land use cover (%)**				
					Native	**Cryptomeria**	**Exotic forest**	**Pasture**	**Artificial**
Tributary 1, Ribeira do Guilherme	Nat1	37°47'57.30"	25°12'10.83"	584	100.0	0.0	0.0	0.0	0.0
Tributary 2, Ribeira do Guilherme	Nat2	37°48'02.09"	25°12'07.71"	576	100.0	0.0	0.0	0.0	0.0
Ribeira Grande	Nat3	37°46'36.58"	25°27'29.94"	597	100.0	0.0	0.0	0.0	0.0
Ribeira da Achada	Crypt1	37°48'46.99"	25°14'45.74"	776	18.1	67.0	0.0	14.9	0.0
Ribeira do Folhado	Crypt2	37°48'50.37"	25°14'35.37"	762	6.3	93.7	0.0	0.0	0.0
Ribeira da Mulher	Crypt3	37°48'42.87"	25°14'22.51"	770	4.9	95.1	0.0	0.0	0.0
Ribeira da Lomba Grande	Past1	37°46'12.20"	25°13'21.18"	244	0.0	0.0	9.0	87.8	3.2
Ribeira dos Lagos	Past2	37°46'07.01"	25°14'55.85"	207	0.0	0.0	22.9	71.5	5.7
Ribeira Quente	Past3	37°46'12.20"	25°17'47.29"	201	0.0	0.0	45.0	52.3	2.7

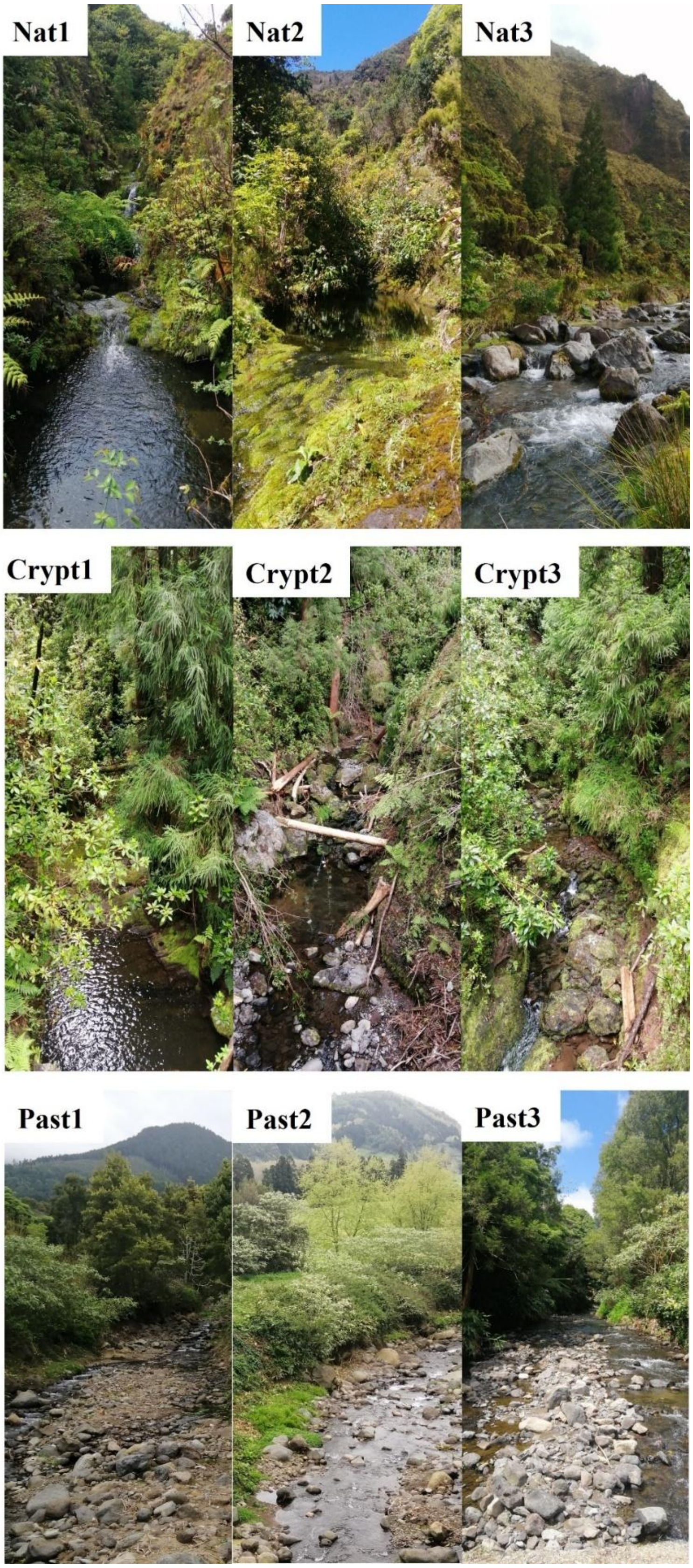

Figure 2.8. Selected study streams in São Miguel Island, Azores archipelago, in April 2022. Streams were categorized into three types according with the dominant surrounding vegetation: native streams (n=3; Nat1 – Nat3), cryptomeria streams (n=3; Crypt1 – Crypt3), and pasture streams (n=3; Past1 – Past3).

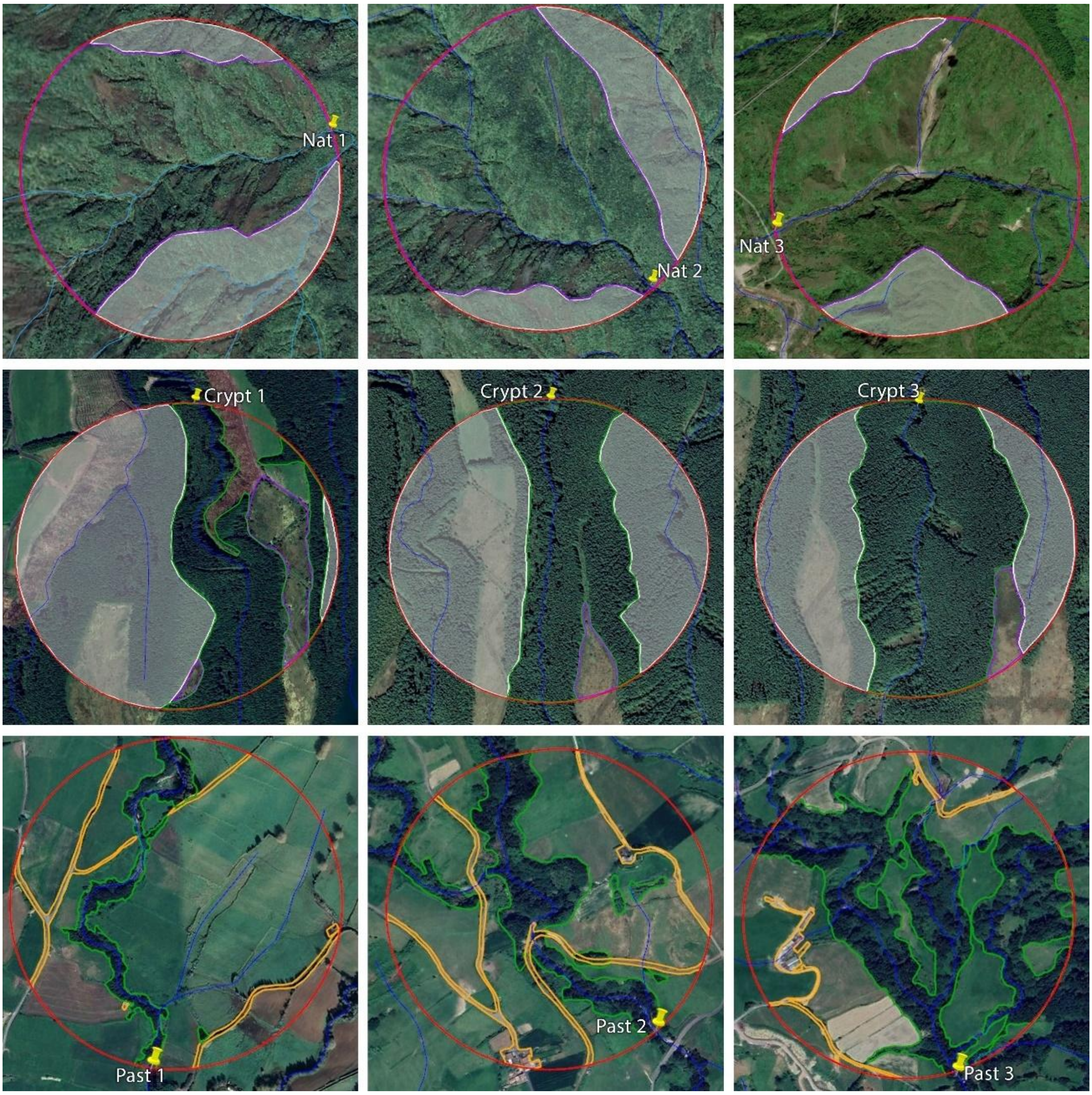

Figure 2.9. Land use cover in the watershed of the selected study streams within a 300-m radius area upstream of the sampling site (red polygon). White coloured polygons correspond to areas of adjacent basins, which were excluded. Purple polygons correspond to native vegetation; green polygons correspond to forested areas (cryptomeria plantations or exotic forest); orange polygons correspond to artificial land uses (urbanization, roads). Pasture areas were estimated by subtracting the area of other land uses and adjacent basins from the total area (red polygon). Images were acquired from Google Earth (accessed in May 2022). Nat1 – Nat3, Native streams; Crypt1 – Crypt3, Cryptomeria streams; Past1 – Past3, Pasture streams.

Water Variables

Water temperature, pH, conductivity, total dissolved solids, and nitrate and phosphate concentrations significantly differed between stream types (two-level nested ANOVA, $P<0.001$; Tables 2.2 and S1 and S2). Water temperature was overall cool, but significantly lower for cryptomeria streams than for native and pastures streams, which did not significantly differ (Table 2.2). Values of pH varied from circumneutral to slightly alkaline, and were significantly lower for native streams, intermediate for cryptomeria streams, and higher for pasture streams (Table 2.2). Conductivity and total dissolved solids were overall low, but significantly lower for cryptomeria streams, intermediate for native streams, and higher for pasture streams (Table 2.2). Nitrate concentrations ranged from moderate to high across stream types, with high variation within native and pasture stream types (Table S1). Nitrate concentrations were significantly higher for pasture streams than for native and cryptomeria streams, which did not significantly differ (Table 2.2). Phosphate concentrations were overall high, being significantly lower for cryptomeria streams, intermediate for native streams, and higher for pasture streams (Table 2.2).

Table 2.2. Physical and chemical characteristics of the stream water during the experiment (9th May–12th July, 2022). Streams were classified in three types according with the dominant surrounding vegetation (native, cryptomeria and pasture; see Table 1 and Figure S2). Values are mean ± SE of three streams (n=5 per stream, except for water temperature where n=63). Stream types with different letters significantly differ (two-level nested ANOVA followed by Tukey's HSD test). TDS, total dissolved solids.

Water variables	Native				Cryptomeria				Pasture			
Temperature (°C)	14.33	±	0.28	a	12.35	±	0.01	b	15.53	±	0.11	a
pH	7.07	±	0.66	a	7.37	±	0.18	b	7.96	±	0.12	c
Conductivity (µS/cm)	85.80	±	18.79	a	53.80	±	1.10	b	130.10	±	5.77	c
TDS (mg/L)	42.80	±	9.46	a	27.00	±	0.70	b	65.00	±	2.91	c
N-total (µg/L)	564.03	±	2.21	a	569.91	±	1.26	a	562.39	±	3.54	a
NO_3^- (µg/L)	83.68	±	51.35	a	24.11	±	7.35	a	755.63	±	249.87	b
$NH_3 + NH_4^+$ (µg/L)	23.61	±	1.68	a	18.47	±	2.03	a	25.59	±	1.14	a
P_2O_5 (µg/L)	81.47	±	12.96	a	30.06	±	5.14	b	127.15	±	43.49	c

Litter Decomposition

Litter mass remaining decreased steadily over the incubation period for all substrates in pasture streams (reaching 19.6% – 42.4% after 63 days) and for clethra leaves in coarse-mesh bags in cryptomeria streams (30.1%) (Figure 2.10). In contrast, balsa wood in native and cryptomeria streams changed little in mass for the first 45 days and reached 80.1% and 85.4% of mass remaining after 63 days, respectively. Also, clethra leaves in coarse-mesh bags in native streams and clethra leaves in fine-mesh bags in native and cryptomeria streams reached ~83% mass remaining within the first 15-days incubation but then the decomposition rate decreased, and after 63-days incubation it had 61.8% – 71.6% mass remaining (Figure 2.10). Litter mass remaining significantly differed between stream types and substrates (four-level nested ANOVA, P=0.001 and P<0.001, respectively) and there was a significant interaction between both factors (four-level nested ANOVA, P=0.006) (Table S3). Clethra leaves in coarse-mesh bags decomposed significantly faster in pasture streams, followed by cryptomeria streams and slower in native streams, while clethra leaves and balsa wood in fine-mesh bags decomposed significantly faster in pasture streams than in cryptomeria streams and native streams, which did not significantly differ (Figure 2.10 and Table S3). Decomposition was faster for clethra leaves in coarse-mesh bags, followed by clethra leaves in fine-mesh bags and slower for balsa wood in fine-mesh bags, except in pasture and native streams where decomposition of leaves in coarse- and fine-mesh bags was similar (Figure 2.10).

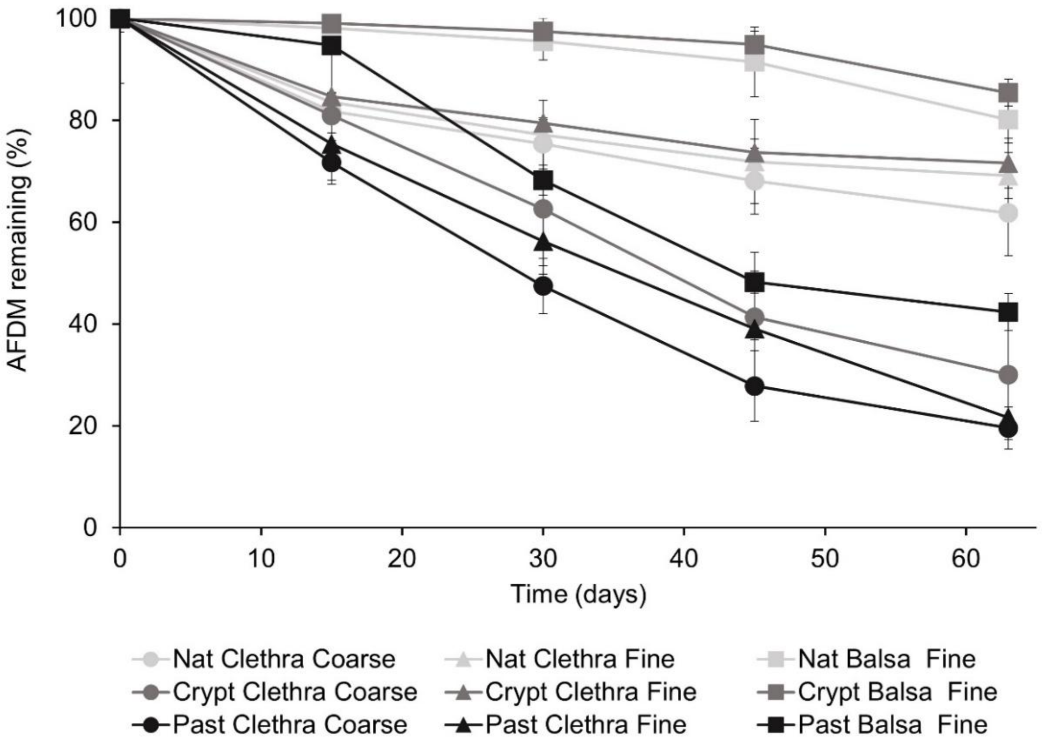

Figure 2.10. Ash-free dry mass (AFDM) remaining (mean ± SE) of clethra leaves enclosed in coarse- and fine-mesh bags and balsa wood enclosed in fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Decomposition rates on a per day (*k*, /d) and on a per degree-day (*k*, /dd) basis for leaves and wood in fine-mesh bags were higher in pasture streams than in native and cryptomeria streams, which did not significantly differ (Table S4 and S5). However, decomposition rates of leaves in coarse-mesh bags were higher in cryptomeria and pastures streams than in native streams. Therefore, relative differences were maintained when expressing results on a per day or a per degree-day basis (Table S4 and S5).

Aquatic Hyphomycetes

A total of 41 aquatic hyphomycete taxa were found associated with clethra leaves in coarse- and fine-mesh bags (Table S6). Fungal community structure was affected by all tested factors and most of their interactions (PERMANOVA, P≤0.042; Table S7). Overall, taxa richness was higher in native (34 and 30 species in coarse- and fine-mesh bags, respectively) and cryptomeria streams (29 and 30 species in coarse- and fine-mesh bags, respectively) than in pasture streams (20 species in both mesh sizes) (Figure 2.11 and Table S6).

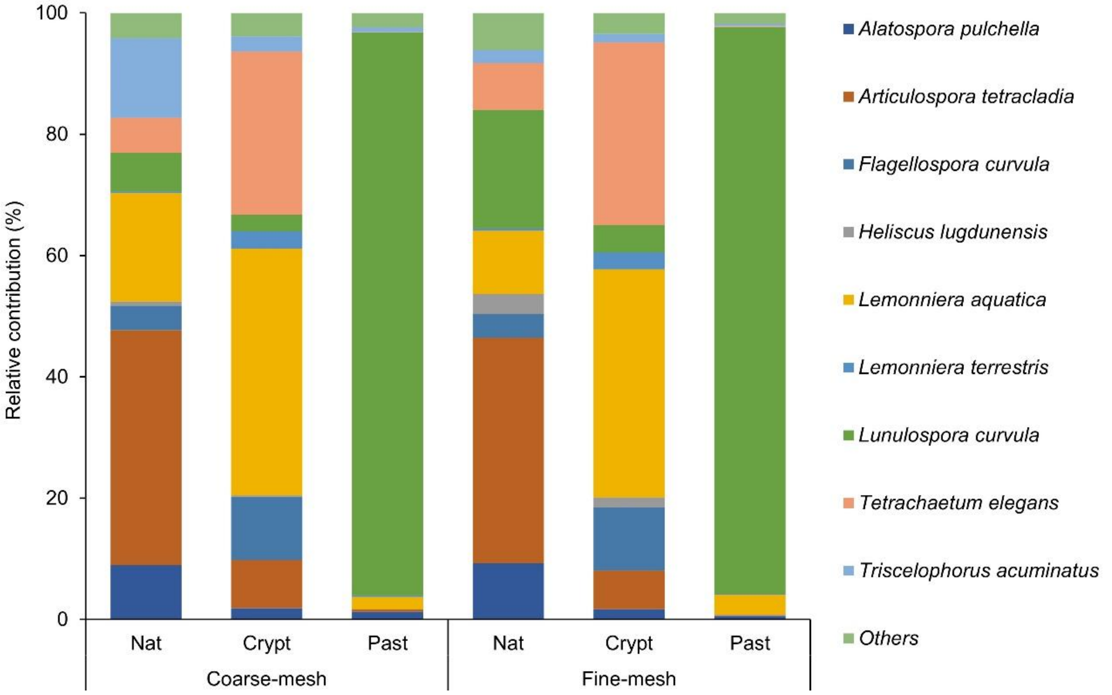

Figure 2.11. Mean relative contribution (across streams and dates, based on spore production) of aquatic hyphomycete taxa associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Articulospora tetracladia Ingold was the most abundant species in native streams (39% and 37% of relative contribution in coarse- and fine-mesh bags, respectively), *Lemonniera aquatica* De Wild. (41% and 38%) and *Tetrachaetum elegans* Ingold (27% and 30%) were the most abundant species in cryptomeria streams, and *Lunulospora curvula* Ingold was the most abundant species in pasture streams (93% and 94%) (Figure 2.11 and Table S6).

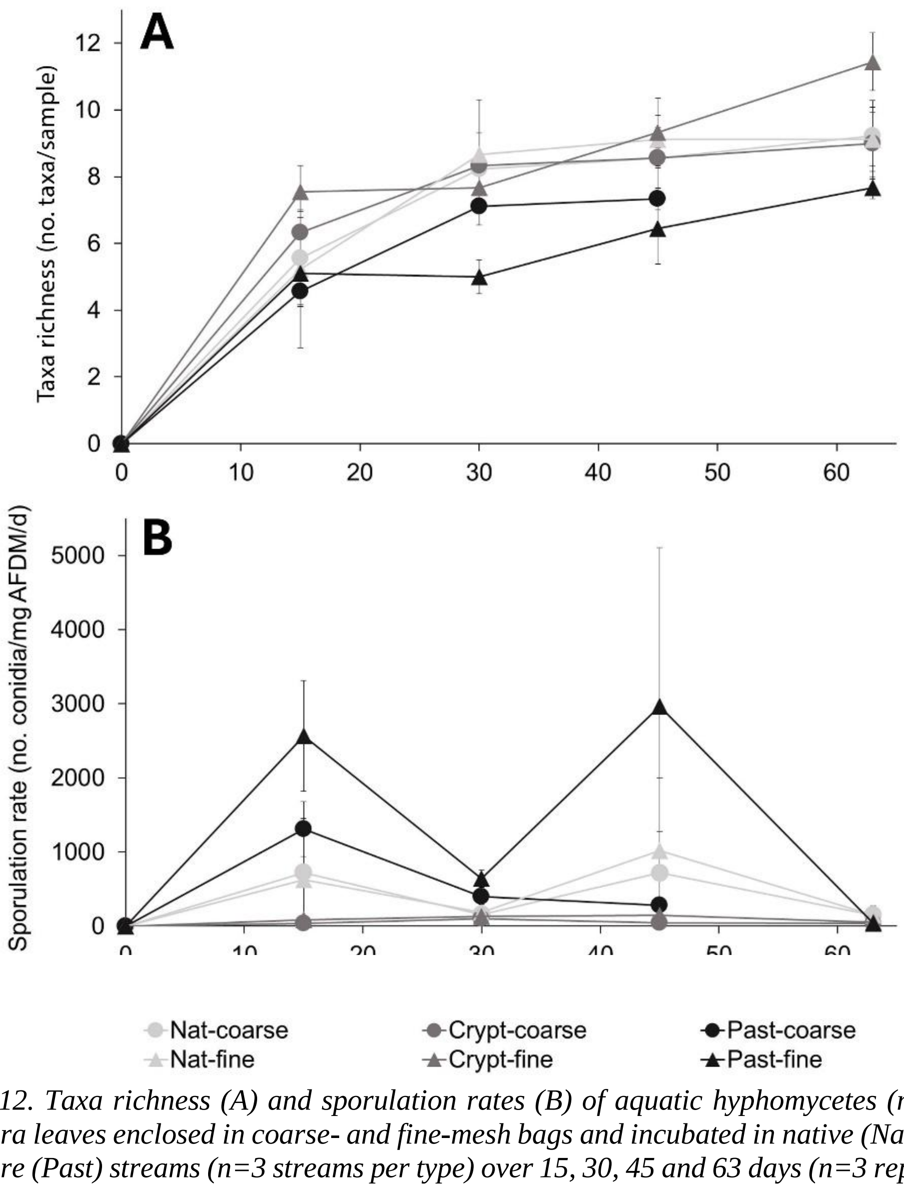

Figure 2.12. Taxa richness (A) and sporulation rates (B) of aquatic hyphomycetes (mean ± SE) associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date). Taxa richness and sporulation rates were not determined at day 63 in pasture streams due to low amount of leaf mass remaining.

Aquatic hyphomycete taxa richness associated with clethra leaves increased over the incubation period for all stream types and both mesh sizes (four-level nested ANOVA, $P<0.001$) but did not significantly differ between stream types, streams or mesh sizes (four-level nested ANOVA, $P \geq 0.052$) (Table S8), reaching 8 – 11 taxa/sample on day 63 (Figure 2.12).

Aquatic hyphomycetes sporulation rates associated with clethra leaves in native and pasture streams were higher on days 15 and 45 than on day 30, while they remained low throughout the incubation period in cryptomeria streams (<150 conidia/mg AFDM/d) (Figure 2.12). However, sporulation rates did not differ significantly among stream types, streams or mesh sizes (four-level nested ANOVA, $P \geq 0.087$; Table S8)

Benthic and Litter Macroinvertebrates

Overall, 46 macroinvertebrate taxa were found in the benthos of native, cryptomeria and pasture streams (Table S9). Benthic communities significantly differed between stream types and streams (PERMANOVA, $P=0.007$ and $P=0.003$, respectively), but did not change during the incubation period (PERMANOVA, P=0.0.654) (Table S10). In native streams, the worm *Nais* sp., Orthocladiinae midges and the caddisfly *Oxyethira falcata* Morton, 1893 were the predominant taxa, with a relative abundance of 31%, 23% and 19 %, respectively. Cryptomeria streams were characterized by the black fly *Simulium azorense* (Carlsson, 1963) (20%), the caddisfly *L. atlanticus* (17% relative abundance), the worm *Lumbriculus variegatus* (Müller, 1774) (16%) and Orthocladiinae midges (16%). The dominant species in pasture streams were the worm *Nai*s sp. (37 % relative abundance), Orthocladiinae midges (29%) and the caddisflies *Hydroptila* sp. (14%).

Dispersion of principal coordinates analysis (PCO) showed that the X axis explained 52.2% of variation in the benthic macroinvertebrate community (Figure 2.13). Shredders were the main functional feeding group responsible for explaining the dissimilarity between cryptomeria streams and the other two stream types. Filter-feeders and predators seemed to be the dominant functional feeding groups in native streams, while dominated by grazer/scrapers and gatherer/collectors predominated in pasture streams (Figure 2.13).

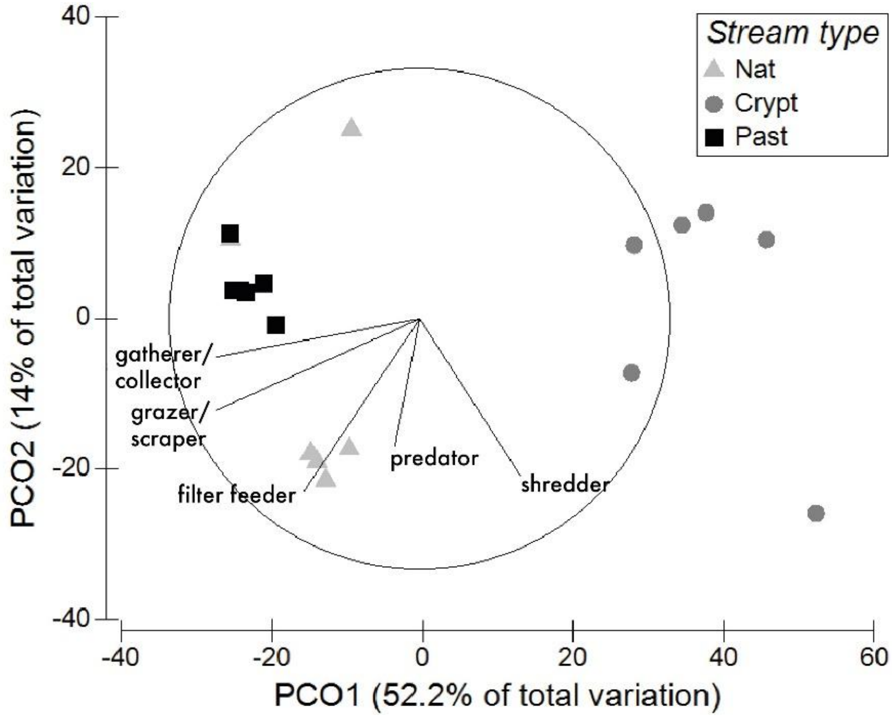

Figure 2.13. Dispersion of principal coordinates analysis (PCO) done on benthic macroinvertebrate community of native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type), sampled on two dates (d0 and d63), with the main functional feeding groups responsible for similarities within stream types. Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Only four shredder taxa were collected across streams (*Dicranomyia* sp., *Jaera nordmanni subsp. insulana Veuille, 1976*, *L. atlanticus* and *Tipula* sp.). Each stream had at least one shredder taxa, but mean relative abundance was generally low for most of them (<5%), except for *L. atlanticus* that was one of the codominant taxa in the benthic macroinvertebrate community in cryptomeria streams (17% relative abundance). Shredder density in the benthos did not significantly differ among stream types (one-way ANOVA, P=0.054), but *L. atlanticus* density did (P<0.001) (Table S11), being significantly higher in cryptomeria streams than in the other stream types (Table S11).

Macroinvertebrate communities associated to decomposing leaves were affected by all tested factors, differing among stream types, streams and along time (PERMANOVA, P≤0.032; Table S12). The most abundant taxa found in native and pasture streams were the worm *Nai*s sp. (25% and 32% relative abundance, respectively) and Orthocladiinae midges (21% and 26%, respectively). The caddisfly *O. falcata* was a codominant taxon in native streams (26%) and the endemic caddisfly *L. atlanticus* was a codominant taxon in cryptomeria streams (50%). Shredder density associated to decomposing leaves significantly differed among stream types (two-way ANOVA, P<0.001; Table S14), being highest in cryptomeria streams (Figure 2.14). Pasture and native streams had very low shredder density associated with leaves (Figure 2.14).

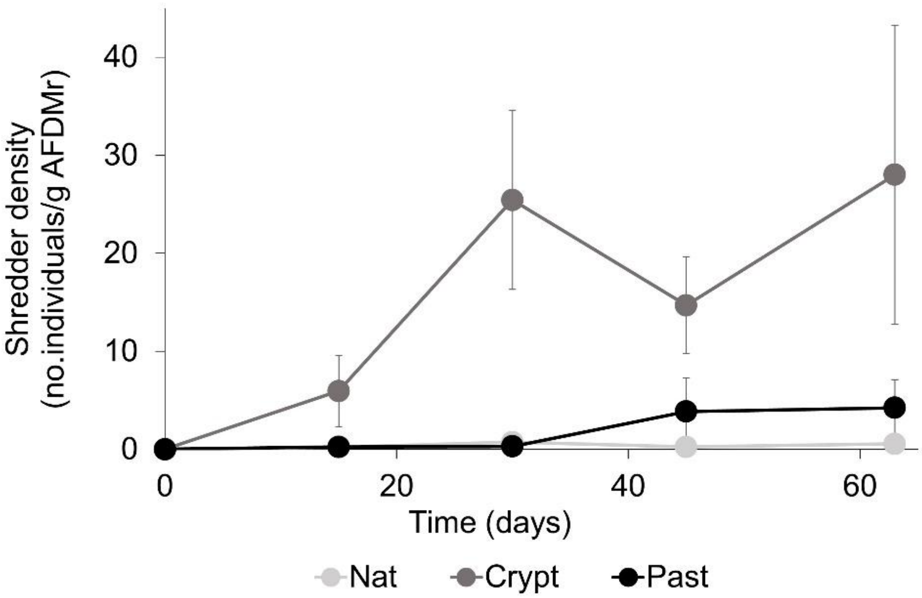

Figure 2.14. Shredder density (mean ± SE) on clethra leaves enclosed in coarse-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

2.5 DISCUSSION

Organic matter decomposition was highly influenced by land uses. The differences in organic matter decomposition between stream types and substrates were most likely due to differences in dissolved nutrient concentrations and water temperature, microbial decomposer activity and macroinvertebrate shredders, as discussed below.

Streams flowing through cryptomeria plantations and native laurel vegetation showed similar microbial-driven decomposition for leaves and wood substrates. This supports our hypothesis 1 and aligns with previous findings from the Azores (Ferreira et al., 2017; Raposeiro et al., 2014). Similar microbial activity could reflect similar water characteristics between cryptomeria and native streams. This was not the case as dissolved nutrient concentration and water temperature differed among stream types. However, aquatic hyphomycete communities also differed among stream types, suggesting some functional redundancy between decomposer communities as communities in cryptomeria streams kept organic matter decomposition at similar rates to those in native streams, despite the lower water temperature and dissolved phosphorus concentrations (Ferreira et al., 2017). This result agrees with results from a few studies that also did not find significant differences in organic matter decomposition between streams in conifer plantations and native forest (Ferreira et al., 2017; Riipinen et al., 2010). Other studies found higher (Riipinen et al., 2009; Whiles & Wallace, 1997) or lower (Hisabae et al., 2011; Martínez et al., 2013) organic matter decomposition rates in conifer streams (see Introduction). The multiple responses of organic matter decomposition to conifer plantations across studies suggests that the effect depends on a complex set of factors such as the identity of plantation species, type and identity of the substrate, decomposer community and water characteristics, complicating generalizations.

Total organic matter decomposition differed between cryptomeria and native streams, contradicting our hypothesis 1. These differences were due to higher shredder densities associated with coarse-mesh bags in cryptomeria streams. In our study, *L. atlanticus* was the shredder species most abundant in cryptomeria streams, being present in very low abundances in native and absent from pasture streams. According to previous studies (Balibreaet al., 2020; Raposeiroet al., 2018) the preferred habitats of this endemic caddisfly are streams at high elevation flowing through native vegetation or commercial conifer plantations with gravel and sand depositional areas with organic matter accumulations. Thus, the restricted ecological niche to lotic systems with circumneutral pH, high oxygen concentration and moderate nutrient concentration may explain abundance differences of this species between natives and cryptomeria streams. Moreover, our results showed an association between benthic invertebrates functional feeding groups and stream type, with shredders predominating in cryptomeria streams, filter-feeder in native streams and gatherer-collectors and grazer-scrappers in pasture streams, which support our results.

Streams surrounded by pastures for livestock use exhibited significantly faster microbial-driven and total organic matter decomposition compared to streams flowing through cryptomeria plantations or native vegetation, in agreement with hypothesis 2. It is well known that an increase in dissolved inorganic nutrient availability may often stimulates microbial activity (Ferreira et al., 2015). In our study, the faster organic matter decomposition in pasture streams can be attributed to higher dissolved nutrient concentrations in the water, probably derived from the leaching of cattle manure. Some other studies that assessed the effects of agricultural land use on stream ecosystems also showed that elevated nutrient inputs, particularly nitrogen and phosphorus promoted microbial activity and thus, enhanced organic matter decomposition (Hladyz et al., 2011; Quinn et al., 2000). However, not always is organic matter decomposition higher in agricultural streams compared with streams with native vegetation as the stimulatory effect of nutrient increase can be counteracted by sedimentation (Niyogi et al., 2003).

Higher decomposition rates in pasture streams may also be attributed to higher water temperatures owing to the lower elevation of these streams. Several studies underscored the pivotal role of water temperature as a key abiotic factor influencing organic matter decomposition rates (reviewed by Amani et al., 2019). Moderate increases in water temperature may promote organic matter decomposition by stimulating the leaching of secondary compounds, thus facilitating decomposer colonization (Mas-Martí et al., 2015), metabolic rates of microbial decomposers and consumption rates of shredders (Irons et al., 1994; Buzby & Perry, 2000; González & Graça, 2003;

Azevedo-Pereira et al., 2006; Ferreira & Chauvet, 2011a, 2011b). Previous studies found that higher water temperatures stimulate fungal biomass and the sporulation of aquatic hyphomycetes, thereby accelerating decomposition rates (Dang et al., 2009; Ferreira & Chauvet, 2011a, 2011b; Martínez et al., 2013; Taylor & Chauvet, 2014). Microbial activity may be particularly stimulated by the simultaneous increase in both water temperature and dissolved nutrients (Ferreira & Chauvet, 2011a; Martínez et al., 2013). However, sporulation rates did not significantly differ among stream types in our study, contradicting our hypothesis 5. However, unexpectedly, sporulation rates declined at day 30 before rising again. This decline may be linked to intense precipitation events, resulting in a strong punctual stream flow on the days before the sample collection (Figure S1). These flow spikes, which may last for a few days, are very often in the archipelago and contribute to the torrential regime of Azorean streams (Gonçalves et al., 2015). Torrential events can potentially disrupt sporulation rates due to water strength, which may damage fungal conidiophores at the leaf surface. We should expect that strong water flow could also mean an increase in substrate decomposing due to physical abrasion. However, our findings did not show a strong increase in organic matter decomposition at that time, which may be explained by the fact that substrates were still in the first stages of decomposition. Nevertheless, aquatic hyphomycete community structure differed between stream types, being less diverse in pasture streams, where it was dominated by *Lunulospora curvula*, which is known to be a warm-water species (Canhoto et al., 2016).

As temperature and land use covary (i.e., agriculture streams are mostly at lower elevations while plantation and native streams are at higher elevations), decomposition rates were also expressed per degree-day, which corrects for the direct effects of differences in temperature (Rowe et al., 1996; Griffiths & Tiegs, 2016; Gessner & Peeters, 2020), to assess if land use effects were in fact only due to differences in temperature (i.e., elevation). This adjustment revealed that organic matter decomposition in pastures remained elevated relative to native streams suggesting that differences between stream types are not only due to differences in temperature and in their position in the elevation gradient.

It is widely known that intrinsic litter characteristics have a strong influence on organic matter decomposition (Ostrofsky, 1997; Jabiol et al., 2019; Ramos et al., 2021) . In our study leaves decomposed faster than wood in all three stream types, in agreement with our hypothesis 3. This difference between organic matter types was due to differences in their structural and chemical properties; clethra leaves are softer and richer in nutrients compared to the harder, nutrient-poor balsa wood (Balibrea et al., 2020; Ferreira et al., 2006), making it a more appealing substrate to decomposers. Moreover, differences in decomposition between leaves and wood were more obvious in pasture streams where decomposition progressed to a more advanced phase.

We also hypothesized that organic matter in coarse-mesh bags would have faster decomposition than in fine-mesh bags due to shredders access and the possible higher physical abrasion in the former. However, we only found significant differences between mesh sizes in cryptomeria streams. Azorean stream macroinvertebrate communities are characterised by few shredder taxa, which are generally present in low abundances or even absent (Balibrea et al., 2020; Borges et al., 2010; Ferreira et al., 2016; Raposeiro et al., 2012; Raposeiro, et al., 2011). Other studies in the Azores found higher leaf litter decomposition rates where *L. atlanticus* occurred at higher densities (Balibrea et al., 2020; Raposeiro et al., 2018). In our study, shredders occurred at low densities in coarse-mesh bags in pasture and native streams, which translated into negligible differences in leaf decomposition between mesh sizes. However, higher densities of *L. atlanticus* associated to leaves occurred in cryptomeria streams, suggesting that this species could be the main decomposer in our study streams. Other studies also found that a single species acted as a key shredder responsible for driving the decomposition process of leaf litter (Encalada et al., 2010; Piscart et al., 2009).

Physical abrasion is also an important factor to consider when comparing organic matter decomposition among streams or between microbial-driven or total organic matter decomposition (Balibrea et al., 2020; Sabatino et al., 2020; dos Santos Fonseca et al., 2013; Graça, 2001). However, the slight difference in leaf decomposition between mesh sizes in native and pasture streams suggests that physical abrasion was likely not a relevant factor in our study. Thus, when comparing leaf decomposition between mesh sizes, our results suggest that shredders had an important role in leaf decomposition in coarse-mesh bags in cryptomeria streams, but not in native and pasture streams where decomposition was mainly driven by microbes. Our results also agreed with previous studies done in Azorean streams showing that when shredders occur in low density, microbes are the main players in leaf litter decomposition (Balibrea et al., 2020; Ferreira et al., 2016; Raposeiro et al., 2014; Raposeiro et al., 2018). However, when shredders were present in higher densities, they strongly contributed to leaf litter decomposition.

Pasture streams had the lowest aquatic hyphomycete taxa richness. Some studies have also shown that aquatic hyphomycete communities differed between native streams and streams flowing through monospecific plantations because high litter species richness allows higher aquatic hyphomycete species richness (Bärlocher & Graca, 2002; Ferreira et al., 2006). This finding agrees with our hypothesis 6 that a reduction in riparian vegetation diversity would reduce riparian habitat diversity and consequently lead to lower taxa diversity. Moreover, in our study, the similar aquatic hyphomycete taxa richness in native and cryptomeria streams, agreed with previous observations reported by Ferreira et al. (2017) where streams in cryptomeria plantation and native forest had similar aquatic hyphomycetes taxa richness associated to decomposing leaves substrate. The quantity and quality of organic matter inputs into pasture streams is probably too low, due to the absence or scarce riparian vegetation, to support a fungal community as diverse as those in native and cryptomeria streams. The similarity between aquatic hyphomycete taxa richness between native and cryptomeria streams may result from a trade-off between substrate diversity (higher in native streams) and litter quantity (higher in cryptomeria streams) (pers. obs.).

Unexpectedly, pasture streams had the highest macroinvertebrate taxa richness and abundance in the benthos among stream types, which contradicted our hypothesis 6. The predominant abundance of gatherer/collectors and grazer/scrapers in pasture streams seemed to be correlated with high solar exposure, which translated into high primary production, due to the absence of vegetation. These findings showed that benthic invertebrate's composition was shaped either by streams characteristics, or by the diversity of riparian vegetation, suggesting that both may be important factors controlling the observed differences in richness and abundance of invertebrate communities in our study. Moreover, pasture streams had a thin strip two- to ten-meters wide of exotic vegetation that may have acted as a small buffer that minimized the effects of bank erosion, sediment inputs, temperature and light increase and may have supplied some organic matter inputs. Similar results were observed in other studies where higher benthos richness was found in pastures streams than in native forest probably due to the establishment of some riparian vegetation in the former ones (Quinn et al., 1997; Scarsbrook & Halliday, 1999).

In conclusion, organic matter decomposition in pasture and native streams was mostly microbial-driven, while macroinvertebrate shredders played an important role in leaf litter decomposition in cryptomeria streams. Higher water nutrient concentrations and temperature explained the faster organic matter decomposition in pasture streams than in native or cryptomeria streams. Comparisons among stream types were partially confounded by differences in elevation due to the observed zonation in land uses that is common in oceanic islands with sharp slopes. Nevertheless, our results suggest that decomposition of leaves in coarse-mesh bags was sensitive to the different conditions among the three stream types, while decomposition of leaves and wood in fine-mesh bags did not discriminate between native and cryptomeria streams. Knowing the interaction between substrate type (i.e., litter species and mesh size) and stream type (i.e., environmental conditions) may inform future studies interested in addressing stream functioning across stream types or in using organic matter decomposition as a tool to address stream functional integrity.

Chapter 3

Effects of leaf litter naturally enriched with metals on invertebrate decomposers.

3.1 ABSTRACT

Streams naturally enriched with metals due to runoff from lava aquifers are a common feature of active volcanic islands. In the Azores archipelago, some streams exhibit inherent acidity and elevated concentrations of metals such as Fe, Al, and Mn. This study examines how metal exposure affects leaf litter decomposition and the performance of shredders decomposers by submerging three types of leaf litter, the exotic *Alnus glutinosa* (high quality substrate and used as a control) and the recalcitrant native *Ilex perado* and *Laurus azorica*, in both a metal-enriched stream and a reference stream with low metal concentration for a two-week period. After incubation, metal-enriched and reference leaf litter were used to evaluate feeding preferences, consumption rates, growth, and survival of the endemic Azorean shredder caddisfly, *Limnephilus atlanticus*. In feeding trials where only reference leaves were available, shredders significantly preferred *A. glutinosa* over the other two species. When given metal-enriched leaves, they exhibited a preference for *I. perado* over *L. azorica*. Additionally, when given a choice between reference and metal-enriched leaves, *L. atlanticus* significantly preferred reference leaves, except in the case of *I. perado*, where no significant preference was observed. During long-term trials (three-weeks), larvae demonstrated a higher consumption rate for metal-enriched *A. glutinosa* leaves compared to reference leaves, whereas no significant difference was detected for *I. perado* or *L. azorica*. Relative growth rates remained similar across metal-enriched and reference leaves. Overall, larvae consumption and growth were higher on *A. glutinosa* and *I. perado* than on *L. azorica* leaves. Survival rates did not significantly differ between leaf types or species. These results indicate that the quality of leaf litter had a greater impact on shredder performance than metal exposure. This suggests that the presence of recalcitrant native litter may buffer the negative effects of metal enrichment on shredders decomposers in Azorean streams.

3.2 INTRODUCTION

Streams impacted by metal drainages are found across the globe (Harbrow, 2001; Harding, 2005; Batty & Hallberg, 2010), especially in regions affected by acid rain, urbanization, and mining activities (Niyogi et al., 2002a; Pascoal et al., 2005; Batty & Hallberg, 2010). Additionally, streams with naturally acidic waters and elevated metal concentrations are widespread (Hogsden, 2013), particularly in areas of volcanic origin (Hurwitz et al., 2010; Schopka & Derry, 2012; Freire et al., 2013). These naturally acidic streams are predominantly located in volcanic regions such as New Zealand, Japan, Russia, Iceland, and the United States (Brock, 1980; Hedenquist et al., 1994; Carey et al., 2002; Singh & Mosley, 2003; Prada et al., 2005; Wilson et al., 2009; Cabral Pinto & Ferreira da Silva, 2019). In Azores archipelago, a set of young oceanic islands in the middle of the North Atlantic, belonging to Portugal, streams naturally enriched with metals are frequent. These streams are characterized by low pH, elevated concentrations of iron (Fe), aluminium (Al), manganese (Mn), copper (Cu), and zinc (Zn), and substrata coated with metal oxides and hydroxides precipitates (Louvat et al., 1998; Cruz et al., 1999; Terroso et al., 2006; Gonçalves et al., 2016). Such high metal concentrations can arise from effluents and springs linked to active volcanism (Cruz, 2003; Quintela et al., 2013), soil leaching by organic acids or metal-enriched groundwater due to the weathering of bedrock or soils (Kelley & Hudson, 2007; Batty & Hallberg, 2010) and runoffs from lava flow aquifers (Cruz & França, 2006; Cabral Pinto & Ferreira da Silva, 2019).

The ecological effects of high metal concentrations in streams have been widely studied, particularly their impact on key ecosystem processes such as organic matter decomposition (Carlisle & Clements, 2005; Hogsden & Harding, 2012; Peters et al., 2013; Ferreira et al., 2016b). Organic matter decomposition is predominantly driven by biological processes mediated by microbes and invertebrate shredders (Cummins et al., 1973; Hieber & Gessner, 2002; Cornut et al., 2010). In temperate continental streams, shredders play an important role in organic matter decomposition by breaking down leaf litter, incorporating organic carbon and nutrients into secondary production, and releasing fine particles that serve as food for collector invertebrates (Hieber & Gessner, 2002; Gulis et al., 2006; Cornut et al., 2010). However, the decomposition of leaf litter in oceanic island streams remains poorly understood (Larned, 2000; Ferreira et al., 2016d; Raposeiro et al., 2018). Studies conducted in streams in the Azores indicated that microbial communities, where aquatic hyphomycetes are the main decomposers, dominate litter decomposition due to the low diversity and abundance of shredders (Raposeiro et al., 2013; Ferreira et al., 2016d). Nonetheless, experiments in high-elevation streams have revealed that when shredders are present in high densities, they assume a dominant role in decomposition processes (Raposeiro et al., 2018; Balibrea et al., 2020a). Despite these findings, how naturally metal-enriched conditions may affect shredders (e.g., feeding and growth) remains largely unknown.

Elevated metal concentrations in stream water can negatively impact shredders by contaminating leaf litter, which can inhibit consumption, reduce growth rates, and increase mortality (Abel & Barlocher, 1988; Gonçalves et al., 2011; Batista et al., 2012; Campos et al., 2014; Ferreira et al., 2016b). Microbial conditioning of metal-enriched litter can be constrained because metals can be adsorbed into leaf litter surfaces inhibiting microbial colonization (Duarte et al., 2008; Roussel et al., 2008; Sridhar & Bärlocher, 2011; Funck et al., 2013a), leading to reduced litter palatability and subsequently inhibiting shredder consumption (Schlief & Mutz, 2006; Gonçalves et al., 2011; Batista et al., 2012; Funck et al., 2013b; Niyogi et al., 2013).

The extent of these effects may also depend on the intrinsic characteristics of the litter. Shredder consumption is often influenced by the physical and chemical properties of leaf litter, such as nutrient content, toughness, and the presence of structural (e.g., lignin) or secondary compounds (e.g., polyphenols) (Abelho and Graça 1996; Graça and Cressa 2010; Ferreira et al. 2017). Thus, soft leaves with low levels of structural (such as lignin) and secondary compounds (such as polyphenols) and high nutrient concentrations are generally more palatable and consumed at higher rates than tough or recalcitrant leaves (Ferreira et al. 2016b; Raposeiro et al. 2018; Balibrea et al. 2020).

The feeding activity of shredders is a reliable indicator of environmental stress, being particularly sensitive to acidity and elevated concentrations of dissolved metals in water (Collier & Winterbourn, 1990; Gonçalves et al., 2011; Hogsden et al., 2013). Moreover, it is an important ecological parameter linked to organismal growth, reproduction, and survival, making it a valuable measure for assessing the impacts of metal enrichment on stream ecosystems through metal-enriched food resources (Irving et al., 2003; Casimiro & Fidalgo, 2007; Gonçalves et al., 2011).

In this study, we incubated leaf litter with distinct initial characteristics (*Alnus glutinosa* (L.) Gaertn, *Ilex perado* Aiton, and *Laurus azorica* (Seub) Franco) in a stream with low metal concentrations and a stream with naturally high metal concentrations. The incubated leaf litter was then used to feed larvae of the Azorean endemic shredder caddisfly, *Limnephilus atlanticus* Nybom, 1948 (Trichoptera, Limnephilidae), to assess their feeding preferences, consumption, growth and survival.

We hypothesized that (1) metal concentrations would be higher in leaf litter exposed in the high-metal stream compared to the low-metal stream; and (2) larvae of *L. atlanticus* would exhibit a preference for consume more of, grow better on, and have higher survival rates when fed with leaves incubated in the low-metal stream compared to the high-metal stream. Additionally, we hypothesized that (3) larvae would prefer, consume more of, grow better on, and have higher survival rates when fed with high-quality leaf litter compared to recalcitrant leaf litter. Finally, we predicted that (4) differences in larvae consumption and growth rates between leaf litter incubated into high vs. low metal concentrations would be more pronounced for high-quality leaf litter than for recalcitrant leaf litter, as the superior performance of larvae on high-quality litter would be more strongly inhibited by elevated metal concentrations in the litter than the performance on low quality litter.

3.3 MATERIALS AND METHODS

Larvae

This study utilized early-instar larvae of the endemic shredder caddisfly *Limnephilus atlanticus* (Figure 3.1), collected during early summer 2015 from the benthic zone of a first-order reach of Ribeira do Folhado, São Miguel Island (37°48’48’’N, 25°14’47’’W, 729 m a.s.l.). The stream is characterized by circumneutral pH, low conductivity, and minimal nutrient concentrations

(Balibrea et al., 2017), with low dissolved metal content (Gonçalves et al., 2016). The riparian vegetation consists of native broadleaf evergreen trees, including *Ilex perado* and *Laurus azorica*, alongside exotic species such as *Clethra arborea* Aiton and *Cryptomeria japonica* (L. f.) D. Don. Larvae were collected using a kick net (0.5 mm mesh), sorted in the field, and transported in a cooler to the University of the Azores, Ponta Delgada, São Miguel. Water, sediment, and conditioned leaf litter were also collected from the stream at the time of sampling. In the laboratory, larvae were maintained in aerated stream water with sediment at 12°C under a 10 h light:14 h dark photoperiod for two days prior to experimentation. During this period, they were fed *Alnus glutinosa* leaf litter, a higher-quality nutritional substrate than naturally available in the stream, except for the 12 h preceding the experiment, when they were fasted. Only larvae with a wet mass of 40.0–50.0 mg (body + case; Balibrea et al. 2017) were selected for the experiments (Figure 2.1).

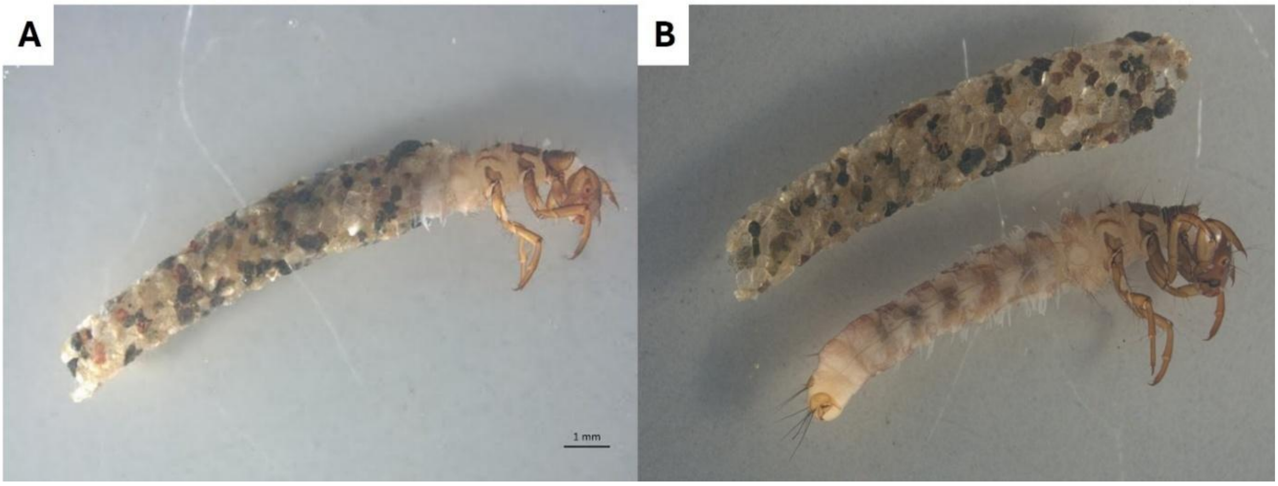

Figure 3.1. Larvae of Limnephilus atlanticus used in the microcosms trials (A); larvae body and case separately, dried larvae body used at the end of the experiments to estimate growth rates (B).

Leaf Litter

Leaves from three tree species exhibiting distinct physical and chemical properties were used: *Ilex perado, Laurus azorica,* and *Alnus glutinosa*. *I. perado* and *L. azorica* are native broadleaf evergreen species commonly found in Azorean riparian zones, with leaves collected from trees in winter 2014–2015 (Planalto dos Graminhais, São Miguel; 37°48’30’’N, 25°14’54’’W, 837 m a.s.l). *A. glutinosa*, a broadleaf deciduous species, was selected due to its frequent use in feeding behavior studies (Graça, 2001; Graça & Cressa, 2010); senescent leaves were collected in autumn 2014 from Lake Furnas, São Miguel (37°45’16’’N, 25°19’31’’W, 294 m a.s.l). Although *A. glutinosa* is an exotic species in the Azores, it occurs naturally along some lakeshores. Collected leaves were air-dried and stored in darkness until use.

Chemical analyses of the leaves were conducted to determine polyphenol, lignin, and nutrient (phosphorus, nitrogen, and carbon) concentrations. Leaf powder (<5 mm) was obtained from oven-dried (60°C) leaves. Polyphenols were extracted with acetone and quantified using the Folin-Ciocalteu method via spectrophotometry (Jenway 6715 UV/Vis, U.K.) (Bärlocher et al., 2020). Additional samples with known concentrations of tannic acid (0 – 500 μg/L) were processed as the experimental samples (Folin Ciocalteu method) and used to establish a linear regression model between absorbance and tannic acid concentrations (R2 = 0.98). Lignin was extracted from leaf powder (500 mg, 0.1 mg precision) by acid digestion in the autoclave. The extract was filtered through ceramic crucibles, and lignin concentrations on the residue were determined gravimetrically. Phosphorus was extracted from leaf powder (3.6 mg, 0.1 mg precision) by basic digestion in the autoclave, and phosphorus concentrations were determined on liquid samples (ascorbic acid method) by spectrophotometry (Bärlocher et al., 2020). Additional samples with known phosphorus concentrations (0 – 1000 mg/L) were processed as the experimental samples (basic digestion in the autoclave and ascorbic acid method) and used to establish a linear regression model between absorbance and phosphorus concentrations (R^2 = 1.00). Nitrogen and carbon concentrations were determined directly from leaf powder (500 μg, 0.1 μg precision) with an Elemental Analyzer/Isotope Ratio Mass Spectrometer (EA/IRMS; IRMS Thermo Delta V advantage with a Flash EA-1112 series, Thermo Fisher Scientific Inc., U.S.A.). Calibration of the IRMS occurs regularly, and current measures match international and laboratory standards to within ± 1‰. Acetanilide standards (71.09% C, 10.36% N) are included at the start, at every 20 samples, and at the end of each ‘run’ (i.e., a discrete set of samples analyzed continuously) for quality assurance. Replicate reference material has a variation ≤ 0.2 ‰, which determines acceptable precision. Leaf toughness was determined using a penetrometer after leaves had been soaked in distilled water for one hour, and results were expressed as the mass needed to penetrate the leaf with an iron rod (0.79 mm in diameter) (g) (Bärlocher et al., 2020).

Leaf litter exposure in streams

For the experiments, 12-mm diameter leaf discs were extracted with a cork borer, avoiding the main vein, from leaves previously moistened with distilled water (Figure 3.2). Leaf discs were then oven-dried (60 °C for 48 h) and weighed (0.1 mg precision) to determine initial discs DM. Leaf discs were enclosed in 0.5-mm mesh bags (Figure 3.2) and exposed for two weeks (May 2015) in two small tributaries of Ribeira Grande, located in the northeast hillside of Lake Fogo (São Miguel Island, Azores), that differ in metal concentration, to ensure the leaching of leaf soluble compounds, colonization by microbial decomposers and adsorption of metals (Figure 3.3). After the exposure period, leaf discs were retrieved, gently rinsed with distilled water and placed in each microcosm (see below). Extra leaf discs from each species were exposed in both tributaries simultaneously and for the same period to be used for metal concentration analyses; after collection, discs were gently rinsed with distilled water and maintained oven-dried (60 °C) until analyzed.

Figure 3.2. Moistened leaves of Alnus glutinosa (A); leaf discs of A. glutinosa cut with corkborer (B); fine-mesh bags for leaf discs incubation (C).

The tributary with low metal concentration (reference stream; 37°46’29’’N, 25°27’31’’W, 594 m above sea level) is circumneutral, has low conductivity and low nutrient concentration (Gonçalves et al., 2015b). The tributary with high metal concentration originating from volcanic emissions (metal-enriched stream; 37°46’31’’N, 25°27’33’’W, 586 m above sea level) is slightly acidic, poorly mineralized, and has low nutrient concentration (Gonçalves et al. 2015, Table S15)(Figure 3.3).

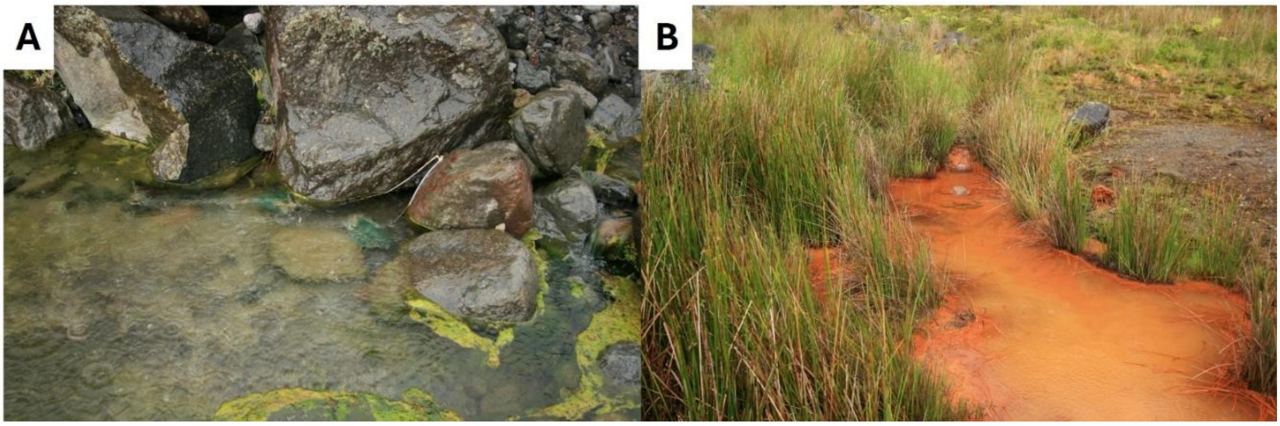

Figure 3.3. Incubation sites in a stream with low metal concentration (reference stream, A); and in a stream naturally enriched with metals (metal-enriched stream, B).

Water samples were collected from both sites, transported to the laboratory, filtered using fiber glass filters (47 mm diameter, 1.2 µm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, U.K.) and frozen until analyzed. Aluminum, Fe, and Mn concentrations in water (µg/L) and in leaves (µg/g of leaf DM) were determined by atomic absorption spectrometry with stoichiometric flame atomization (Willis, 1975). Metals considered for analyses were those most abundant in the metal-enriched stream (Gonçalves et al., 2015b). The precision of the analytical method was based on the determination of the repeatability (intra-day assays) and of the intermediary precision (inter-day assays) following the recommendations of the International Conference of Harmonisation (CPMP/ICH/281/96) and the International Union of Pure and Applied Chemistry (ISO 17025:2018 and ISO 3534:2006 validation guidelines)(ICH, 1996; Thompson et al., 2002; IPAC, 2018). Precision ranged from 3.0 to 5.0% and accuracy from 81.0% to 124.0% for the calibration concentrations.

Experimental setup

Trials to determine feeding preferences and leaf consumption by *L. atlanticus* larvae and its growth and survival when fed with leaf litter previously exposed in the reference stream and in the metal-enriched stream were run in laboratory microcosms. Microcosms consisted of 8.5 × 8.0 × 6.5

cm containers supplied with a layer of 10 g ignited (8 h at 500 °C) sand (200 μm grain size), and 250 mL of filtered stream water (0.7 μm-pore glass microfiber filter; Whatman GF/F, GE Healthcare Europe GmbH, Little Chalfont, U.K.), both from Ribeira do Folhado. One single pre-weighed larva (wet mass at 0.1 mg precision) was added to each experimental microcosm. Larvae wet mass varied between 40.0 – 50.0 mg, corresponding to 4.0 – 5.2 mg DM (Balibrea et al., 2017) across microcosms. Experimental trials were run inside a Sanyo versatile Environmental Test Chamber, MLR-351-H (Japan), where microcosms were kept with constant aeration at 12 ± 0.5 °C, with a 10 h light:14 h dark photoperiod.

Feeding preferences of *L. atlanticus* larvae

To assess feeding preferences of *L. atlanticus* larvae, individuals were faced with two choice situations: (i) a choice among the three leaf species previously exposed either in the reference stream (reference leaves) or in the metal-enriched stream (metal-enriched leaves); and (ii) a choice between reference and metal-enriched leaves for each leaf species (Figure 3.4). For the first situation (i), there were 15 microcosms with reference leaves and 15 microcosms with metal-enriched leaves; each microcosm receiving one disc per leaf species, individually pinned into the sand. For the second situation (ii), 15 microcosms were set up for each leaf species; each microcosm received two discs from reference leaves and two discs from metal-enriched leaves, pinned into the sand in pairs of the same type. Additionally, small 0.5-mm mesh bags containing discs similar to those pinned into the sand were attached to the top of the microcosms with a clip; these discs were not accessible to the larvae, and mass loss was due to leaching and microbial activity (control discs) (Figure 3.4). Experiments ran until at least one of the exposed discs was consumed to half (approximately 72 h). Then, leaf discs exposed to larvae and control leaf discs were oven-dried (60 °C for 48 h) and weighed (0.1 mg precision) to determine the remaining DM. After being oven-dried (60 °C for 48 h), final larval DM was also determined (0.1 mg precision).

Relative consumption rate (RCR) was calculated as: (DM control disc (g) – DM accessible disc (g)) / larvae final DM (g) / time (days). Results were expressed as g leaf DM/g individual DM/day (Balibrea et al., 2017).

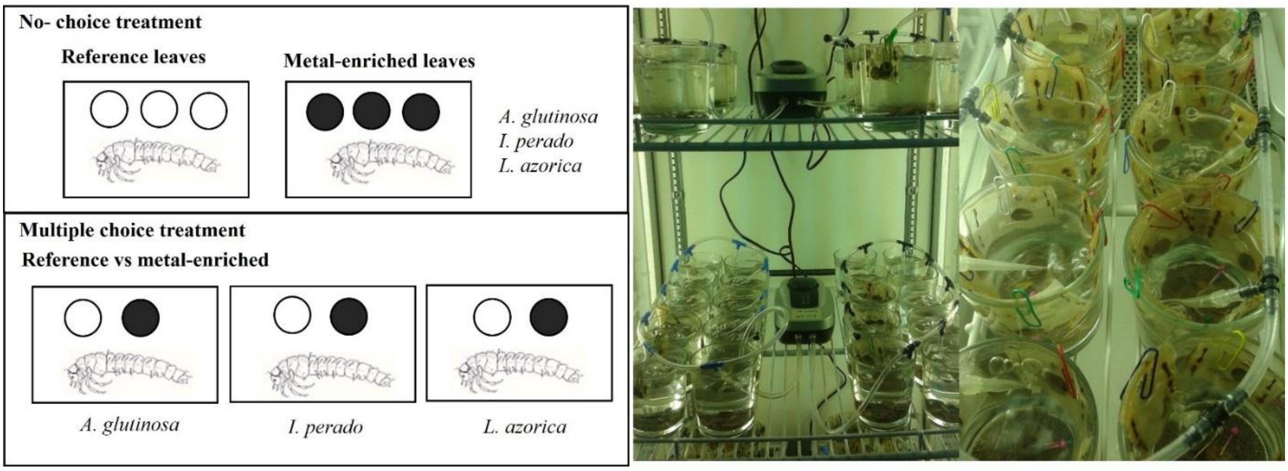

Figure 3.4. Short-term trials scheme of no-choice treatment and multiple-choice treatment where larvae were exposed to reference and metal-enriched leaves at the same time.

Consumption, growth and survival of *L. atlanticus* larvae

Another trial was carried out over three weeks to assess the consumption, growth, and survival of *L. atlanticus* larvae. Ten microcosms per leaf species and leaf origin (reference and metal-enriched leaves) were set up (total n = 60). Each microcosm received four discs of a given treatment that were pinned into the sand and a small litter bag with similar discs that were not accessible to the larvae (control discs), as above (Figure 3.5). At the end of every week, water, sand, and leaf discs from each microcosm were replaced with new ones. Leaf discs were oven dried (60 °C for 48 h) and weighed (0.1 mg precision) at the end of each week to determine the remaining DM. Larval DM (mg) was estimated at the beginning of each week from wet mass (WM, mg) by the regression model DM = –0.963 + 0.124 × WM (n = 120, R^2 = 0.87, P < 0.001; Balibrea et al. 2017). At the end of the trial, larvae were dried at 60 °C for 48 h and weighed (0.1 mg precision).

Relative consumption rate (RCR) was determined as described above. Relative growth rate (RGR) was calculated as: (final larvae DM (mg) – initial larvae DM (mg) / final larvae DM (g) / time (days)). Results were expressed as mg individual DM/g individual DM/day (Balibrea et al., 2017). Survival was expressed as the percentage of individuals alive at the end of the experiment.

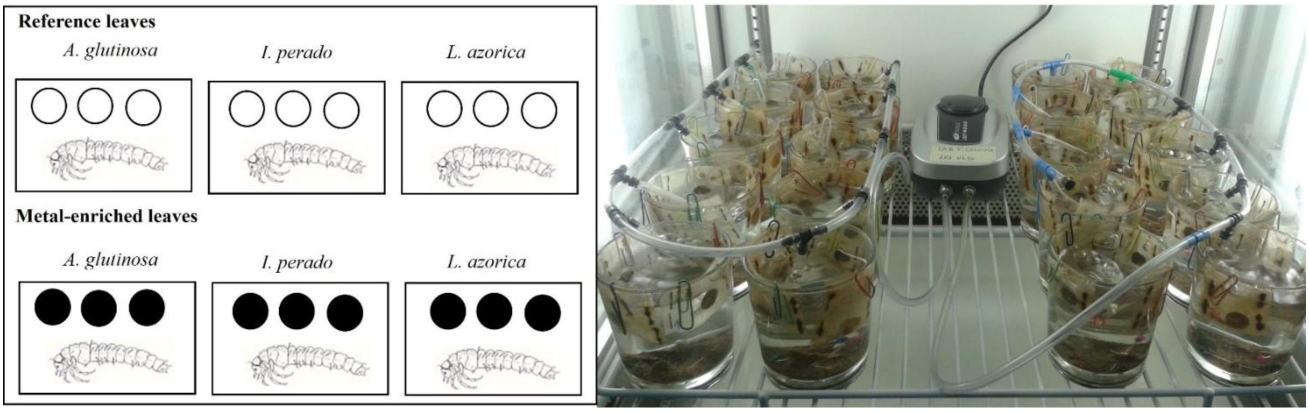

Figure 3.5. Long-term trials scheme where larvae were exposed to reference or metal-enriched leaves of each different leaf species.

Data analysis

Comparisons of physical and chemical characteristics among leaf species were done by one-way analyses of variance (ANOVAs), followed by Tukey's Honest Significant Difference (HSD) test when necessary. Comparisons of metal concentrations in water between streams were done by one-way ANOVAs, and comparisons of metal concentrations in leaf litter among the three leaf species exposed in the two streams were done by two-way ANOVAs, followed by Tukey's HSD test. RCRs were compared among the three leaf species for reference and for metal-enriched leaves (situation (i)) and between reference and metal-enriched leaves for each leaf species (situation (ii)) using Friedman's test, followed by Wilcoxon Signed Rank test. Comparisons of RCR and RGR among treatments were done by three-way repeated measures ANOVAs (leaf species, leaf origin and time as factors). Fisher's test was applied for post hoc multiple comparisons. Survival was analyzed using the Kaplan-Meier test, and comparison of survival curves was done by Log Rank (Mantel-Cox) test. Data conformed to assumptions of ANOVA (normality checked with Shapiro–Wilk test and homoscedasticity checked with Bartlett's test). Alpha was 0.05 for all tests. Analyses were performed using STATISTICA 7 (StatSoft, Tulsa, OK, U.S.A.).

3.4 RESULTS

Leaf litter and stream water characteristics

The three leaf species exhibited distinct initial physical and chemical properties (Table 3.1 and S15). *Alnus glutinosa* leaves were the softest, with the lowest polyphenol content, highest nitrogen concentration, and intermediate lignin levels. *Ilex perado* leaves had the lowest concentrations of lignin and nutrients (nitrogen and phosphorus) and moderate polyphenol levels. In contrast, *Laurus azorica* leaves displayed the highest concentrations of polyphenols and lignin, with intermediate nitrogen content (Table 3.1).

Concentrations of Fe and Mn in the metal-enriched stream were significantly higher than in the reference stream, increasing approximately 5× for Fe and 90× for Mn. However, Al concentrations did not significantly differ between streams due to considerable within-stream variation (Table 3.2 and S16).

*Table 3.1. Initial leaf litter chemical and physical characteristics (mean ± SE, n = 3) for the three species used in the experiments. For each characteristic (line), leaf litter species with different letters significantly differ (one-way ANOVA followed by Tukey's HSD test; *P<0.05, **P<0.01, ***P<0.001). DM, dry mass.*

Litter characteristics	*Alnus glutinosa*				*Ilex perado*				*Laurus azorica*			
P (% DM) **	0.10	±	<0.01	a	0.07	±	<0.01	b	0.16	±	0.01	a
N (% DM) ***	2.50	±	0.10	a	0.80	±	0.02	b	1.53	±	0.04	c
C (% DM) *	47.50	±	1.00	a	49.45	±	0.34	ab	50.62	±	0.59	b
Lignin (% DM) ***	35.80	±	0.40	a	22.38	±	1.43	b	40.98	±	0.72	c
Polyphenols (% DM) ***	3.50	±	0.30	a	6.63	±	0.23	b	12.26	±	0.71	c
Toughness (g) ***	107.20	±	8.60	a	276.81	±	6.49	b	266.15	±	10.68	b

*Table 3.2. Metal concentrations (mean ± SE, n = 3) in a stream with low metal concentration (reference stream) and in a stream naturally enriched with metals (metal-enriched stream). Comparisons between streams were made with one-way ANOVA (*P<0.05).*

Metal	Reference stream			Metal-enriched stream		
Al (µg/L)	278	±	1889	11975	±	2776
Fe(µg/L) *	104	±	11	516	±	64
Mn (µg/L) *	2	±	1	181	±	26

After the incubation period, leaves from the metal-enriched stream accumulated significantly higher metal concentrations than those from the reference stream (Table 3.3 and S17). The most pronounced differences were observed for Fe, which was 37 times more concentrated in *L. azorica* metal-enriched leaves compared to reference leaves, 24 times higher in *I. perado*, and 12 times higher in *A. glutinosa* (Table 3.3). Across all treatments, Fe concentrations followed the order: *A. glutinosa* > *I. perado* > *L. azorica*, whereas Mn concentrations followed the order: *I. perado* > *A. glutinosa* > *L. azorica*. No significant differences in Al concentrations were found among leaf species (Table 3.3 and S17).

*Table 3.3. Metal concentrations (mean ± SE, n = 3) in leaf litter previously exposed (two weeks) in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves). For each metal (columns), leaf litter species with different letters significantly differ (two-way ANOVA followed by Tukey's HSD test; *P<0.05, **P<0.001).*

Leaf type	Leaf species	Al (µg/g) *				Fe (µg/g) **				Mn (µg/g) **			
Reference	*A. glutinosa*	1239	±	229	a	389	±	7	a	65	±	2	a
leaves	*I. perado*	1733	±	204	a	162	±	0	b	124	±	2	b
	L. azorica	1198	±	83	a	88	±	5	c	24	±	0	c
Metal	*A. glutinosa*	1988	±	67	b	4794	±	28	d	70	±	1	d
enriched	*I. perado*	2063	±	48	b	3836	±	79	e	142	±	3	e
leaves	*L. azorica*	2311	±	483	b	3255	±	40	f	43	±	2	f

Feeding preferences of *L. atlanticus* larvae

Shredders consumed all the leaf species offered. When given a choice among reference leaves, individuals significantly preferred *A. glutinosa* over the other two species (Wilcoxon Signed Rank test, P = 0.008; Figure 3.6 A). When given a choice among metal-enriched leaves, individuals preferred *I. perado* over *L. azorica* (Wilcoxon Signed Rank test, P = 0.008; Figure 3.6 B).

When individuals were allowed to choose between reference and metal-enriched leaves, they preferred the former in the case of *A. glutinosa* and *L. azorica* (Friedman's test, P < 0.001 and P = 0.021 respectively; Table S18 and Figure 3.7), while no significant preference was found for *I. perado* (Friedman test, P = 0.109; Table S18 and Figure 3.7).

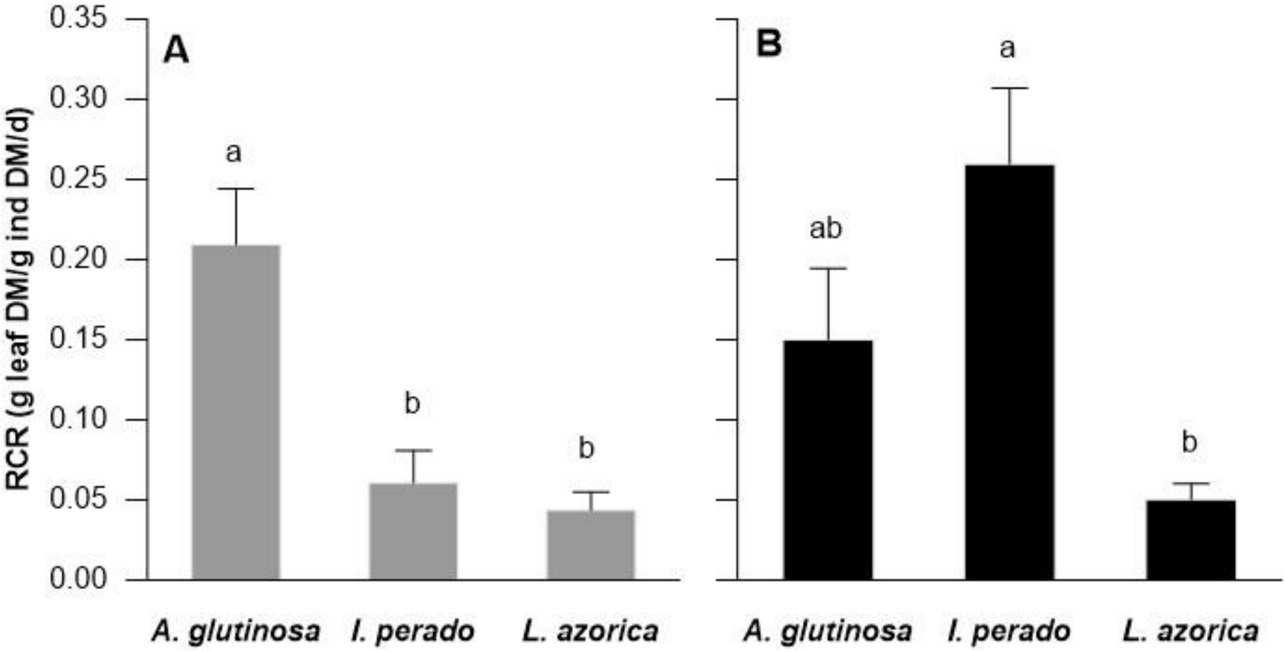

Figure 3.6. Relative consumption rates (RCR; mean ± SE, n = 15) of L. atlanticus individuals on each leaf species, when given a choice between the three leaf species previously exposed in a stream with low metal concentration (reference leaves; A) and in a stream naturally enriched with metals (metal-enriched leaves; B). Different letters indicate significant differences (Wilcoxon Signed Rank test, P<0.05). Adapted from Balibrea et al. (2023).

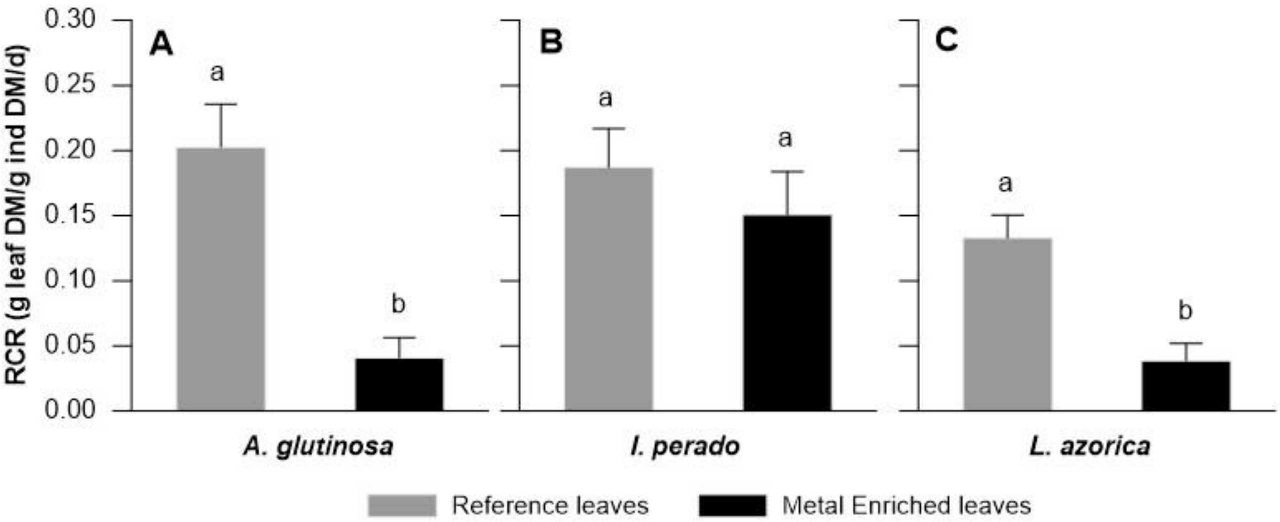

Figure 3.7. Relative consumption rate (RCR; mean ± SE, n = 15) of L. atlanticus individuals on each leaf species when given a choice between leaves previously exposed in a stream with low metal concentration (reference leaves) or exposed in a stream naturally enriched with metals (metal-enriched leaves) for A. glutinosa (A), I. perado (B) and L. azorica (C). Different letters indicate significant differences (Wilcoxon Signed Rank test, P < 0.05). Adapted from Balibrea et al. (2023).

Consumption, growth and survival of *L. atlanticus* larvae

Leaf species and stream significantly affected relative consumption rates (three-way repeated measures ANOVA, $P < 0.001$ and $P = 0.028$, respectively; Table S19), with a marginal significant interaction between both factors ($P = 0.086$). Overall, RCR were in the order *A. glutinosa* ~ *I. perado* > *L. azorica* for reference leaves and in the order *A. glutinosa* > *I. perado* > *L. azorica* for metal-enriched leaves (Figure 3.8 A). Relative consumption rates were significantly higher for metal-enriched than for reference *A. glutinosa* leaves (Fisher's test, $P = 0.004$). Moreover, there was a significant interaction between leaf species and time (three-way repeated measures ANOVA, $P = 0.004$; Table S19). Relative consumption rates increased over time for *A. glutinosa* reference and metal-enriched leaves (Fisher's test, $P = 0.026$), while RCR for *I. perado* and *L. azorica* reference and metal-enriched leaves remained constant among the three weeks ($P = 0.985$ and $P = 0.843$, respectively) (Figure 3.9 A).

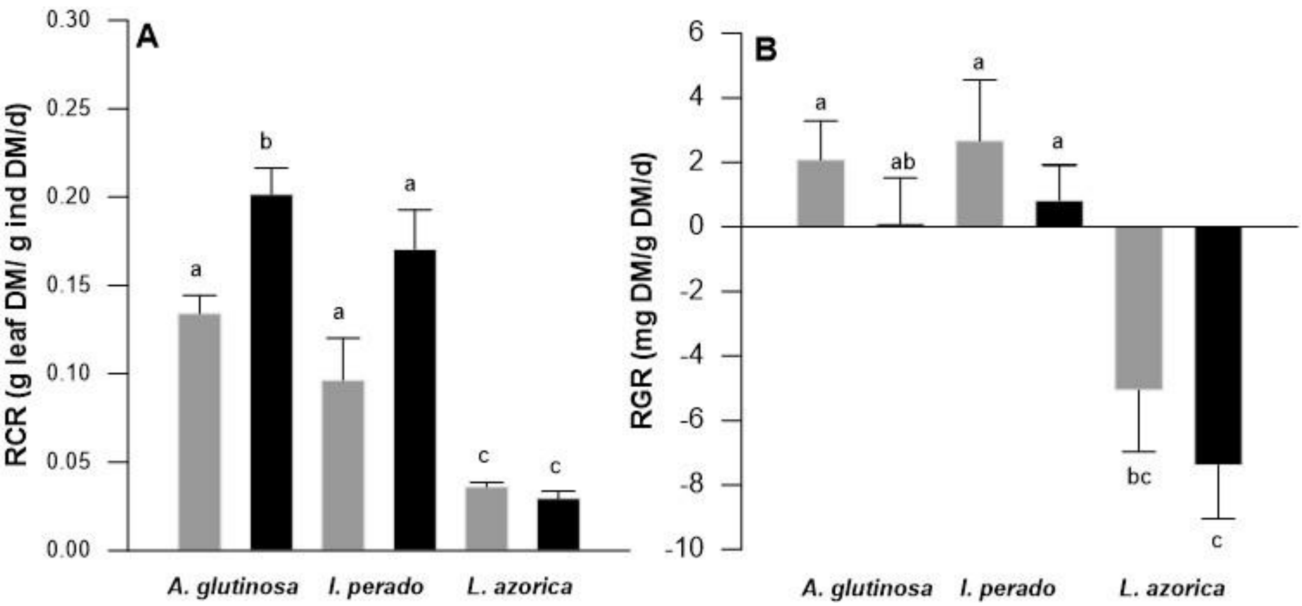

Figure 3.8. Relative consumption rate (RCR; A) and relative growth rate (RGR; B) (mean ± SE, n = 10) of L. atlanticus individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves). Different letters indicate significant differences (three-way repeated measures ANOVA followed by Fisher's test, P<0.05). Adapted from Balibrea et al. (2023).

Relative growth rates significantly differed among leaf species (three-way repeated measures ANOVA, $P < 0.001$; Table S20), with overall RGR in the order: *A. glutinosa* ~ *I. perado* > *L. azorica* (Figure 3.8 B). Relative growth rates did not significantly differ between reference and metal-enriched leaves for any leaf species (three-way repeated measures ANOVA, P = 0.593; Table S20) (Figure 3.8 B). Growth rates differed over time (three-way repeated measures ANOVA, P < 0.001; Table S20), with values increasing over the incubation period (Figure 3.9 B). Negative growth rates (i.e., individuals lost mass) occurred during the first two weeks when larvae consumed *L. azorica* leaves (Figure 3.9 B). Survival was higher when individuals fed on *A. glutinosa* and *I. perado* leaves (90 %) than on *L. azorica* (80 %). Survival did not differ when shredders fed on reference or metal-enriched leaves (80% – 90% across treatments; Log Rank (Mantel-Cox) test, P = 0.911).

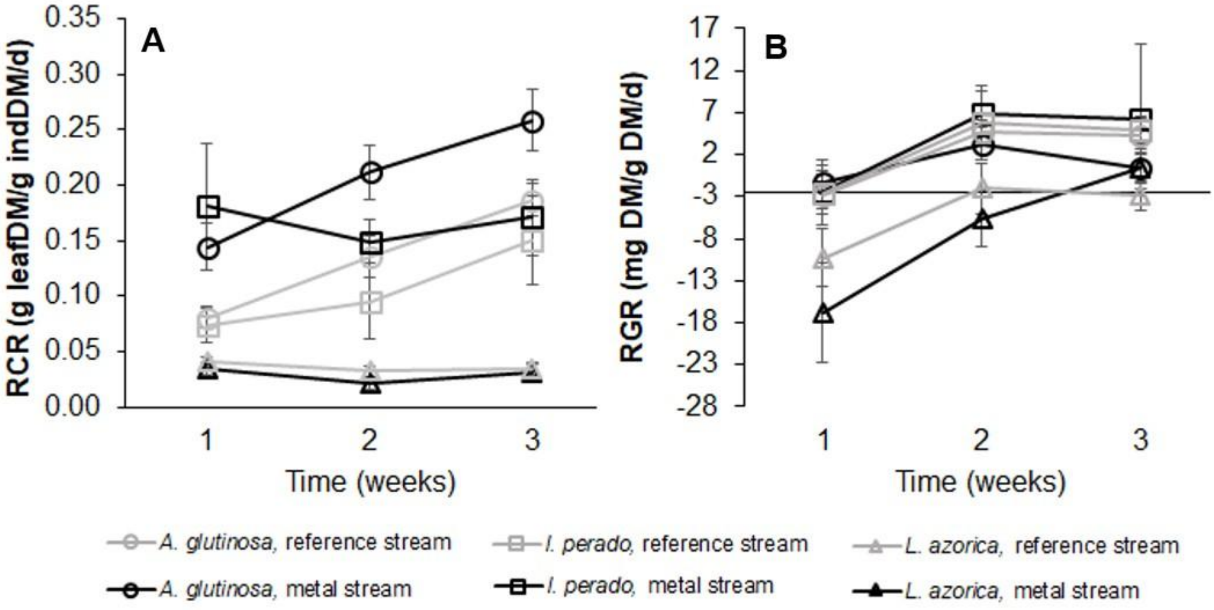

Figure 3.9. Relative consumption rate (RCR; A) and relative growth rate (RGR; B) (mean ± SE, n = 10) of L. atlanticus individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves). Adapted from Balibrea et al. (2023).

3.5 DISCUSSION

Leaf litter that had been previously exposed to a metal-enriched stream exhibited higher metal concentrations than reference leaves, confirming our first hypothesis. After the two-week incubation period, leaves from the metal-enriched stream were observed to be coated with precipitated metal oxides (A. Balibrea, personal observation). This pattern is consistent with previous studies, which have also reported elevated metal accumulation in coarse organic matter collected from streams receiving metal-rich effluents compared to pristine environments (Breteler et al., 1981; Schaller et al., 2008; Sridhar et al., 2008; Liu et al., 2021). The variation in metal concentrations among different leaf species suggests that intrinsic litter characteristics may influence metal incorporation processes such as adsorption and absorption. Studies have shown that surface topography of substrates plays a key role in biological colonization (Mechaber et al., 1996; Dang et al., 2007; Kearns & Bärlocher, 2008) and also in metal accumulation (Little, 1970). Consequently, rougher surfaces and the presence of cuticle appendages such as trichomes may increase the area available for metal adsorption. The fact that *L. azorica* exhibited the largest difference in Al, Fe, and Mn concentrations between metal-enriched and reference leaves may be associated with the dense trichomes on the lower leaf surface and its relatively rougher texture. Conversely, *A. glutinosa* displayed the smallest difference in metal concentrations between metal-enriched and reference leaves, which is likely due to its smoother cuticle and fewer appendages. Future research should focus on quantifying the characteristics of leaf surfaces and mesophyll structure to better understand the metal adsorption and absorption capacities of different leaf species. Additionally, Fe exhibited the highest difference in concentration between metal-enriched and reference leaves across all three leaf species, which is likely attributed to its strong precipitation capacity.

After stream incubation, leaf litter was analysed exclusively for metal concentrations. While nutritional quality was assessed before conditioning, it remains possible that differences in nutrient content, structural components, and secondary compounds between streams arose due to variations in leaching and microbial conditioning. However, if such differences were present, they were likely more pronounced in the reference stream than in the metal-enriched stream, as metal precipitates could have inhibited leaching and microbial colonization in the latter. As a result, following the incubation period, the nutritional quality of leaf litter was likely lower in the metal-enriched stream than in the reference stream. Investigating the effects of leaf litter conditioning on changes in nutritional quality and microbial colonization in naturally metal-enriched streams would enhance our understanding of the trophic interactions between detrital resources and consumers. Future studies should explore the broader implications of metal contamination on the trophic and functional ecology of stream ecosystems.

In the short-term feeding preference trials, *L. atlanticus* larvae exhibited a clear preference for reference over metal-enriched leaves when feeding on *A. glutinosa* and *L. azorica*, supporting our second hypothesis. A similar feeding pattern has been observed in *Sericostoma vittatum* Rambur, which preferred reference *Quercus robur* L. leaves over uranium-enriched ones in laboratory experiments (Gonçalves et al., 2011). The observed feeding preferences likely reflect both the negative impact of metal contamination and the reduced microbial colonization of metal-enriched leaves, which together lower the palatability of these resources compared to reference leaves. When leaf litter is well colonized, microbial enzymatic activity weakens its structural integrity, microbial biomass accumulation enhances its nutrient content, and exoenzymes facilitate digestion for consumers (Duarte et al., 2008; Fernandes, 2008; Ferreira et al., 2015; Raposeiro et al., 2018). These microbial processes generally increase litter palatability for shredders (Arsuffi & Suberkropp, 1988; Chung & Suberkropp, 2009). In contrast, when larvae were presented with a choice between reference and metal-enriched *I. perado* leaves, no significant preference was observed. This unexpected result is likely due to the detachment of the cuticle from the mesophyll in *I. perado* leaf discs (Figure S2), which enabled larvae to feed directly on the mesophyll, bypassing the potential inhibitory effects of the cuticle and metal deposits. Cuticle detachment, which occurred consistently across all experiments, was likely an artifact caused by damage from the cork borer used to extract the leaf discs. However, in natural stream conditions, small fragments of *I. perado* leaves that undergo perimeter damage from invertebrate feeding or physical abrasion may also experience cuticle loss, making them more palatable to shredders.

During the long-term (three-week) experiment, larvae exhibited higher consumption rates for metal-enriched *A. glutinosa* leaves than for reference leaves, which was unexpected. Despite this increased consumption, larval growth remained similar between treatments, suggesting that compensatory feeding may have occurred on metal-enriched leaves. This behavior, in which organisms consume greater amounts of a lower-quality food source to meet their nutritional needs, has been documented in previous studies (Carvalho & Graça, 2007). Although larvae consumed more metal-enriched leaves, their growth was likely constrained by metal toxicity. Therefore, these results did not support our second hypothesis. Similar compensatory feeding behaviors have been reported in *Deleatidium* spp. exposed to low-quality food in acidic New Zealand streams (Collier & Winterbourn, 1990). Additionally, *Nemurella picteti* Klapalek and other shredders have been shown to adjust their consumption rates to offset poor resource quality in acidic streams (Winterbourn et al., 1985). This feeding plasticity may be an adaptive strategy in resource-limited environments, allowing consumers to adopt a more generalist or opportunistic feeding behavior that includes lower-quality food sources (Iversen, 1974; Arsuffi & Suberkropp, 1989). In contrast, other studies have demonstrated that metal accumulation in leaf litter can inhibit shredder consumption, ultimately reducing decomposition rates (Quinn et al., 2000; Irving et al., 2003; Gonçalves et al., 2011; Johnson et al., 2014). Metal toxicity may directly affect shredder physiology (Carvalho and Graça, 2007) and/or inhibit microbial colonization of leaf litter (Schlief & Mutz, 2006; Niyogi et al., 2013), thereby reducing food quality. No significant differences in consumption or growth were detected for larvae fed on *I. perado* and *L. azorica* leaves, which was also surprising. For *I. perado*, this lack of difference is likely due to the previously discussed cuticle detachment, which facilitated direct feeding on the mesophyll and mitigated potential inhibitory effects of metal adsorption. For *L. azorica*, the absence of significant effects of metal enrichment on consumption and growth likely results from its inherently poor nutritional quality (high lignin and polyphenol content), which limited larval feeding regardless of metal presence. This species is known for its high concentration of essential oils (Pedro et al., 2001; Rosa et al., 2010; Furtado et al., 2014), which likely further constrained its palatability.

Survival rates over the three-week period did not differ significantly between larvae fed on reference and metal-enriched leaves, suggesting a certain degree of tolerance to metal exposure through food ingestion. However, additional research is necessary to determine whether *L. atlanticus* has developed physiological adaptations to cope with high metal concentrations or whether the duration of the experiment was insufficient to detect chronic toxicity effects. Future studies should examine metal bioaccumulation in larvae and assess sublethal effects on physiological parameters to better understand the ecological consequences of metal contamination.

Larvae exhibited a clear preference for *A. glutinosa* and *I. perado* over the more recalcitrant *L. azorica* leaves, which aligns with our third hypothesis. It is well established that shredder feeding preferences are primarily influenced by the physical and chemical properties of leaf litter, with soft, nutrient-rich leaves being favored over tougher, chemically defended, and nutrient-poor alternatives (Canhoto & Graça, 1995; Balibrea et al., 2017; Simões et al., 2021). Among the three species, *A. glutinosa* leaves were the softest, had the highest nitrogen content, and the lowest polyphenol concentration, making them a highly palatable resource for *L. atlanticus*. This is consistent with previous research demonstrating that shredders exhibit higher consumption and growth rates when fed *A. glutinosa* compared to other leaf species (Irons et al., 1988; Friberg & Jacobsen, 1994; González & Graça, 2003). Despite *I. perado* leaves being the toughest and having the lowest nutrient concentrations, they were well consumed and supported high larval growth rates, likely due to the accessibility of the mesophyll following cuticle detachment, as discussed previously. In contrast, the poor performance of larvae feeding on *L. azorica*, in terms of both consumption and growth, can likely be attributed to the high concentration of secondary compounds, including polyphenols and essential oils (Rosa et al., 2010; Furtado et al., 2014). This suggests that *L. azorica* is not a suitable long-term food source for *L. atlanticus* larvae. These findings are in line with prior studies indicating that secondary metabolites in leaf litter can negatively impact larval growth and reduce nutrient assimilation efficiency in shredders (Friberg & Jacobsen, 1994; Carvalho & Graça, 2007). Streams inhabited by *L. atlanticus* are characterized by the presence of recalcitrant leaf litter from endemic species such as *I. perado* and *L. azorica*. While larvae may be accustomed to these leaf species, this did not influence their preference for the higher-quality *A. glutinosa* litter. The concept of a home-field advantage, where consumers perform better on locally available leaf litter, typically applies when native leaf litter is of comparable or superior quality to alternative sources, which was not the case in this study. Lower survival rates were recorded for larvae feeding on *L. azorica*, although differences were not statistically significant between treatments. However, mortality occurred from the first week of the experiment in this treatment, suggesting that intrinsic properties of *L. azorica*, particularly its high concentrations of secondary compounds, may have toxic effects on larvae. Supporting this, essential oils extracted from *L. azorica* leaves have been shown to exert strong insecticidal effects on the larvae of *Pseudaletia unipuncta* Haworth (Lepidoptera) (Rosa et al., 2010) and the adults of *Ceratitis capitata* Wiedemann (Diptera) (Furtado et al., 2014). This pattern may be representative of Azorean streams, where riparian vegetation is predominantly composed of sclerophyllous evergreen species rich in secondary metabolites that could be detrimental to invertebrates (Dias et al., 2007; Rosa et al., 2010; Balibrea et al., 2017). This could help explain the relatively low diversity and abundance of shredder species observed in these stream ecosystems (Raposeiro et al., 2012).

To conclude, this study suggests that, in Azorean streams, the impact of metal enrichment on shredder performance is relatively minor compared to the influence of the intrinsic characteristics of the leaf litter. Exposure of native *I. perado* and *L. azorica* leaves to metals, and the subsequent increase in their metal concentrations, did not significantly affect larval feeding or growth. However, caution is needed when interpreting results for *I. perado*, as experimental artifacts related to cuticle detachment may have influenced larval consumption patterns. The most notable effects of metal enrichment on shredders were observed in larvae fed on *A. glutinosa*, an exotic species not commonly found in Azorean stream riparian vegetation. Despite this, including *A. glutinosa* in the study provided valuable insights into the interplay between metal enrichment and leaf litter quality. Overall, our findings indicate that the recalcitrant nature of native leaf species is a stronger determinant of shredder performance than metal contamination. The higher consumption and growth rates observed in larvae feeding on high-quality litter, such as *A. glutinosa*, reinforce the idea that leaf litter quality plays a central role in consumer-resource interactions. In this context, the presence of recalcitrant native leaf litter appears to buffer the effects of metal enrichment on detrital consumers, reducing the overall impact of metal contamination in these stream ecosystems.

Chapter 4

Effects of leaf litter naturally enriched with metals on microbial decomposers activity and community structure.

4.1 ABSTRACT

Streams naturally enriched with metals are common in regions with active volcanism, where geochemical processes release high concentrations of metals into the water. The high acidity and metal levels of these streams can significantly affect aquatic communities and processes. However, the impact of metal enrichment on microbial decomposers and leaf litter decomposition remains poorly understood in these environments. In this study, we incubated leaf litter of *Clethra arborea* for two weeks in two streams differing in metal concentrations: a reference stream with low metal concentration and a metal-enriched stream with high metal concentrations. After the incubation period in the streams, leaf litter was incubated for 90 days in laboratory microcosms with six water treatments, representing a gradient of metal concentrations: 100% (only metal-enriched stream water), 75%, 50%, 25%, 10%, and 0% (only reference stream water). Microbial decomposer response to leaf litter type (two levels) and water treatments (six levels) was assessed through leaf litter mass remaining and aquatic hyphomycetes sporulation rate, taxa richness and community structure. Results showed that leaf litter mass remaining did not differ between reference and metal-enriched leaves, however leaf decomposition declined with increasing metal concentrations in water, for both leaf types. Sporulation rates of aquatic hyphomycetes were significantly reduced in metal-enriched than reference leaves and in high-metal water treatments, indicating that fungal reproductive activity was negatively affected by metal enrichment. Taxa richness did not differ significantly across leaf and water treatments, though community composition was altered in response to metal exposure, with some fungal species showing greater resilience to high-metal conditions. These findings highlight the detrimental effects of metal enrichment on microbial-driven organic matter decomposition and on microbial decomposer community structure, suggesting that ecosystem functioning processes in naturally metal-enriched streams may differ from those in reference streams due to potentially lower decomposer efficiency.

4.2 INTRODUCTION

Riparian habitats are important linkages for energy flow between terrestrial and aquatic ecosystems, channelling essential organic inputs such as leaves, twigs, and dissolved organic compounds into water bodies (Gregory et al., 1991; Naiman & Décamps, 1997). The decomposition of this terrestrial organic matter is a fundamental ecosystem process that fuels aquatic food webs and drives nutrient cycling (Wallace et al., 1997; Webster & Meyer, 1997; Hieber & Gessner, 2002). These allochthonous inputs not only provide the primary energy source but also help maintain the structural and functional integrity of stream ecosystems (Richardson & Moore, 2010; Ferreira et al., 2023a). Organic matter inputs quality is essential for sustaining aquatic communities, as both microbial decomposers (mainly aquatic hyphomycetes) and shredders select substrates based on their chemical and physical properties (Abelho & Graça, 1996; Graça & Cressa, 2010). Thus, high-quality organic matter, typically characterized by high nutrient concentration and low recalcitrance, is preferentially consumed thereby facilitating efficient nutrient cycling and energy transfer within aquatic systems (Graça, 2001; Ferreira et al., 2016a; Balibrea et al., 2020a).

Once in, water chemistry can affect organic matter quality and consequently its decomposition mediated by decomposer communities. Parameters such as pH, redox conditions, CO_2 concentrations, nutrient availability may alter the inherent quality of allochthonous inputs even before they are processed by aquatic organisms (Ferreira & Chauvet, 2011a; Suseela et al., 2014; Zhang et al., 2019). Metal contamination of water, which may arise from industrial, mining or agricultural activities (Batty & Hallberg, 2010; Hogsden & Harding, 2012), can profoundly affect aquatic decomposers activity, particularly that of microbial decomposers (Ferreira et al., 2016b; Burdon et al., 2020; Niu et al., 2024). Natural metal enrichment in streams often occur in regions with active volcanism (Juncos et al., 2016; Viaroli et al., 2016). This is the case of the Azores archipelago, where streams are characteristically acidic and contain elevated levels of metals like iron (Fe), aluminium (Al), manganese (Mn), copper (Cu), and zinc (Zn) (Cruz et al., 1999; Gonçalves et al., 2016; Balibrea et al., 2023). In these environments, metal oxide and metal hydroxide precipitates can be adsorbed onto leaf litter surfaces during stream conditioning, altering both the physical structure and chemical reactivity of the substrate (Roussel et al., 2008; Duarte et al., 2008; Sridhar and Bärlocher, 2011; Funck et al., 2013a). These modifications may reduce litter palatability and inhibits microbial colonization, ultimately affecting shredder consumption (Schlief and Mutz, 2006; Gonçalves et al., 2011; Batista et al., 2012; Niyogi et al., 2013; Funck et al., 2013b). High metal concentrations in water and the presence of metal oxides precipitates on substrates typically inhibit microbial activity (Sridhar et al., 2001; Niyogi et al., 2002b), while low concentrations of certain metals dissolved in water may enhance microbial decomposition by acting as enzymatic cofactors (Johnson, 1998; Pu et al., 2014; Batista et al., 2017; Batista et al., 2020). Additionally, metals can indirectly affect microbial activity by disrupting enzymatic pathways essential for decomposition, interfering with enzymatic activity by inducing oxidative stress and disrupting cell membranes (Sridhar et al., 2001; Krauss et al., 2005; Azevedo et al., 2007; Duarte et al., 2008). The long exposure to elevated metal concentration can inhibited reproduction of aquatic fungi and reduce species richness by favouring opportunistic taxa over more sensitive species (Baudoin et al., 2008; Moreirinha et al., 2011; Bergmann & Graça, 2020; Bertol et al., 2022). It has been shown that aquatic hyphomycete communities can be used as sensitive and integrative indicators of freshwater quality. However, the effects of metal enrichment on aquatic hyphomycete decomposer activity, growth and reproduction are complex and nonlinear (Sridhar et al., 2005; Solé et al., 2008; Ferreira et al., 2012; Ferreira et al., 2016; Run et al., 2022). Such changes not only hinder decomposition processes but also compromise the overall function and resilience of freshwater ecosystems. These variable responses underscore the importance of understanding the interactions between metal concentrations in substrates and water, substrate quality and microbial-driven decomposition.

This study provides a comprehensive overview of how natural metal enrichment in streams affects microbial-driven organic matter decomposition and the structure of aquatic hyphomycetes communities. For that, we incubated leaf litter of *Clethra arborea* Aiton for two weeks in two streams differing in metal concentrations, a stream with low metal concentrations (reference stream) and in a stream naturally enriched with high metal concentrations (metal-enriched stream). Subsequently, a microcosm experiment was run under laboratory conditions where two leaf treatments (reference leaves and metal-enriched leaves) were crossed with six water treatments (100% –only metal-enriched stream water, 75%, 50%, 25%, 10% and 0%– only reference stream water) to assess microbial decomposer response through leaf litter mass remaining, aquatic hyphomycetes sporulation rate, taxa richness and community structure. We hypothesized that (1) metal concentrations would be higher for leaf litter incubated in the metal-enriched stream than in the reference stream; (2) leaf litter mass remaining would be higher in metal-enriched than in reference leaves; and that (3) faster decomposition would occur at lower than higher water metal concentration. Furthermore, we hypothesized that microbial activity and taxa richness would be affected by metal concentration in leaves and water and thus (4) aquatic hyphomycete sporulation rates would be higher at reference than metal-enriched leaves and at lower than higher water metal concentration; (5) taxa richness would be higher in reference than metal-enriched leaves and at lower than higher water metal concentration, and also that (6) aquatic hyphomycetes community structure would differ between leaf and water treatments.

4.3 MATERIALS AND METHODS

Leaf litter

For the experiment, leaves from *Clethra arborea* Aiton, an exotic perennial tree species commonly found in Azorean riparian vegetation, were selected as substrate. In autumn 2021, leaves were directly collected from trees in Planalto dos Graminhais (37º49’04.09’’N, 25º14’28.94’’W; 692 m above sea level). They were then transported to the laboratory, air-dried and stored in the dark. Nutrients contents (phosphorus, nitrogen, and carbon) were quantified before the beginning of the experiment from oven-dried (60 °C) leaf powder (< 5 mm) and results were expressed as a percentage of dry mass (DM). To determine phosphorus concentration, ca. 3.6 mg of leaf powder (0.1 mg precision) was incubated with sodium persulphate and sodium hydroxide in the autoclave.

Phosphorus concentrations were assessed from liquid samples using the ascorbic acid method and spectrophotometry (Bärlocher et al., 2020). To create a calibration curve, additional samples with known phosphorus concentrations ranging from 0 to 1000 mg/L were processed similarly and used to establish a linear regression model correlating absorbance with phosphorus concentration, with a R^2 value of 1.00. Nitrogen and carbon concentrations were measured directly from ca. 500 µg of leaf powder (with a precision of 0.1 µg) using an Elemental Analyzer/Isotope Ratio Mass Spectrometer (EA/IRMS; IRMS Thermo Delta V advantage with a Flash EA-1112 series, Thermo Fisher Scientific Inc., USA). The IRMS calibration was performed regularly, ensuring that current measurements align with international and laboratory standards within ± 1‰ accuracy. For quality assurance, acetanilide standards (71.09% C, 10.36% N) were included at the start, after every 20 samples and at the end of each analytical run (a continuous sequence of sample analyses). Replicate reference materials exhibited a variation of ≤ 0.2 ‰, ensuring acceptable precision (Bärlocher et al., 2020).

Leaf litter conditioning

Leaf discs of 12-mm diameter were cut with a cork borer, avoiding the main vein, from leaves pre-moistened with distilled water for 30 min (Figure 4.1). Leaf discs were oven-dried (60 °C for 48 h) and weighed (0.1 mg precision) in sets of 20 discs to determine initial discs DM. Sets of leaf discs were enclosed in 0.5-mm mesh bags (Figure 4.1) and incubated for two weeks in two streams differing in metal concentrations (reference leaves and metal-enriched leaves) (Figure 4.2). The incubation process ensured the leaching of leaf soluble compounds, colonization by microbial decomposers and adsorption of metals.

The selected incubation sites were two tributaries of Ribeira Grande stream, located in Lombadas valley on the northeast hillside of Lake Fogo, in São Miguel Island, Azores. The site with low metal concentration (reference site; 37°46’28’’N, 25°27’32’’W; 601 m above sea level) was slightly acidic, had low conductivity and low nutrient concentration. The site with high metal concentration originating from volcanic emissions (metal-enriched site; 37°46’32’’N, 25°27’34’’W;

582 m above sea level) was slightly acidic, poorly mineralized, had high conductivity and low nutrient concentration (Balibrea et al., 2023)(Figure 4.2).

Figure 4.1. Moistened leaves of Clethra arborea(A); leaf discs of C. arborea cut with corkborer (B); mesh bags for leaf discs incubation (C).

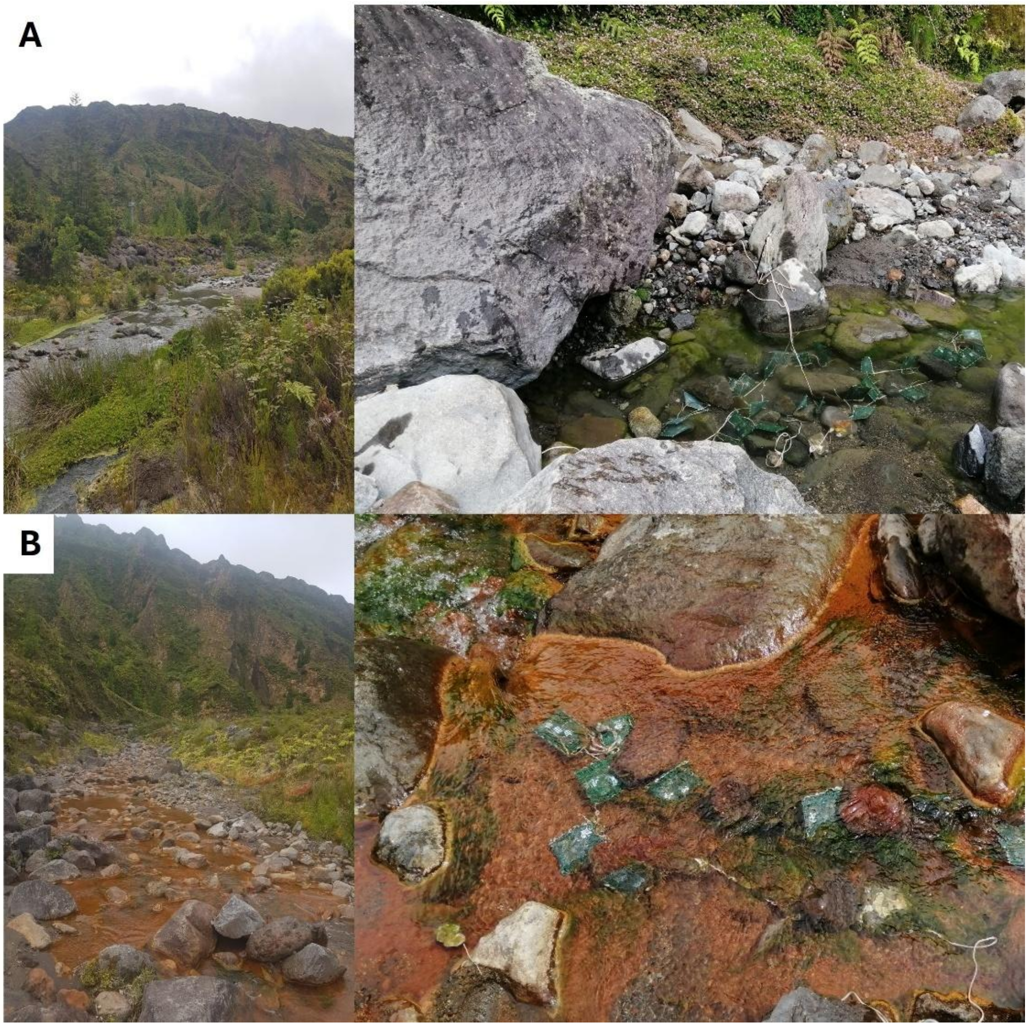

Figure 4.2. Incubation sites in the stream with low metal concentration (reference stream, A) and in a stream naturally enriched with metals (metal-enriched stream, B). Photos were taken on 1st February 2022.

After the incubation period, leaf disc sets deployed in the streams were transported to the laboratory in a cooler for experimental setup (explained below). Moreover, metal concentrations in leaves were determined before and after the conditioning period and expressed in μg/g of leaf DM. Metals considered for analyses were those most abundant in the metal-enriched stream (aluminum, iron and manganese; Gonçalves et al. 2015b) and were determined by atomic absorption spectrometry with stoichiometric flame atomization. The precision of the analytical method was based on the determination of the repeatability (intra-day assays) and of the intermediary precision (inter-day assays) following the recommendations of the International Conference of Harmonisation (CPMP/ICH/281/96) and the International Union of Pure and Applied Chemistry (ISO 17025:2018 and ISO 3534:2006 validation guidelines) (ICH, 1996; Thompson et al., 2002; IPAC, 2018). Precision ranged from 3.0 to 5.0% and accuracy from 81.0% to 124.0% for the calibration concentrations.

Water variables

Temperature, electrical conductivity, pH and dissolved oxygen were measured with a multiparametric field probe (Horiba model U-52G, Horiba Instruments, UK) at the beginning and at the end of conditioning period (1st and 15th February 2022). Water samples were collected from both sites at the beginning and at the end of the conditioning period, transported to the laboratory, filtered (47-mm diameter, 1.2-μm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK) and frozen until analyzed.

Nutrient concentrations (nitrate, ammonium, total nitrogen, phosphate and total phosphorous) were determined using a Continuous Flow Analyser Skalar San++ (Skalar Analytical B.V., Breda, The Netherlands) with segmented flow analysis (SFA) according to Skalar methods M461-318 (EPA 353.2) for nitrate and total nitrogen, M155-008R (EPA 350.1) for ammonium and M503-555R (Standard Method 450-P I) for phosphate and total phosphorus. Metal concentrations (aluminum, iron and manganese) were determined as explained above for leaf samples. Water and leaf analyses were done at MARINNOVA—Marine and Environmental Innovation, Technology and Services, Porto. Technical problems resulted in the loss of water replicates for nutrients and metal analyses.

Experimental setup

Trials to assess the effects metal concentration on aquatic hyphomycetes (methods adapted from Biasi et al. 2017; Pazianoto et al. 2019) were run in microcosms under controlled laboratory conditions. Microcosm consisted of 100-mL Erlenmeyer flasks supplied with 40 mL of stream water representing a gradient of metal concentration (n=144). This gradient was obtained by dilution of water from the stream site with high metal concentration (metal-enriched stream) with water of low metal concentration (reference stream). There were six water treatments in total: 100% (only metal-enriched stream water), 75%, 50%, 25%, 10% and 0% (only reference stream water). Before dilutions, stream water for each site was filtered (47-mm diameter, 1.2-µm pore size; Whatman GF/C, GE Healthcare Europe GmbH, Little Chalfont, UK).

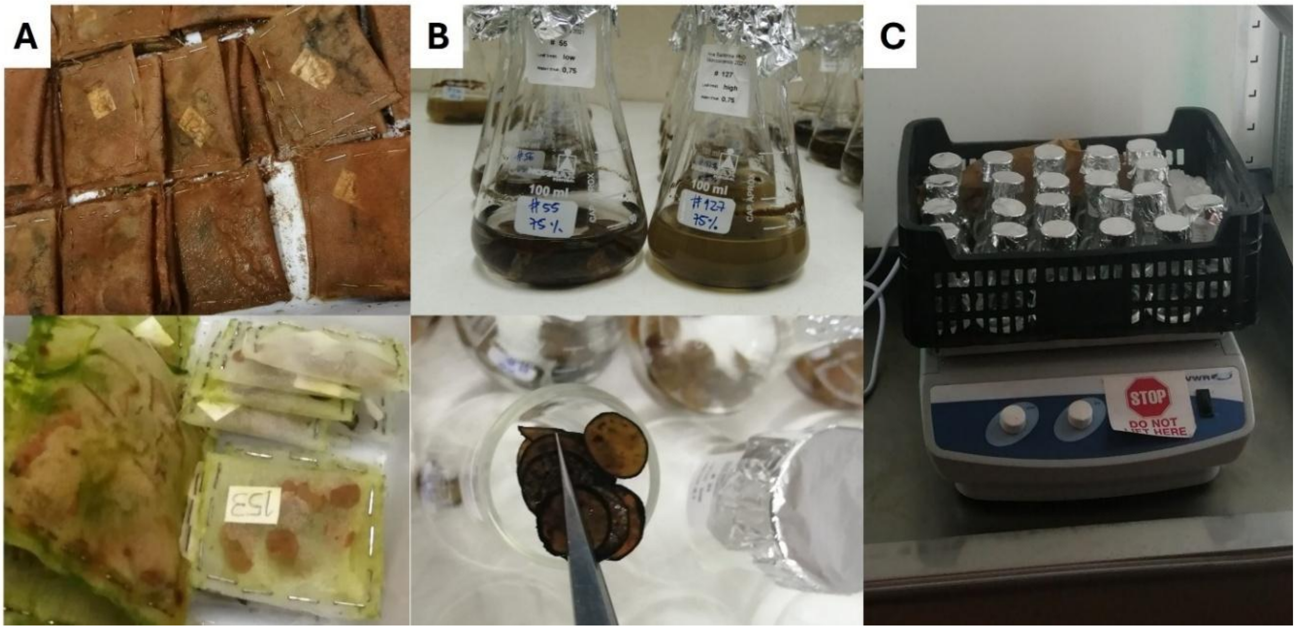

Figure 4.3. Leaf discs incubated in metal- enriched and reference stream after incubation period (A); microcosms consisted of Erlenmeyer flasks with different stream water treatments and leaf discs incubated in metal-enriched and reference streams (B); microcosms deployed in an orbital shaker with continue agitation inside an environmental test chamber (C).

Each microcosm received a group of 20 leaf discs, incubated in enriched or reference stream sites. Therefore, there were two litter treatments (reference leaves and metal-enriched leaves) crossed with six water treatments. Microcosms were randomly displayed on orbital shakers (100 rpm) (VWR Standard 3500 Orbital Shaker, VWR, USA) and experimental trials were run inside an Environmental Test Chamber (Economic Lux Chamber EC01-094, Snijders Scientific B.V., Tilburg, Holland) set at 13 °C and with a 10 h light:14 h dark photoperiod (Figure 4.3). Water was replaced twice a week. On each of three sampling dates (day 14, 43 and 90), 4 microcosms for each water and leaf treatment combination (n=48) were retrieved for analysis.

Leaf litter decomposition

At each sampling date, leaf discs from sacrificed microcosms were oven dried (60 °C for 48 h) and weighed (0.1 mg precision) to determine final DM. Dry leaf discs were ignited in a muffle (500 °C for 4h) and ashes were weighed. Ash-free dry mass (AFDM) remaining was calculated as the difference between DM and ash mass (Figure 4.4). The percentage of AFDM remaining was calculated as final AFDM/initial AFDM × 100. Initial AFDM was estimated by multiplying the initial DM of leaves before conditioning by a conversion factor derived from additional sets of discs deployed in each stream for incubation process.

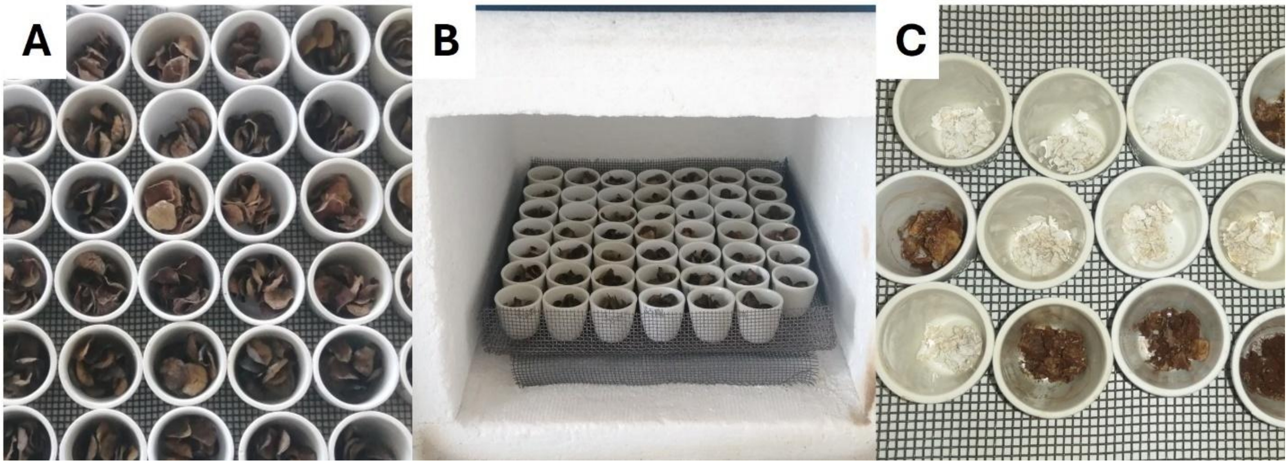

Figure 4.4. Leaf discs deployed in pre-weighed pans at the end of each sampling dates for oven-dried inside and oven (A); leaf discs inside a muffle for ignition process (B); ashes from metal-enriched and reference leaf discs after ignition (C).

Conidial production

Conidial suspensions from sacrificed microcosms were preserved with 2 mL of 37% formalin in graduated tubes and stored in the dark until processed (Figure 4.5). The exact time that conidial suspensions were in the microcosms (from last water replacement to the sampling date) was recorded. Before conidial counting and identification, suspensions were gently shaken, transferred into a beaker with 100 mL of Triton X-100 solution (0.5%) and homogenized with a magnetic stirring bar. Aliquots of the suspensions were filtered with cellulose nitrate filters (25-mm diameter, 5-µm pore size; SMWP, Merk Millipore Ltd. Cork, Ireland). Cotton blue in 60% lactic acid (0.05%) was used to stain the filters that were mounted on a microscope slide (Figure 4.5). Spores were identified and counted under a microscope (Leica DM2500 Led, Leica Microsystems CMS GmbH, Wetzlar, Germany) at 200× magnification (Descals, 2020). Sporulation rates by aquatic hyphomycetes were expressed as the number of spores released per mg AFDM per day. Aquatic hyphomycete taxa richness was expressed as number of taxa per sample.

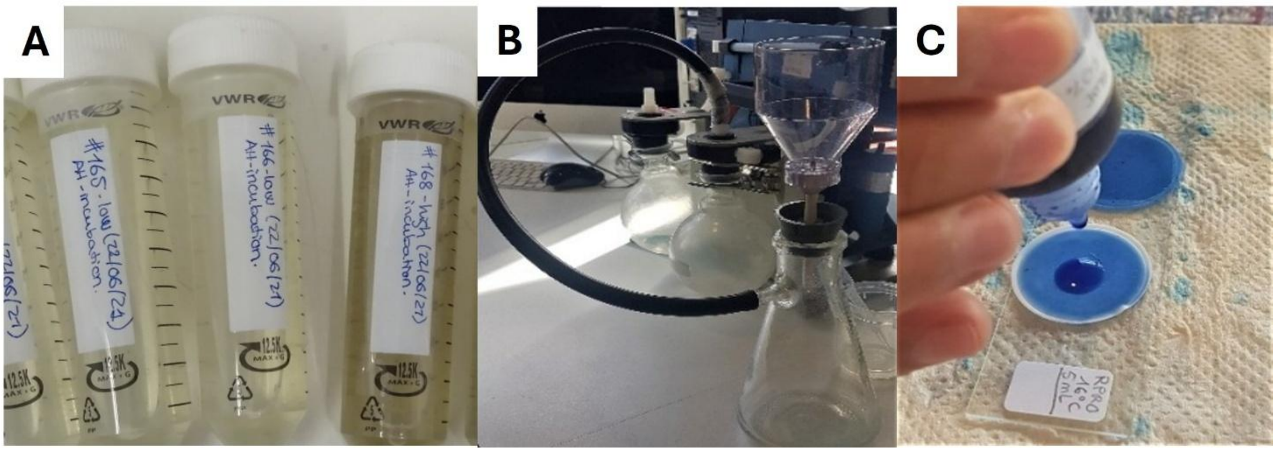

Figure 4.5. Conidial suspension from each microcosm fixed with 37% formalin and storage inside graduated tubes (A); system for filtering aliquots of conidial suspension with cellulose nitrate filters (B); filters stained with cotton blue in lactic acid for microscope slides preparation for spores counting and identification under microscope (C).

Statistical analysis

Water characteristics measured with field probe were compared between incubation sites using one-way analysis of variance (ANOVA). Metal concentrations in leaf litter were compared between leaf treatments (reference leaves and metal-enriched leaves) and sampling occasions (between before and after leaf litter incubation) using one-way ANOVA, followed by Tukey´s HSD test when significant effects were detected.

Comparisons of leaf litter decomposition among water treatments (100%, 75%, 50%, 25%, 10% and 0%), leaf treatments (reference leaves and metal-enriched leaves) and sampling dates (day 14, 43 and 90) were done based on proportion of AFDM remaining using two-way repeated measures ANOVA, followed by Tukey's HSD test when significant effects were detected.

Aquatic hyphomycete sporulation rates and taxa richness were compared among water treatments, leaf treatments and sampling dates by two-way repeated measures ANOVA, followed by Tukey's HSD test. Aquatic hyphomycete communities (based on log (x + 1)-transformed specific spore production; fourth root-transformed) were compared among water treatments, leaf treatments and sampling dates by permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis similarity matrix followed by pairwise tests. Similarity analysis (SIMPER) was also used to identify the species contributing more to dissimilarities between groups.

Data were checked for homoscedasticity (Bartlett's test) and normality (Shapiro-Wilk test) before analyses. Univariate analyses were performed using STATISTICA 7 (StatSoft, Tulsa, OK, USA) and IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA); community analyses were performed using PRIMER 6 v6.1.11 & PERMANOVA + v1.0.1 (Primer-E Ltd, Plymouth, UK).

4.4 RESULTS

Characterization of stream water and leaves

During leaf litter incubation in the streams, water temperature, pH, conductivity, total dissolved solids and dissolved oxygen concentration significantly differed between reference and metal-enriched streams (one-way ANOVA, $P<0.05$; Table 4.1 and S21). Water temperature was cool but significantly lower for the reference than the metal-enriched stream (one-way ANOVA, $P<0.001$; Table 4.1 and S21). pH values were acidic but significantly higher for the reference than the metal-enriched stream (one-way ANOVA, $P=0.009$; Table 4.1 and S21). Conductivity and total dissolved solids were low in the reference stream and moderate in the metal-enriched, and the difference was significantly (one-way ANOVA, P<0.001; Table 4.1 and S21). Dissolved oxygen concentrations were moderate and significantly higher in the reference site (one-way ANOVA, P=0.029; Table 4.1 and S21). Concentration of total nitrogen, ammonium and total phosphorous seemed similar in both reference and metal-enriched streams, while nitrate concentrations were higher in the reference stream (Table 4.1). Metal concentrations were higher in the metal-enriched than in the reference stream, however significant differences were only found in aluminum (4×) (one-way ANOVA, P=0.009) and iron (10×) (one-way ANOVA, P=0.007) (Table 4.1 and S21).

Metal concentration significantly differed between leaf types (one-way ANOVA, P<0.001 for Al, Fe and Mn; Table 4.2 and S22). Metal concentrations were significantly higher in metal-enriched than in reference leaves (4× for Al, 111× Fe and 9× Mn) or leaves before incubation (48× for Al, 1944 × Fe and 22× Mn) (Table 4.2 and S22). Metal concentrations did not significantly differ between reference leaves and leaves before incubation (Tukey test, P=0.124 for Al, P=0.990 for Fe and P=0.597 for Mn), although concentrations tended to be higher in reference leaves (Table 4.2).

Table 4.1. Physical and chemical characteristics, nutrient and metal concentration of reference stream and metal-enriched stream water at first and fourteenth day of incubation. For physical and chemical parameters values are mean ± SE of each stream site and date (n=5). For nutrient concentration samples were only collected at one date. For metal concentration values are mean± SE of each stream site and date (n=2). TDS, total dissolved solids

Water parameters	Reference stream				Metal-enriched stream			
Temperature (°C)	11.72	±	0.18	a	15.24	±	0.40	b
pH	6.06	±	0.14	a	5.63	±	0.03	b
Conductivity (µS/cm)	84.90	±	3.80	a	217.20	±	24.50	b
TDS (mg/L)	42.40	±	1.98	a	108.40	±	12.20	b
DO (%)	74.75	±	2.14	a	69.76	±	3.86	a
DO (mg/L)	7.67	±	0.18	a	6.65	±	0.39	a
N total (µg/L)	313				321			
NO_3^- (µg/L)	167				74			
$NH_3^+ + NH_4^+$ (µg/L)	47				48			
P total (µg/L)	2719				2960			
P_2O_5 (µg/L)	21.90				x			
Al (µg/L)	2345.50	±	123.50	a	9819.50	±	715.50	b
Fe (µg/L)	349.50	±	113.50	a	2325.50	±	125.50	b
Mn (µg/L)	16.00	±	1.00	a	238.00	±	118.00	a

Table 4.2. Metal concentrations (mean ± SE, n = 3) in leaves before incubation period and after incubation (two weeks) in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves).

Leaves	Al (µg/g DM)				Fe (µg/g DM)				Mn (µg/g DM)			
Leaves before incubation	30.2	±	1.0	a	31.0	±	4.2	a	31.6	±	1.0	a
Reference leaves	338.3	±	26.9	a	540.5	±	69.0	a	74.4	±	8.1	a
Metal-enriched leaves	1444.5	±	182.7	b	60274.4	±	3838.6	b	683.8	±	53.0	b

Leaf mass remaining

Leaf mass remaining decreased gradually over the incubation period for all treatments (two-way repeated measures ANOVA, P<0.001; Table S23 and Figure 4.6). Mass remaining was significantly affected by water treatment (two-way repeated measures ANOVA, P<0.001; Table S23 and Figure 4.6) but it did not significantly differ between reference and metal-enriched leaves (P=0.361; Table S23 and Figure 4.6). Moreover, there was a significant interaction between leaf and water treatments (two-way repeated measures ANOVA, P<0.001; Table S23). Along the experiment leaf mass remaining was significantly lower in reference than in metal enriched leaves at 0% and 25% water treatments (Tukey test, P= 0.019 and P=0.009, respectively). Leaf mass remaining was also significantly lower in 0% water treatment than the75% and 100% water treatments for reference leaves (Tukey test, P=0.002 and P=0.002, respectively).

At the end of the experiment (day 90), mass remaining was significant affected by the leaf treatment (two-way ANOVA, P=0.032), water treatment (P<0.001) and the interaction between both (P=0.006) (Table S24). Mass remaining of reference leaves tended to increase from low-metal to high-metal water treatments, showing significant differences between the lowest metal concentration (0%) and 25%, 75% and 100% water treatments (Tukey test, P<0.001; Figure 4.7). Metal enriched leaves had higher mass remaining at the 100% than at the 25% water treatment (Tukey test, P=0.01; Figure 4.7). Moreover, reference leaves showed significant lower mass remaining than metal-enriched leaves at 0% water treatment (Tukey test, P=0.016; Figure 4.7).

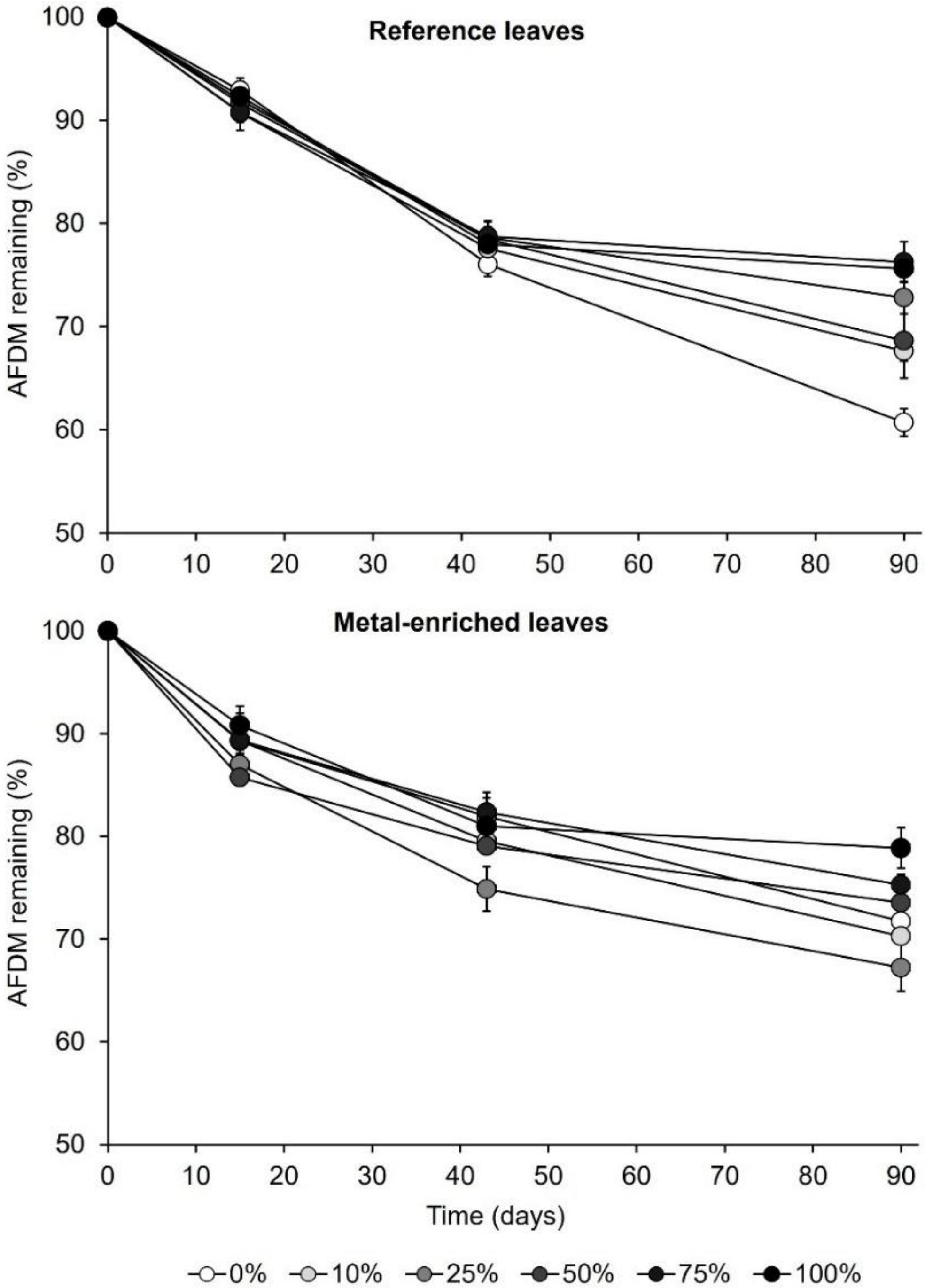

Figure 4.6. Ash-free dry mass (AFDM) remaining (mean ± SE, n=4) of clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) after 15, 43 and 90 days.

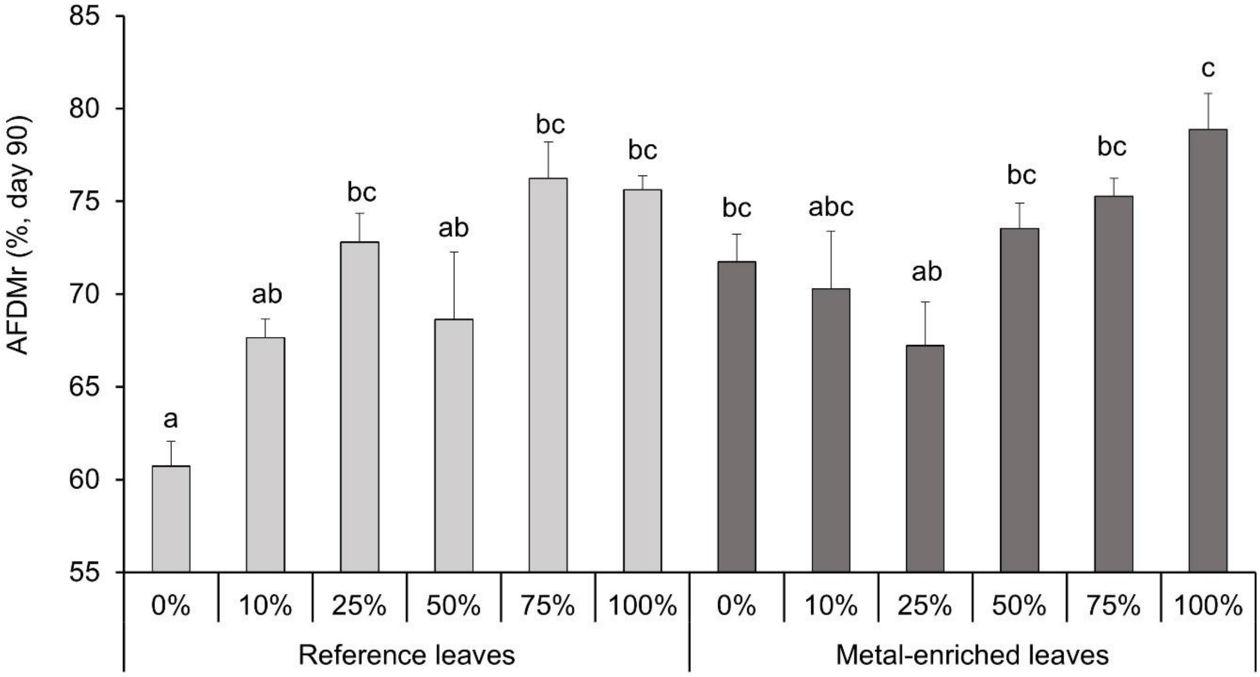

Figure 4.7. Ash-free dry mass (AFDM) remaining (mean ± SE, n=4) of clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) at the end of the experiment (day 90).

Aquatic hyphomycete communities

Sporulation rates peaked at day 43 for all treatments, with maximum values of 468 – 584 conidia/mg AFDM/d for 0%–25% water treatments across leaf treatments (Figure 4.8). Sporulation rates were significantly affected by leaf treatment, water treatment, sampling dates, and the interaction between time × leaf treatment and time × water treatment (two-way repeated measures ANOVA, $P \leq 0.012$; Table S25). Higher sporulation rates occurred in reference leaves with maximum values of 402–584 conidia/mg AFDM/d at low water treatments (0%–25%) and of 215–252 conidia/mg AFDM/d at higher water treatments (50%–100%) (Figure 4.8). In metal-enriched leaves, higher sporulation rates ranged 259–468 conidia/mg AFDM/d at low water treatments (0%–25%) and 62–98 conidia/mg AFDM/d at higher water treatments (50%–100%) (Figure 4.8). Aquatic hyphomycete taxa richness was not significantly affected by leaf treatment, water treatment, or their interaction (two-way repeated measures ANOVA, $P \geq 0.162$; Table S25). Time was a significant factor ($P < 0.001$; Table S25) with higher taxa richness at day 15 for both reference (at water treatments 10%, 25%, 50% and 100%) and metal-enriched leaves (at water treatments 10%–75%) ranging from 9.50–11.75 and 8.75–9.75 number of taxa per sample, respectively (Figure 4.8).

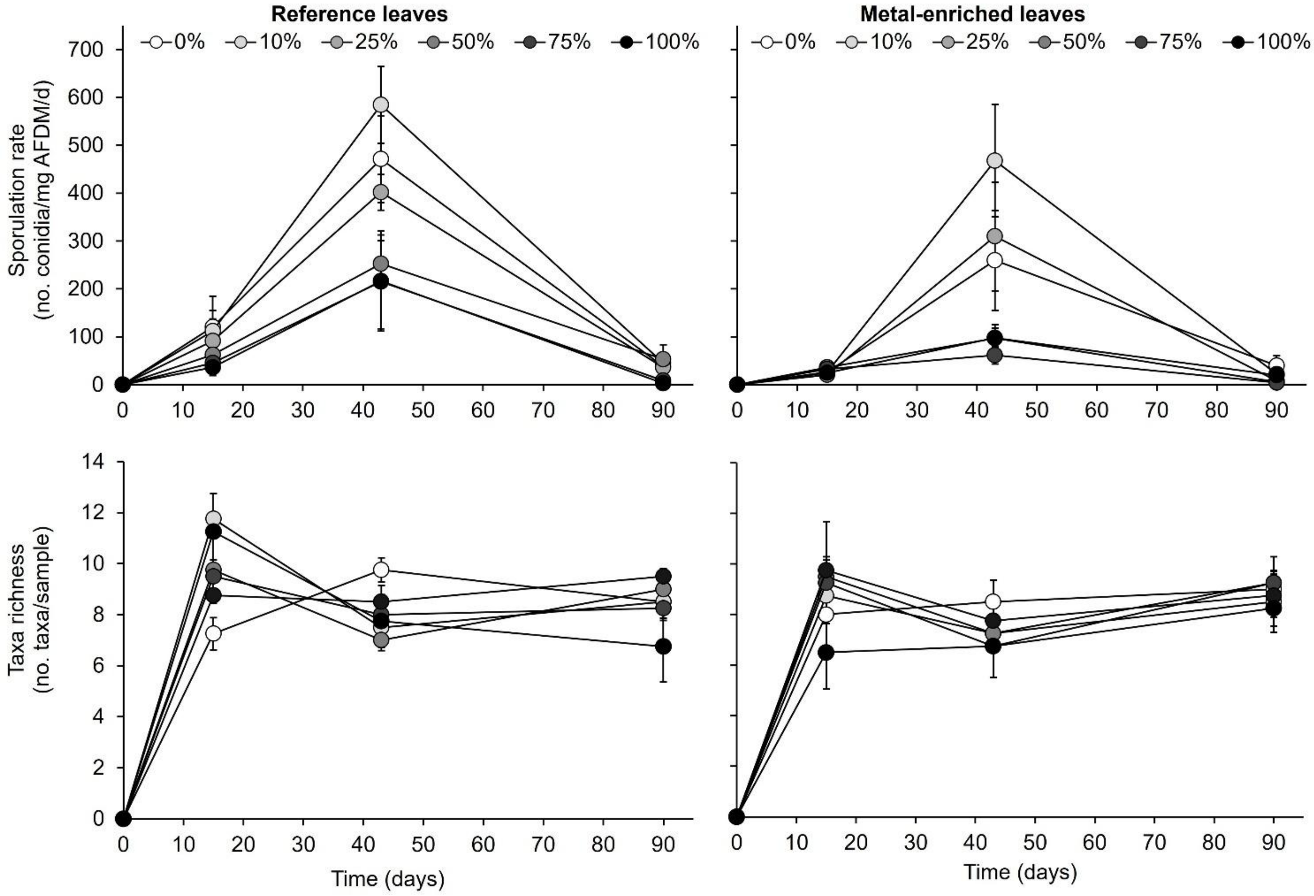

Figure 4.8. Sporulation rates and taxa richness of aquatic hyphomycetes (mean ±SE, n=3) associated with clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) after 15, 43 and 90 days.

Thirty-nine aquatic hyphomycetes taxa were found associated with leaf discs (Table S26). Thirty-five taxa were identified up to species level, four of which were considered as different species due to consistent morphological alterations, such as extra arms (Table S26). Aquatic hyphomycete communities were significantly affected by leaf treatment, water treatment, time, and all interactions (PERMANOVA, P = 0.001; Table S27). *Flagellospora curvula* Ingold (34–69% of relative contribution to conidial production across leaf and water treatments), *Lemonniera aquatica* De Wildeman (5–25%), *Lunulospora curvula* Ingold (10-19%), *Tricladium chaetocladium* Ingold (1–14%), *Articulospora tetracladia* Ingold (1–12%) and *Alatospora pulchella* Marvanová (2–6%) were the most abundant species across treatments (Figure 4.9).

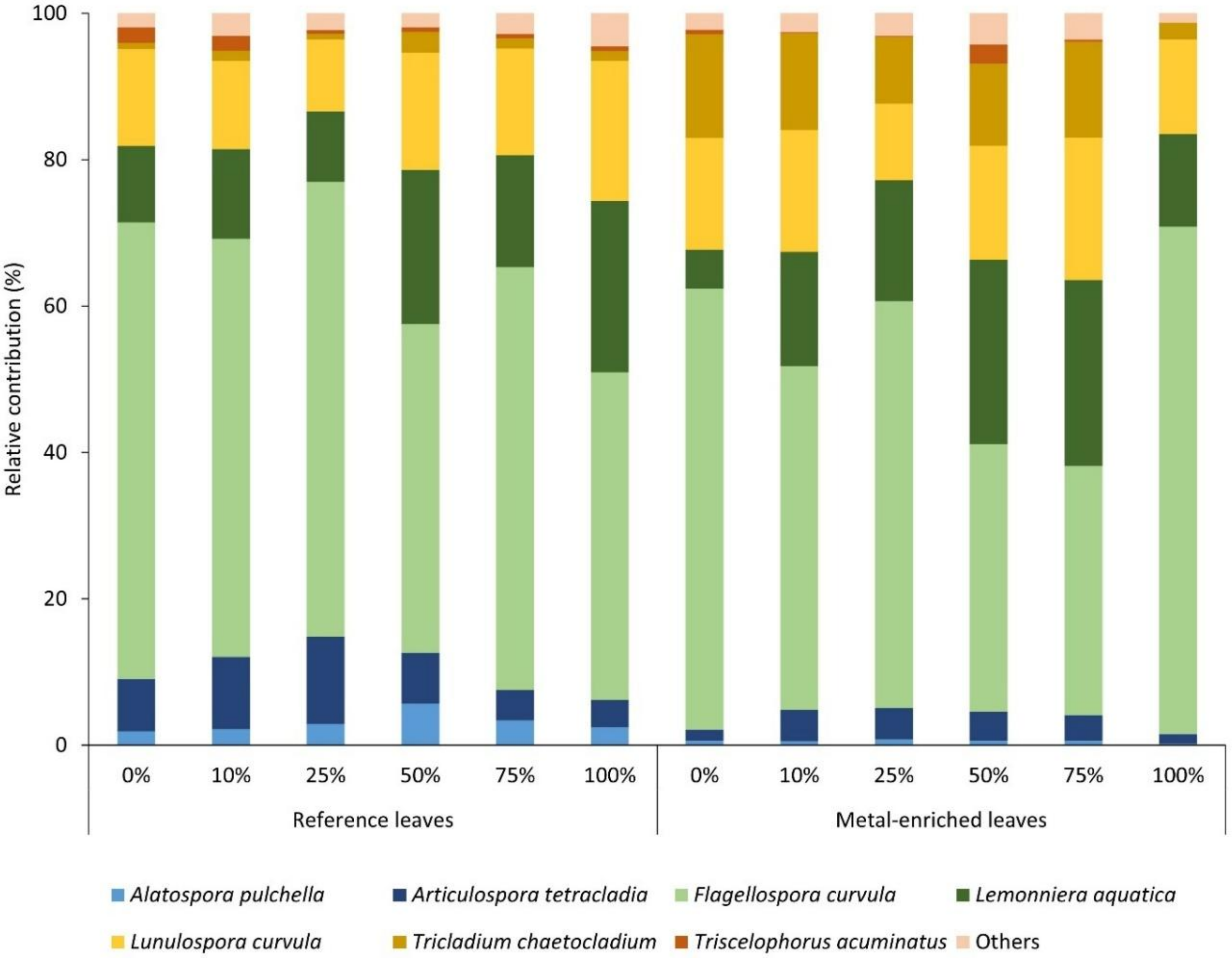

Figure 4.9. Mean relative contribution (based on spore production, across the three sampling dates) of aquatic hyphomycete taxa associated with clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) across six water treatments (0%, 10%, 25%, 50%, 75% and 100%). Only species contributing at least 2% to total conidial production in at least one treatment are shown; rare species are included within "Others".

The percentage of dissimilarity in aquatic hyphomycete communities between reference and metal-enriched leaves was 34%, and the species that contribute most to this dissimilarity were *T. chaetocladium*, *A. tetracladia*, *L. curvula*, *A. pulchella* and *F. curvula* (Table S28).

4.5 DISCUSSION

Metal concentrations in leaf litter and stream water significantly influenced microbial decomposer responses, including microbial-driven organic matter decomposition, aquatic hyphomycetes activity and community composition.

Consistent with our first hypothesis, metal concentrations were significantly higher in leaf litter incubated in the metal-enriched stream compared to the reference stream. Leaf discs incubated in the metal-enriched stream exhibited visible coating of metal precipitates on their surface. A similar layer of metal oxides was observed in leaves from other plant species incubated in streams naturally enriched with metals in the same region (Balibrea et al., 2023). Moreover, leaf discs had rust-brown hues indicative of metal adsorption, darkened edges and concentric discoloration derived from localized metal deposition and possible chemical interactions, probably derived from oxidative processes from organic matter interaction with metals (Figure S3). Metal oxides, including iron, aluminium and manganese are known to disrupt aquatic ecosystem balance through complex interactions with organic matter (Kolya & Kang, 2024; Xue et al., 2024). Some studies showed that these oxides may alter the degradation pathways of organic substrates by promoting organic matter decomposition through surface transformation by oxidation processes (Wang et al., 2016; Li et al., 2021). For instance, iron and manganese have been shown to promote organic matter degradation through aerobic and anaerobic oxidation processes in groundwater contaminated with metals from anthropogenic origin (Pierri & Czop, 2020). In contrast, other studies showed that metal interactions with organic matter may reduce its bioavailability inhibiting further microbial decomposition. For instance, iron and manganese were found to catalyse reactions leading to the transformation of organic matter into organo-mineral associations promoting long-term carbon preservation (Li et al., 2021; Moore et al., 2023).

Our results also showed higher accumulation of iron over aluminium in metal-enriched leaves, despite the greater abundance of aluminium in metal-enriched stream water. This may result from higher affinity of iron for organic matter, driven by its chemical properties reflected in its ability to form stable complexes and iron oxides and iron hydroxides precipitates that adhere strongly to leaf surfaces (Wagai et al. 2020; Figure S3). Moreover, the solubility and speciation of both aluminium and iron are highly pH-dependent; while the solubility of aluminium tends to increase under acidic conditions, iron tends to precipitate and adsorb more readily onto surfaces under slightly acidic to neutral conditions (Nierop et al., 2002; Botté et al., 2022), leading to the observed iron oxides precipitates in the metal-enriched stream.

Our results provided partial support for our second hypothesis, which suggested that leaf litter mass remaining would be higher in metal-enriched leaves than in reference leaves. Reference leaves decomposed faster than metal-enriched leaves at low-metal water treatments (0%, 10%, and even 50%). The faster decomposition of reference than metal-enriched leaves in low-metal water treatments suggests that the intrinsic quality of the leaf litter (reference leaves had low metal concentration) is an important factor controlling leaf decomposition when environmental condition (water quality) are not too harsh. In contrast, in high-metal water treatments (75% and 100%), the decomposition of both leaf types converged, which may suggest that the negative effects due to external metal accumulation overwhelmed any differences in intrinsic litter quality. This result is in line with previous studies that have found higher differences in leaf litter decomposition between species in reference than in nutrient-enriched streams (Gulis & Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006; Biasi et al., 2017). The absence of microbial-driven decomposition differences between leaf types at high-metal water treatments may be related with the dark edges found around metal-enriched leaves, where metal accumulation may interact with leaf compounds through oxidation processes promoting the degradation of organic matter on those areas, potentially overcoming the reduced microbial activity. Thus, organic matter decomposition that occurred in metal-enriched leaves was probably not only attributed to microbial community activity but also to external processes derivate from metal-substrate interactions.

The observed pattern of higher mass remaining in reference leaves at higher than lower water metal treatments agreed with our third hypothesis, highlighting that elevated concentration of metals in water may constrain microbial-driven organic matter decomposition. These findings agree with previous studies that found slower decomposition of leaf litter in metal-enriched stream water (Niyogi et al., 2001; Duarte et al., 2004, 2008; Fernandes et al., 2009; Moreirinha et al., 2011; Pradhan et al., 2011; Hogsden & Harding, 2013; Du et al., 2017; Loureiro et al., 2024). Higher metal concentrations in water may have changed the physical structure of leaf tissues (e.g., metal coating) and limited the availability of oxygen and inorganic nutrients for the mycelia, constraining microbial colonization and decomposition (Sridhar et al., 2001; Ehrman et al., 2008; Ding et al., 2012). Moreover, similar reduction in decomposition rates for both leaf types in high-metal water treatments may suggest that the negative effects of elevated metal concentration in water on microbial activity are stronger than those resulting from metal adsorption on substrate surface. On the contrary, some studies showed that metal oxides and metal hydroxides deposition on leaves had stronger negative effects of organic matter decomposition than metal dissolved in water (Niyogi et al., 2013). Run et al. (2022) showed that litter decomposition did not differ significantly between the metal contaminated and reference streams, however, metal-contaminated leaf litter decomposed faster than reference litter. These previous studies suggest that the indirect effects of metal contamination, such as changes in leaf litter quality, have a greater influence on decomposition processes than the direct effects of altered water quality. However, this is not supported by our results. The discrepancy between studies is likely due to the higher nutrient concentrations in water in previous studies, which may have counteracted the negative effects of metal toxicity in metal-contaminated streams (Woodward et al., 2012; Pu et al., 2014).

Microbial-driven decomposition patterns observed in metal-enriched leaves across some water treatments aligned with the concept of hormesis, where low levels of a stressor, in this case metal concentration, stimulate biological activity, while higher levels inhibit it (Calabrese & Baldwin, 2002). Specifically, in low-metal water treatments (0% and 25%) the mass remaining of metal-enriched leaves was relatively low, suggesting that the microbial decomposers may have been stimulated by the presence of metals at these lower concentrations. This stimulatory effect could result from the role of trace metals such as manganese and iron as cofactors in enzymatic reactions essential for microbial activity (Chapman, 2002; Lefcort et al., 2008; Shen et al., 2009). However, as the metal concentration in the water increased (75% and 100% treatments) an inhibitory effect on decomposition was observed where the toxic effects masked any initial stimulatory responses. High levels of metals are well-known to disrupt microbial activities (Sridhar et al., 2001; Krauss et al., 2005; Duarte et al., 2008; Moreirinha et al., 2011) and enzymatic processes, either by directly inhibiting metabolic pathways or by increasing oxidative stress through the generation of reactive oxygen species (Azevedo et al., 2007, 2009). Hormesis effect has been also found in litter decomposition in laboratory microcosms exposed to different metals, where growth and conidial production was stimulated under low uranium concentration in some aquatic hyphomycetes species (Bergmann & Graça, 2020) and activity of fungal decomposers was stimulated at low concentration of cadmium (Batista et al., 2012). Moreover, Du et al. (2020) found that zinc oxide may also have hormesis effect at very low concentrations increasing aquatic fungi biomass on leaf litter. The hormesis-like pattern observed in our study may also be influenced by the capacity of microbial communities to adapt or acclimate to metal-enriched conditions. At lower metal concentrations, decomposers colonizing metal-enriched leaves may benefit from higher nutrient availability due to less contaminated substrate or by reduced competition due to the exclusion of more sensitive species. For instance, it has been showed that aquatic fungi mycelia growing within the leaf matrix may be protected from silver exposure, as a significant portion of silver was retained on the leaf surface (Funck et al., 2013a). This protective effect is likely more pronounced at lower silver concentrations, when adsorption sites on the leaf surface are not yet saturated (Krauss et al., 2011; Pradhan et al., 2011; Funck et al., 2013a). A similar process may have occurred in our experiment where metal precipitates at low water concentrations may promote microbial driven-decomposition due to substrate and nutrient availability. However, as metal concentrations increase, the accumulation of metals on leaf surfaces and in microbial cells likely reaches a threshold where toxicity outweighs any potential benefits (Niyogi et al., 2001; Krauss et al., 2003; Azevedo et al., 2009). The hormesis effect observed in metal-enriched leaves highlighted the complex and nonlinear responses of microbial decomposers to metal enrichment. Our findings suggest that decomposition dynamics through the different leaf and water treatments may be explained by complex interactions between intrinsic (leaf quality) and extrinsic substrate characteristics (metal-substrate interaction), environmental variables (water characteristics and metal concentrations) and structure of microbial decomposer community.

Aquatic hyphomycete sporulation rate was higher in reference than metal-enriched leaves and in low-metal compared to high-metal water treatments supporting our fourth hypothesis. Sporulation rates peaked at intermediate sampling date (day 43), with higher rates in reference leaves and low-metal water treatments (0% and 25%). In contrast, sporulation rates were markedly lower in high-metal water treatments (75% and 100%) for both leaf types. Reference leaves consistently supported higher sporulation rates than metal-enriched leaves, highlighting the detrimental effect of metal adsorption onto leaf surfaces. Metal accumulation on leaves may have constrained aquatic hyphomycetes colonization and then inhibited the spore production (Krauss et al., 2003; Sridhar et al., 2005). Moreover, metals coating of metal-enriched leaves likely restricted microbial growth and reproduction due to metal accumulation in cell-walls or aquatic hyphomycetes mycelium (Chamier & Tipping, 1997; Duarte et al., 2004). Metal deposits may also constrain bioavailability of essential nutrients on organic matter resource and alter the leaf matrix, making it less accessible to fungal decomposers (Sridhar & Bärlocher, 2011; Funck et al., 2013a). The observed decline in fungal reproductive output, at higher metal concentration in water treatments, likely reflects the direct toxic effects on their enzymatic processes, such as oxidative stress and enzyme inhibition (Sridhar et al., 2001; Azevedo et al., 2007; Baudoin et al., 2008; Bergmann & Graça, 2020). Our results are consistent with others showing that fungal reproduction appears to be easily disrupted under metal stress when exposed to a single metals or mixtures (e.g., Abel and Barlocher 1984; Sridhar et al. 2001; Duarte et al. 2008; Medeiros et al. 2010; Batista et al. 2012; Jain et al. 2019; Bergmann and Graça 2020).

Contrary to our fifth hypothesis, aquatic hyphomycete taxa richness did not significantly differ between reference and metal-enriched leaves or among water treatments. However, taxa richness varied significantly over time, with higher values observed at earlier sampling dates (day 15). This temporal variation may be explained because at the initial colonization stage diverse fungal taxa rapidly established at the available substrate before competitive exclusion; then, in later stages, metal stress selectively favours tolerant species while suppressing sensitive taxa (Fernandes et al., 2009). Shifts in fungal community composition had been reported from numerous of studies under metal exposure stress where the development of a more adapted community may differ significantly from those of the original community due to better tolerance and mechanisms to overcome high metals concentrations (e.g. (Sridhar et al., 2005; Azevedo et al., 2007; Fernandes et al., 2009; Niyogi et al., 2009; Medeiros et al., 2010; Moreirinha et al., 2011; Batista et al., 2017; Bertol et al., 2022).

Despite the lack of significant differences in richness, community composition was influenced by both leaf and water treatments, supporting our sixth hypothesis. Our results showed shifts in dominant aquatic hyphomycetes species abundance between reference and metal-enriched leaves, suggesting that metals may selectively influence microbial assemblages, where certain species may exhibit resilience under this stressor (Pradhan et al., 2011; Sridhar & Bärlocher, 2011; Du et al., 2017; Yue et al., 2019; Run et al., 2022).

This study highlighted the complex responses of microbial decomposers and organic matter decomposition in freshwater ecosystems naturally enriched with metals from volcanic origin. The results demonstrated that metal concentrations in both water and leaf substrates significantly affect microbial-driven decomposition, aquatic hyphomycete activity and community composition. While decomposition of reference leaves at low-metal water treatments suggested that intrinsic leaf quality facilitates microbial activity, higher metal concentrations in water and on leaf surfaces constrained microbial-driven leaf decomposition, likely through toxic effects on microbial communities and physical alterations to leaf substrates. The complexity of microbial responses observed in our study can be attributed to the innovative nature of our experimental design. Although this was a microcosm experiment, where conditions are typically controlled, we used water sourced from natural streams to better simulate realistic environmental conditions where complex mixtures of metals generally occur. This highlights both the challenges and the ecological relevance of studying natural systems, even within the controlled framework of microcosm experiments, and emphasizes the importance of accounting for environmental heterogeneity in experimental designs.

Chapter 5

General discussion and conclusions.

As anthropogenic activities pressures on land and natural resources grow, the freshwater ecosystems of the Azores face complex challenges due to human disturbances (Triantis et al., 2010; Connor et al., 2012; Rull et al., 2017; Raposeiro et al., 2021). A main stressor in Azores Island streams is the rapid transformation of native riparian zones into pastures for livestock uses and commercial plantations, altering habitat structure and ecosystem function (Constância, 1963; Silva & Smith, 2004; Calado et al., 2015; Castanho et al., 2021). Moreover, the inherent natural metal enrichment linked to volcanic activity may also affect stream ecosystem health imposing unique geochemical constraints on biotic communities (Cruz, 2003; Freire et al., 2013; Quintela et al., 2013). These pressures not only disrupt the physical and chemical characteristics of streams but also alter the structure and functioning of decomposer communities that act as key players in organic matter decomposition and nutrient cycling, essential processes for ecosystems stability (Allan, 2004; Hladyz et al., 2011c; Ferreira et al., 2016c; Silva-Junior, 2016). Several studies have focused on the effect of these stressors to either the community composition of individual decomposer groups or on isolated chemical parameters that may affect organic matter process (e.g. Hisabae et al. 2011; Quintaneiro et al. 2016; Ferreira et al. 2017; Tagliaferro et al. 2018; Balibrea et al. 2023). However, in order to fully understand how shifts in riparian vegetation and natural geochemical process affect organic matter decomposition, it is necessary to adopt an integrative approach that combines both structural and functional perspectives (Elosegi et al., 2006; Young et al., 2008; Ferreira et al., 2020; Harrison et al., 2021; Cheung & Burrows, 2024). Moreover, understanding how these stressors may affect both microbial decomposers and macroinvertebrate shredders, a more comprehensive knowledge of energy flow and nutrient cycling in these highly stressed systems may be developed (Chauvet et al., 2016; Ferreira et al., 2020).

This thesis, together with other studies conducted on the same topic in the Azores (Raposeiro et al., 2014b, 2018; Ferreira et al., 2016d, 2017; Balibrea et al., 2017, 2020a, 2023), provides a deeper understanding of the functional process of organic matter decomposition in Azorean streams. By integrating both field experiments and microcosm trials, the present thesis provides a comprehensive evaluation of organic matter decomposition in Azorean streams, revealing how both anthropogenic land-use changes and natural metal enrichment can influence ecosystem functioning. Our findings reveal that changes in riparian vegetation, water quality and substrate characteristics not only impact the process of organic matter decomposition but also alter the structure and performance of decomposer communities. Consequently, these changes have important implications for freshwater ecosystem health in islands with active volcanism. However, it is important to recognize at the outset that while the first part of the thesis predominantly explores the influence of land-use changes on stream ecosystems, these findings must not be applied in the context of naturally high metal concentrations in other streams. This dual perspective underscores that streams are influenced by multiple stressors, not only those arising from human land use but also by inherent geochemical conditions, which may lead to differing decomposition dynamics.

Our results from Chapter 2 showed that organic matter decomposition in streams is highly influenced by shifts in land uses, particularly the replacement of native riparian vegetation with exotic plantations or pasture. Both biotic and abiotic factors played key roles in shaping decomposition dynamics. When comparing streams surrounded by cryptomeria plantations to those with native laurel vegetation, we found that microbial-driven decomposition of both leaves and wood was similar, despite lower water temperature and dissolved phosphorus concentrations in cryptomeria streams. This outcome suggested that variations in the aquatic hyphomycete community composition might be counteracted by functional redundancy, ensuring that decomposition remains consistent despite contrasting environmental conditions. Similar patterns were also found in other studies where no significant differences in organic matter decomposition occurred between streams in conifer plantations and native forest (Riipinen et al., 2010; Ferreira et al., 2017). However, total organic matter decomposition was higher in cryptomeria streams due to an increased abundance of shredders, such as the endemic caddisfly *Limnephilus atlanticus*, which underscores the importance of macroinvertebrate activity in these systems. Previous studies in Azorean streams showed that microbes are the key players in litter decomposition due to low shredder diversity and density

(Raposeiro et al., 2013; Ferreira et al., 2016b). However, when shredders are present at high densities, normally found in streams located at higher elevations and surrounded by cryptomeria plantations, they become important decomposers (Raposeiro et al., 2018; Balibrea et al., 2020a). In contrast, in native and pasture streams, the lower density of shredders resulted in microbial processes being the primary drivers of decomposition. These results indicate that the role of macroinvertebrates is stream-specific and that variations in shredder abundance across stream types might be linked more to the habitat preferences of these taxa than to riparian vegetation disturbances alone (Balibrea et al., 2020b).

Streams surrounded by pastures had significantly faster organic matter decomposition, mainly driven by higher water temperatures and dissolved nutrient concentrations, likely due to inputs from cattle manure. These nutrient and temperature conditions stimulated microbial activity, and even when accounting for temperature differences using degree-day corrections, the enhanced decomposition in pasture streams remained evident. This finding emphasizes that nutrient enrichment, along with warmer water temperatures, can synergistically increase decomposition rates (Ferreira & Chauvet, 2011b; Martínez et al., 2013). Similar results were found in other studies where elevated nutrient inputs, particularly nitrogen and phosphorus, to streams surrounded by agricultural land accelerated organic matter decomposition (Quinn et al., 2000; Hladyz et al., 2011a). Moreover, pasture streams had lower aquatic hyphomycete taxa richness, which was likely due to the reduced inputs of organic matter and limited riparian vegetation typical of this stream type (Bärlocher & Graca, 2002; Ferreira et al., 2006). However, pasture streams supported a higher richness and abundance of macroinvertebrates than the other stream types. This divergence suggests that while lower riparian diversity may limit fungal diversity, the absence of riparian buffers and increased solar exposure might promote macroinvertebrate diversity., particularly grazers, and constitute target habitats for invasive species.

In the Azores, land use patterns are inherently linked to the island elevational gradient (Quaternaire-Portugal, 2008). At lower elevations, urban and agricultural activities dominate, often resulting in altered riparian vegetation and increased human impact (Raposeiro et al., 2011; Silva & Smith, 2004). Conversely, streams at higher elevations are more likely to be surrounded by commercial tree plantations or patches of native laurel forest that have remained relatively undisturbed (Borges et al., 2009; DRRF, 2014; Gonçalves et al., 2015; Raposeiro et al., 2011). This spatial arrangement means that streams in agricultural areas, for instance, tend to occur in warmer, lower-elevation environments where temperature is a critical driver of ecological processes. Temperature not only influences the basic metabolic processes in these streams but also affects organic matter decomposition (Rowe et al., 1996; Griffiths & Tiegs, 2016; Gessner & Peeters, 2020). When decomposition rates were expressed per degree-day, which standardizes the effect of temperature, our results revealed that even after adjusting for temperature, decomposition in pasture streams remained elevated compared to that in native streams. This suggested that the accelerated decomposition observed in pasture streams is not only attributed to the warmer conditions associated with lower elevations but also reflects the influence on stream functioning due to altered land use, such as changes in riparian vegetation and nutrient inputs. These results highlight the importance of considering both climatic gradients and anthropogenic disturbances when evaluating ecosystem processes. Thus, the interaction between land use and temperature highlights that managing and conserving stream ecosystems in the Azores may require a dual focus on mitigating human impacts as well as understanding the environmental context in which these streams occur.

A key conclusion from Chapter 2 is that organic matter decomposition is a valuable functional indicator of land-use changes in Azorean streams. Temperature-adjusted decomposition rates allow us to differentiate the effects of water temperature and nutrient levels, thereby providing a clearer assessment of ecosystem condition. These insights are particularly relevant given the ongoing pressures faced by freshwater ecosystems in the Azores, where agricultural intensification, urban expansion, and altered riparian landscapes are common challenges. To mitigate these impacts, several management strategies emerge as potential approaches to safeguard stream integrity. Establishing native riparian buffers could help regulate water temperatures and enhance nutrient retention, thus reducing nutrient loading from agricultural runoff (Osborne & Kovacic, 1993; Schoonover et al., 2005; Cole et al., 2020; Nsenga Kumwimba et al., 2023). Moreover, controlling cattle access along stream corridors could further limit nutrient enrichment (Agouridis et al., 2005; Aarons & Gourley, 2013; McKergow et al., 2016; Grudzinski et al., 2020). In areas with commercial wood plantations, sustainable management practices could be applied to promote diversity enrichment in riparian in vegetation to better support both microbial and macroinvertebrate decomposer communities (Broadmeadow & Nisbet, 2004; González et al., 2015; Stutter et al., 2019). Furthermore, adopting elevation-specific management practices ensures that conservation efforts are appropriately targeted. For instance, where lower-elevation areas may benefit most from enhanced riparian restoration and nutrient control, higher-elevation streams might be focused on preserving the functional redundancy of key decomposer communities through the maintenance of vegetation diversity. Together, these strategies highlight how functional indicators such as organic matter decomposition not only assess the integrity of Azorean stream ecosystems but also may guide effective, targeted management interventions in the face of ongoing anthropogenic pressures. Therefore, integrating functional assessments into conservation planning could enhance the resilience of these island freshwater ecosystems, ensuring the long-term sustainability of their ecological processes.

While Chapter 2 provides clear evidence on how land-use changes, mediated by elevation gradients and vegetation shifts, affect decomposition dynamics, it is critical to note that these results might not fully apply to streams where natural metal enrichment is the dominant stressor. Thus, Chapters 3 and 4 were designed to elucidate the unique mechanisms at play in metal-enrichment streams. By disentangling the effects of natural metal inputs from those of land-use change, these chapters provide a comparative framework for understanding how different stressors shape organic matter breakdown. These results highlights that the patterns observed in Chapter 2 are specific to systems primarily impacted by land use rather than by metal enrichment.

Our results from Chapter 3 and Chapter 4 showed that natural metal enrichment modified the quality of leaf litter and, in turn, influenced the feeding behaviour of invertebrates and microbial activity. This sets the stage for a joint discussion of how altered substrate conditions affect the entire decomposer community. In our short-term trials in Chapter 3, shredders consistently favoured leaves that have been incubated in the reference stream rather than those from the metal-enriched stream, especially those of *Alnus glutinosa* and *Laurus azorica*. These findings agree with previous studies (Quinn et al., 2000; Irving et al., 2003; Gonçalves et al., 2011; Campos et al., 2014; Johnson et al., 2014), which also reported that metal contamination can reduce the palatability of litter through both direct metal toxicity and by inhibiting microbial conditioning on leaf surfaces. On the other hand, microcosms trials from Chapter 4, revealed that leaf litter exposed to metal-enriched conditions developed distinct coatings of metal oxides, particularly of iron, due to its precipitation capacity to adhere strongly to leaf surfaces at this slightly acidic water conditions (Nierop et al., 2002; Wagai et al., 2020; Botté et al., 2022), that visibly altered the substrate. Similar observations have been made by Balibrea et al. (2023), who noted that metal deposition on leaves not only changes the physical appearance but also influences subsequent microbial colonization. When comparing decomposition dynamics in trials from Chapter 4, our findings revealed that under low-metal water treatments, reference leaves decomposed significantly faster than those previously incubated in metal-enriched streams. This indicates that the inherent quality of the substrate is critical under conditions where external metal stress is minimal. In contrast, at high metal concentrations in the water, the substrate decomposition of both leaf types converged. This outcome aligns with previous studies (Gulis & Suberkropp, 2003; Ferreira et al., 2006; Gulis et al., 2006), which have reported stronger differences in decomposition rates between leaf litter species under lower than higher dissolved nutrient concentrations. Moreover, the comparable reduction in decomposition rates for both leaf types under high-metal water treatments suggests that the inhibitory effects of elevated metal concentrations in the water on microbial activity are more pronounced than the impacts of metal adsorption on the substrate surface.

Interestingly, laboratory trials in Chapter 3 suggested that the increased consumption of metal-enriched *A. glutinosa* leaves over longer periods may be due to a compensatory feeding response, a strategy previously reported in other systems (Winterbourn et al., 1985; Carvalho & Graça, 2007), whereby larvae ingested more of a lower-quality resource in an attempt to meet their nutritional needs. However, despite these shifts in feeding rates, larval growth remained similar across treatments, reinforcing that compensatory feeding on metal-enriched leaves may have occurred agreeing with results from Irons et al. (1988) and Friberg and Jacobsen (1994). Thus, this resilience may indicate that while metal deposition alters immediate feeding preferences, the overall performance of shredders is buffered by the intrinsic nutritional quality of the litter. Moreover, our results diverge from some studies on metal-polluted streams where chronic metal exposure was linked to reduced shredder growth and survival (Quinn et al., 2000; Irving et al., 2003), highlighting that the interaction between metal enrichment and litter quality is complex and may further depend on whether metals originate from natural sources or anthropogenic activities.

Metal concentration variations among leaf species in our study from Chapter 3 suggested that leaf surface characteristics, such as roughness and trichome presence, influenced metal absorption. For instance, *L. azorica*, with its rougher surface and dense trichomes, exhibited greater metal accumulation, while *A. glutinosa*, with a smoother surface, showed lower differences in metal concentration between leaves incubated in reference and metal-enriched streams. Among the metals analyzed, iron, in particular, showed the greatest concentration differences, likely due to its strong precipitation capacity. These findings highlight the importance of leaf surface characteristics influencing metal uptake, suggesting that future studies should also focus on the specific role of leaf surface traits in metal retention dynamics. This closer examination of the results from Chapters 3 and 4 reveals a strong interconnection between the responses of macroinvertebrate shredders and aquatic hyphomycetes under metal stress. While Chapter 3 highlights changes in shredder feeding behaviour and compensatory responses to altered litter quality, Chapter 4 demonstrates that metal enrichment also directly impacts microbial decomposers by modifying leaf surfaces and affecting fungal colonization and sporulation. Together, these findings suggest that metal enrichment does not act in isolation on one group of decomposers; rather, it triggers a cascade of responses across the entire decomposer community. This joint discussion reinforces that the interplay between physical substrate modifications and biological responses is crucial to understanding overall decomposition dynamics in metal-rich environments.

Our findings also showed that shredder feeding preferences were largely driven by the physical and chemical properties of leaf litter. Larvae consistently preferred the softer, nutrient-rich *A. glutinosa* over the tougher nutrient-poor *L. azorica*. However *I. perado*, despite its high toughness, also supported good growth rates, likely due to mesophyll accessibility following cuticle detachment. These results align with previous studies (e.g., Irons et al. 1988; Friberg and Jacobsen 1994; Canhoto and Graça 1995) and highlight that the high concentrations of secondary metabolites, such as polyphenols and essential oils in *L. azorica*, may have toxic effects, reducing its suitability as a long-term food source. This pattern is particularly relevant for Azorean streams, where the prevalence of sclerophyllous native vegetation, rich in secondary compounds, could contribute to the low diversity and abundance of shredder species.

Moreover, results from Chapter 4 showed that higher metal concentrations in water can inhibit microbial-driven organic matter decomposition by altering the physical structure of leaf tissues as well reducing the availability of oxygen and inorganic nutrients necessary for microbial colonization. Consistent with this, our results showed slower leaf litter decomposition in higher water metal treatments in reference leaves, which agrees with several studies that found slower decomposition of leaf litter in metal-enriched stream water (Niyogi et al., 2001; Duarte et al., 2004, 2008; Fernandes et al., 2009; Moreirinha et al., 2011; Pradhan et al., 2011; Hogsden & Harding, 2013; Du et al., 2017; Loureiro et al., 2024). Moreover, our results showed a potential hormesis effect in microbial decomposition of metal-enriched leaves subjected to different water treatments. At low metal concentrations (0% and 25%), microbial activity is enhanced, likely due to the beneficial role of trace metals such as manganese and iron in enzymatic processes, resulting in lower leaf mass remaining. In contrast, higher metal concentrations (75% and 100%) exert a toxic effect that inhibits decomposition, overwhelming any initial stimulatory benefits. Similar hormesis responses have been reported previously (Batista et al., 2012; Bergmann & Graça, 2020; Du et al., 2020), reinforcing the idea that the relationship between metal concentration and microbial activity is nonlinear and highly complex.

Laboratory trials from Chapter 4 further highlights the negative effect that metal enrichment has on the sporulation of aquatic hyphomycetes. Our findings showed that sporulation rates were significantly lower on metal-enriched leaves and in high-metal water treatments. This reduction in fungal reproductive can result from metal-induced oxidative stress and enzyme inhibition (Sridhar et al., 2001; Azevedo et al., 2007). Moreover, while overall fungal taxa richness did not significantly differ between treatments, the composition of aquatic hyphomycete communities was substantially altered by metal exposure. Certain species, such as those contributing most to community dissimilarities in our study, appeared more resilient under metal stress, as observed in other studies (Medeiros et al., 2010; Moreirinha et al., 2011; Run et al., 2022). These shifts suggest that metal enrichment may selectively favour the proliferation of tolerant taxa over more sensitive ones, potentially leading to long-term changes in ecosystem functioning (Fernandes et al., 2009; Batista et al., 2017; Bertol et al., 2022).

In conclusion integrating the findings from Chapters 3 and 4, it is evident that natural metal enrichment exerts multifaceted effects on stream decomposer communities. Together, these findings provide a comprehensive view of how metal enrichment from natural volcanic processes can disrupt the balance of microbial and shredders decomposition. While changes in litter quality induced by metal deposition, as demonstrated in Chapter 3, directly influence shredder feeding behaviour, at the same time, the modifications to leaf surfaces not only affect shredder choice but also alter microbial colonization and sporulation, as shown in Chapter 4. Macroinvertebrate shredders respond to metal-enriched litter primarily by modifying their feeding behaviour, with compensatory mechanisms partly offsetting potential negative impacts on growth and survival. In parallel, microbial decomposers displayed a clear sensitivity to metal concentrations, with lower concentrations potentially stimulating activity while higher concentrations inhibited key processes like sporulation. Beyond metal exposure, the intrinsic quality of the litter, its nutrient concentrations, toughness, and secondary compound concentrations, seemed to be important factors that modulated decomposer responses. These findings also highlight the need to consider both the quality of organic substrates and the characteristics of the surrounding water when assessing the ecological impacts of metal enrichment.

These results emphasize that organic matter decomposition is a functional indicator that captures the interactions between abiotic stressors (such as metal enrichment) and biotic processes. Thus, our findings reinforce the need for bioassessment frameworks that combine structural and functional indicators to better inform the management of freshwater ecosystems in the Azores and similar island environments. Consequently, establishing accurate reference conditions in metal-enriched streams, particularly those affected by active volcanism, is fundamental for understanding the dynamics of organic matter decomposition in these naturally stressed ecosystems. Such baselines may provide an important background against which alterations in biological communities and functional processes can be measured, thereby highlighting the subtle shifts induced by metal enrichment. This is especially important because natural metal inputs may often be accompanied by complex ecological interactions that can modify microbial activity and macroinvertebrate behaviour in unpredictable ways. By characterizing these baseline states, there will be better knowledge to discern the extent to which metal enrichment influences aquatic fungi and shredder traits such as, sporulation rates, fungi growth, invertebrate feeding, growth and larvae survival among others. Moreover, these insights not only advance our theoretical understanding of biogeochemical cycles under stress but also inform the development of targeted management and conservation strategies for freshwater ecosystems in regions marked by volcanic activity.

To conclude, given the ecological fragility of riparian and freshwater ecosystems on islands, there is an urgent need for region-specific research and adaptive management strategies. These strategies must account for the unique challenges faced by island ecosystems, including their geographical isolation, limited space, and vulnerability to environmental changes. Protecting and restoring native riparian vegetation, mitigating pollution, and reducing habitat fragmentation are essential steps to safeguard the ecological integrity of island freshwater systems and ensure their long-term sustainability. Due to the limitations of assessments based on structural indicators, there is a growing consensus that bioassessment programs should integrate functional indicators alongside traditional taxonomic approaches to provide a more integrative evaluation of stream ecological status. Functional indicators can help detect early signs of ecosystem dysfunction, offering a more sensitive approach to assessing the impacts of land-use changes, pollution, and climate-driven alterations. Thus, expanding bioassessment frameworks to incorporate functional ecology metrics will enhance the ability to monitor, manage and restor freshwater ecosystems in a way that aligns with both scientific knowledge and conservation priorities. This thesis contributes to scientific knowledge demonstrating that incorporating organic matter decomposition as a functional indicator into bioassessment frameworks can enhance the evaluation of stream health in the Azores. While organic matter decomposition is a highly sensitive, ecologically relevant, and cost-effective functional indicator, its effectiveness in freshwater bioassessment depends on careful methodological standardization and interpretation. Its ability to detect early ecological changes and its direct link to key ecosystem services make it a valuable complement to traditional structural assessments. However, challenges such as abiotic variability, methodological inconsistencies, and ambiguous ecological interpretations must be addressed to maximize its reliability. Thus, our findings provide valuable insights for developing bioassessment frameworks that incorporate functional indicators such as organic matter decomposition and decomposer community dynamics to achieve a more integrative assessments of ecosystem health, ultimately improving conservation and management strategies. Although the research focused on streams in the Azores, the findings of this thesis may have relevance for freshwater ecosystems in other volcanic islands or rapidly changing isolated freshwater ecosystems worldwide. Thus, this work establishes a foundation for developing improved management and restoration approaches aimed at reestablishing the intrinsic functional integrity of these ecosystems in the Azores islands.

It is important to adopt a modest perspective regarding these study's contribution to the development of functional bioassessment tools. While Chapter 2 highlights the covariation between elevation and land use, and Chapters 3 and 4 offer insights from controlled laboratory experiments, these findings should be viewed as complementary steps toward understanding the complex dynamics of stream ecosystems. The laboratory settings used in Chapters 3 and 4, while valuable for elucidating mechanistic responses under metal stress, inherently limit the direct application of these results to natural settings. Field conditions encompass a broader range of variables and interactions that are difficult to replicate entirely in the laboratory. Future research should, therefore, aim to validate these integrated findings under in situ conditions, further refining and calibrating bioassessment frameworks that account for both anthropogenic and natural stressors.

In summary, this thesis demonstrates that while land-use changes significantly influence organic matter decomposition in Azorean streams, natural metal enrichment introduces additional layers of complexity that modify decomposer community dynamics. The integrated insights across Chapters 2, 3, and 4 underscore the need for context-specific bioassessment tools that combine structural and functional indicators. Although the current findings provide a solid foundation, the limitations inherent in laboratory experiments and the covariation of environmental factors in the field suggest that further in situ research is essential to fully develop and standardize these functional indicators for effective ecosystem management and restoration.

BIBLIOGRAPHY

Aarons, S. R., & C. J. P. Gourley, 2013. The role of riparian buffer management in reducing off-site impacts from grazed dairy systems. Renewable Agriculture and Food Systems 28: 1–16. https://doi.org/DOI: 10.1017/S1742170511000548.

Abbasi, T., & S. A. Abbasi, 2011. Water quality indices based on bioassessment: The biotic indices. Journal of Water and Health 9: 330–348. https://doi.org/10.2166/wh.2011.133.

Abel, T. ., & F. Barlocher, 1988. Uptake of cadmium by *Gammarus fossarum* (Amphipoda) from food and water. The Journal of Applied Ecology 25: 223. https://doi.org/10.2307/2403620.

Abel, T. H., & F. Barlocher, 1984. Effects of cadmium on aquatic hyphomycetes. Applied and Environmental Microbiology 48: 245–251. https://doi.org/10.1128/AEM.48.2.245-251.1984.

Abelho, M., & M. A. S. Graça, 1996. Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. Springer. Hydrobiologia 1996 324:3 324: 195–204. https://doi.org/10.1007/BF00016391.

Agouridis, C. T., S. R. Workman, R. C. Warner, & G. D. Jennings, 2005. Livestock grazing management impacts on stream water quality: A review. Journal of the American Water Resources Association 41: 591–606. https://doi.org/https://doi.org/10.1111/j.1752-1688.2005.tb03757.x.

Aguiar, T. R., F. R. Bortolozo, F. A. Hansel, K. Rasera, & M. T. Ferreira, 2015. Riparian buffer zones as pesticide filters of no-till crops. Environmental Science and Pollution Research 22: 10618–10626. https://doi.org/10.1007/s11356-015-4281-5.

Akselsson, C., O. Westling, H. Sverdrup, J. Holmqvist, G. Thelin, E. Uggla, & G. Malm, 2007. Impact of harvest intensity on long-term base cation budgets in Swedish forest soils. Acid Rain - Deposition to Recovery 7: 201–210. https://doi.org/10.1007/978-1-4020-5885-1_22.

Albergaria, I. S., 2000. Quintas, jardins e parques da Ilha de São Miguel. Quetzal Editora.

Alexandratos, N., & H. De Haen, 1995. World consumption of cereals: Will it double by 2025?

Food Policy 20: 359–366. https://doi.org/https://doi.org/10.1016/0306-9192(95)00030-5.

Allan, J. D., 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35: 257–284. https://doi.org/10.1146/ANNUREV.ECOLSYS.35.120202.110122/CITE/REFWORKS.

Alpert, P., T. Ben-Gai, A. Baharad, Y. Benjamini, D. Yekutieli, M. Colacino, L. Diodato, C. Ramis, V. Homar, R. Romero, S. Michaelides, & A. Manes, 2002. The paradoxical increase of Mediterranean extreme daily rainfall in spite of decrease in total values. Geophysical Research Letters 29: 31–34. https://doi.org/https://doi.org/10.1029/2001GL013554.

Amani, M., M. A. Graça, & V. Ferreira, 2019. Effects of elevated atmospheric CO2 concentration and temperature on litter decomposition in streams: A meta-analysis. International Review of Hydrobiology 104: 14–25.

Angier, J. T., G. W. McCarty, C. P. Rice, & K. Bialek, 2002. Influence of a riparian wetland on nitrate and herbicides exported from an agricultural field. Journal of Agricultural and Food Chemistry 50: 4424–4429. https://doi.org/10.1021/jf011057n.

Arroita, M., I. Aristi, L. Flores, A. Larrañaga, J. Díez, J. Mora, & A. Elosegi, 2012. The use of wooden sticks to assess stream ecosystem functioning: Comparison with leaf breakdown rates. Science of the Total Environment 440: 115–122.

Arsuffi, T. L., & K. Suberkropp, 1988. Effects of fungal mycelia and enzymatically degraded leaves on feeding and performance of caddisfly (Trichoptera) larvae. Journal of the North American Benthological Society 7: 205–211. https://doi.org/10.2307/1467420.

Arsuffi, T. L., & K. Suberkropp, 1989. Selective feeding by shredders on leaf-colonizing stream fungi: comparison of macroinvertebrate taxa. Oecologia 79: 30–37. https://doi.org/10.1007/BF00378236.

Azevedo-Pereira, H. V. S., M. A. S. Graça, & J. M. González, 2006. Life history of *Lepidostoma* *hirtum* in an Iberian stream and its role in organic matter processing. Hydrobiologia 559: 183–192.

Azevedo, J. M. ., 1998. Geologia e hidrologia da Ilha das Flores (Açores – Portugal). Universidade de Coimbra.

Azevedo, M. M., B. Almeida, P. Ludovico, & F. Cássio, 2009. Metal stress induces programmed cell death in aquatic fungi. Aquatic Toxicology 92: 264–270. https://doi.org/10.1016/j.aquatox.2009.02.010.

Azevedo, M. M., A. Carvalho, C. Pascoal, F. Rodrigues, & F. Cássio, 2007. Responses of antioxidant defenses to Cu and Zn stress in two aquatic fungi. Science of the Total Environment 377: 233–243.

Aznar-Sánchez, J. A., M. Piquer-Rodríguez, J. F. Velasco-Muñoz, & F. Manzano-Agugliaro, 2019. Worldwide research trends on sustainable land use in agriculture. Land Use Policy 87: 104069. https://doi.org/https://doi.org/10.1016/j.landusepol.2019.104069.

Bahadur, K. K., G. M. Dias, A. Veeramani, C. J. Swanton, D. Fraser, D. Steinke, E. Lee, H. Wittman, J. M. Farber, K. Dunfield, K. McCann, M. Anand, M. Campbell, N. Rooney, N. E. Raine, R. Van Acker, R. Hanner, S. Pascoal, S. Sharif, T. G. Benton, & E. D. G. Fraser, 2018. When too much isn't enough: Does current food production meet global nutritional needs? PloS one 13: 1–16. https://doi.org/10.1371/journal.pone.0205683.

Balibrea, A., V. Ferreira, C. Balibrea, V. Gonçalves, & P. M. Raposeiro, 2020a. Contribution of macroinvertebrate shredders and aquatic hyphomycetes to litter decomposition in remote insular streams. Hydrobiologia 847: 2337–2355. https://doi.org/10.1007/S10750-020-04259-1/FIGURES/6.

Balibrea, A., V. Ferreira, V. Gonçalves, & P. M. Raposeiro, 2023. Effects of leaf litter naturally enriched with metals on consumption, growth, and survival of an endemic Azorean shredder. Aquatic Sciences 85: 65.

Balibrea, A., V. Ferreira, V. Gonçalves, & P. M. P. M. Raposeiro, 2017. Consumption, growth and survival of the endemic stream shredder *Limnephilus atlanticus* (Trichoptera, Limnephilidae) fed with distinct leaf species. Limnologica 64: 31–37. https://doi.org/10.1016/J.LIMNO.2017.04.002.

Balibrea, A., Vi. V. Gonçalves, & P. M. Raposeiro, 2020b. Larval description of *Limnephilus atlanticus* Nybom 1948, morphological comparison with *Limnephilus affinis* Curtis 1834, (Trichoptera: Limnephilidae) and additional notes on their ecology in Azores Islands. Zootaxa 4852: 372–382. https://doi.org/10.11646/zootaxa.4852.3.8.

Bärlocher, F., 2020. Sporulation by aquatic hyphomycetes. In Methods to study litter decomposition: A practical guide. Springer. pp. 241-245.

Bärlocher, F., M. O. Gessner, & M. A. S. Graça, 2020. Methods to study litter decomposition. A practical guide. Springer International Publishing, Switzerland.

Bärlocher, F., & M. A. Graca, 2002. Exotic riparian vegetation lowers fungal diversity but not leaf decomposition in Portuguese streams. Freshwater Biology 47: 1123–1135.

Bärlocher, F., & J. . J. Oertli, 1978. Colonization of conifer needles by aquatic hyphomycetes. Article in Canadian Journal of Botany 56: 57–62. https://doi.org/10.1139/b78-005.

Bastias, E., M. Bolivar, M. Ribot, M. Peipoch, S. A. Thomas, F. Sabater, & E. Martí, 2020. Spatial heterogeneity in water velocity drives leaf litter dynamics in streams. Freshwater Biology 65: 435–445. https://doi.org/https://doi.org/10.1111/fwb.13436.

Batista, D., C. Pascoal, & F. Cássio, 2012. Impacts of warming on aquatic decomposers along a gradient of cadmium stress. Environmental Pollution 169: 35–41.

Batista, D., C. Pascoal, & F. Cássio, 2017. How do physicochemical properties influence the toxicity of silver nanoparticles on freshwater decomposers of plant litter in streams? Ecotoxicology and Environmental Safety 140: 148–155. https://doi.org/10.1016/j.ecoenv.2017.02.039.

Batista, D., A. Tlili, M. O. Gessner, C. Pascoal, & F. Cássio, 2020. Nanosilver impacts on aquatic microbial decomposers and litter decomposition assessed as pollution-induced community tolerance

(PICT). Environmental Science: Nano 7: 2130–2139. https://doi.org/10.1039/d0en00375a.

Batty, L. C., & K. B. Hallberg, 2010. Ecology of industrial pollution. Cambridge Univeristy Press, New York.

Baudoin, J. M., F. Guérold, V. Felten, E. Chauvet, P. Wagner, & P. Rousselle, 2008. Elevated aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf-litter breakdown. Microbial Ecology 56: 260–269. https://doi.org/10.1007/s00248-007-9344-9.

Bello, A. D., N. B. Hashim, & M. R. Mohd Haniffah, 2017. Predicting impact of climate change on water temperature and dissolved oxygen in Tropical rivers. Climate 5: 58. https://doi.org/10.3390/cli5030058.

Benfield, E. F., K. M. Fritz, & S. D. Tiegs, 2017. Leaf-Litter Breakdown. In Lamberti, G. ., & F. . Hauer (eds) Methods in Stream Ecology: Volume 2: Ecosystem Function. Elsevier, Academic Press. pp. 71–82.

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

Bertol, E. C., C. Biasi, R. C. Loureiro, R. M. Mielniczki-Pereira, A. A. Restello, & L. U. Hepp, 2022. The effect of arsenic on the structure and composition of stream hyphomycetes assemblages. Anais da Academia Brasileira de Ciências 94: e20210192.

Bettencourt, M. L., 1979. O clima dos Açores como recurso natural, especialmente em agricultura e indústria de turismo. Instituto Nacional de Meteorologia e Geofísica.

Biasi, C., M. A. S. Graça, S. Santos, & V. Ferreira, 2017. Nutrient enrichment in water more than in leaves affects aquatic microbial litter processing. Oecologia 1: 555–568. https://doi.org/10.1007/S00442-017-3869-5/METRICS.

Bird, G. A., & N. K. Kaushik, 1992. Invertebrate colonization and processing of maple leaf litter in a forested and an agricultural reach of a stream. Hydrobiologia 234: 65–77. https://doi.org/10.1007/BF00010862/METRICS.

Birk, S., W. Bonne, A. Borja, S. Brucet, A. Courrat, S. Poikane, A. Solimini, W. van de Bund, N. Zampoukas, & D. Hering, 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecological Indicators 18: 31–41. https://doi.org/https://doi.org/10.1016/j.ecolind.2011.10.009.

Blanco, H., & R. Lal, 2023. Nutrient erosion and hypoxia of aquatic ecosystems. In Soil Conservation and Management. Springer Nature Switzerland, Cham. pp. 391–415.

Bojsen, B. H., & D. Jacobsen, 2003. Effects of deforestation on macroinvertebrate diversity and assemblage structure in Ecuadorian Amazon streams. Archiv Für Hydrobiologie 158: 317–342. https://doi.org/10.1127/0003-9136/2003/0158-0317.

Borges, P. A. V., & V. K. Brown, 1999. Effect of island geological age on the arthropod species richness of Azorean pastures. Biological Journal of the Linnean Society 66: 373–410. https://doi.org/10.1111/j.1095-8312.1999.tb01897.x.

Borges, P. A. V., A. Costa, R. Cunha, R. Gabriel, V. Gonçalves, A. F. Martins, I. Melo, M. Parente, P. M. Raposeiro, P. Rodrigues, R. S. Santos, L. Silva, P. Vieira, & V. Vieira, 2010. A list of the terrestrial and marine biota from the Azores. Principia Editora Lda., Cascais.

Borges, P. A. V., E. B. Azevedo, A. E. S. de Borba, F. Dinis, R. Gabriel, & E. Silva, 2009. Ilhas Oceânicas. Escolar Editora. Portugal Millenium Ecosystem Assessment : 463–510.

Botté, A., M. Zaidi, J. Guery, D. Fichet, & V. Leignel, 2022. Aluminium in aquatic environments: abundance and ecotoxicological impacts. Aquatic Ecology 56: 751–773. https://doi.org/10.1007/s10452-021-09936-4.

Breteler, R. J., J. M. Teal, A. E. Gffilin, & I. V Aliela, 1981. Trace element enrichments in decomposing litter of *Spartina alterniflora*. Aquatic Botany 11: 111–120.

Broadmeadow, S., & T. R. Nisbet, 2004. The effects of riparian forest management on the freshwater environment: a literature review of best management practice. Hydrology and Earth System Sciences 8: 286–305. https://doi.org/10.5194/hess-8-286-2004.

Brock, T., 1980. Thermophilic microorganisms and life at high temperatures. Springer Science & Business Media.

Burdon, F. J., Y. Bai, M. Reyes, M. Tamminen, P. Staudacher, S. Mangold, H. Singer, K. Räsänen, A. Joss, S. D. Tiegs, J. Jokela, R. I. L. Eggen, & C. Stamm, 2020. Stream microbial communities and ecosystem functioning show complex responses to multiple stressors in wastewater. Global Change Biology 26: 6363–6382. https://doi.org/https://doi.org/10.1111/gcb.15302.

Buttermore, E. N., W. G. Cope, T. J. Kwak, P. B. Cooney, D. Shea, & P. R. Lazaro, 2018. Contaminants in tropical island streams and their biota. Environmental Research 161: 615–623. https://doi.org/https://doi.org/10.1016/j.envres.2017.11.053.

Buzby, K. M., & S. A. Perry, 2000. Modeling the potential effects of climate change on leaf pack processing in central Appalachian streams. Canadian Journal of Fisheries and Aquatic Sciences 57: 1773–1783.

Cabral Pinto, M. M. S., & E. A. Ferreira da Silva, 2019. Heavy metals of Santiago Island (Cape Verde) alluvial deposits: Baseline value maps and human health risk assessment. International Journal of Environmental Research and Public Health 16: 2. https://doi.org/10.3390/IJERPH16010002.

Calabrese, E. J., & L. A. Baldwin, 2002. Defining hormesis. Human & Experimental Toxicology 21: 91–97. https://doi.org/10.1191/0960327102ht217oa.

Calado, H., A. Braga, F. Moniz, A. Gil, & M. Vergílio, 2015. Spatial planning and resource use in the Azores. Mitigation and Adaptation Strategies for Global Change 20: 1079–1095.

Calado, H., C. Bragagnolo, S. Silva, & M. Vergílio, 2016. Adapting environmental function analysis for management of protected areas in small islands – case of Pico Island (the Azores). Journal of Environmental Management 171: 231–242.

Campos, D., A. Alves, M. F. L. Lemos, A. Correia, A. M. V. M. Soares, & J. L. T. Pestana, 2014. Effects of cadmium and resource quality on freshwater detritus processing chains: A microcosm approach with two insect species. Ecotoxicology 23: 830–839. https://doi.org/10.1007/S10646-014-1223-9.

Canhoto, C., A. L. Gonçalves, & F. Bärlocher, 2016. Biology and ecological functions of aquatic hyphomycetes in a warming climate. Fungal Ecology 19: 201–218. https://doi.org/10.1016/j.funeco.2015.09.011.

Canhoto, C., & M. A. S. Graça, 1995. Food value of introduced eucalypt leaves for a Mediterranean stream detritivore: *Tipula lateralis.* Freshwater Biology 34: 209–214. https://doi.org/10.1111/J.1365-2427.1995.TB00881.X.

Carey, A. E., C. A. Nezat, W. B. Lyons, S.-J. Kao, D. M. Hicks, & J. S. Owen, 2002. Trace metal fluxes to the ocean: The importance of high-standing oceanic islands. Geophysical Research Letters 29: 14–1. https://doi.org/10.1029/2002GL015690.

Carlisle, D. M., & W. H. Clements, 2005. Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. Freshwater Biology 50: 380–390. https://doi.org/10.1111/j.1365-2427.2004.01323.x.

Carpenter, S. R., S. G. Fisher, N. B. Grimm, & J. F. Kitchell, 1992. Global change and freshwater ecosystems. Annual Review of Ecology and Systematics 23: 119–139.

Carpenter, S. R., E. H. Stanley, & M. J. Vander Zanden, 2011. State of the world’s freshwater ecosystems: Physical, chemical, and biological changes. Annual Review of Environment and Resources 36: 75–99. https://doi.org/10.1146/annurev-environ-021810-094524.

Carvalho, E. M., & M. A. S. Graça, 2007. A laboratory study on feeding plasticity of the shredder *Sericostoma vittatum* Rambur (Sericostomatidae). Hydrobiologia 575: 353–359. https://doi.org/10.1007/s10750-006-0383-x.

Casas, J. J., A. Larrañaga, M. Menéndez, J. Pozo, A. Basaguren, A. Martínez, J. Pérez, J. M. González, S. Mollá, C. Casado, E. Descals, N. Roblas, J. A. López-González, & J. Luis Valenzuela, 2013. Leaf litter decomposition of native and introduced tree species of contrasting quality in headwater streams: How does the regional setting matter? Science of the Total Environment 458: 197–208. https://doi.org/10.1016/J.SCITOTENV.2013.04.004.

Casimiro, S., & M. L. Fidalgo, 2007. Performance of the freshwater shrimp *Atyaephyra desmarestii* as indicator of stress imposed by textile effluents. Web Ecology 7: 35–39. https://doi.org/10.5194/we-7-35-2007.

Castanho, R. A., G. Couto, P. Naranjo Gómez, J. M. Pimentel, C. Carvalho, Á. Sousa, B. M. G, & L. Loures, 2021. Evolutionary dynamics in Azorean landscapes: The land-use changes in forests and semi-natural areas in the archipelago from 1990 to 2018. In World Conference on Information Systems and Technologies. pp. 244–252.

Chamier, A. C., & E. Tipping, 1997. Effects of aluminium in acid streams on growth and sporulation of aquatic hyphomycetes. Environmental Pollution 96: 289–298. https://doi.org/10.1016/S0269-7491(97)00054-7.

Chapman, P. M., 2002. Ecological risk assessment (ERA) and hormesis. Science of The Total Environment 288: 131–140. https://doi.org/https://doi.org/10.1016/S0048-9697(01)01120-2.

Chauvet, E., V. Ferreira, P. Giller, B. McKie, S. Tiegs, G. Woodward, A. Elosegi, M. Dobson, T. Fleituch, M. Graça, V. Gulis, S. Hladyz, J. Lacoursière, A. Lecerf, J. Pozo, E. Preda, M. Riipinen, G. Rîsnoveanu, A. Vadineanu, L. Vought, & M. O. Gessner, 2016. Litter decomposition as an indicator of stream ecosystem functioning at local-to-continental scales: insights from the European RivFunction Project. In Advances in Ecological Research. Academic Press. pp. 99–182.

Cheung, W. C. D., & R. M. Burrows, 2024. Structural and functional indicators in freshwater ecosystem monitoring programs. Ecological Management & Restoration 25: 3–13. https://doi.org/https://doi.org/10.1111/emr.12599.

Chi, Y., Z. Zhang, Z. Xie, & J. Wang, 2020. How human activities influence the island ecosystem through damaging the natural ecosystem and supporting the social ecosystem? Journal of Cleaner Production 248: 119203. https://doi.org/10.1016/J.JCLEPRO.2019.119203.

Chung, N., & K. Suberkropp, 2009. Contribution of fungal biomass to the growth of the shredder, *Pycnopsyche gentilis* (Trichoptera: Limnephilidae). Freshwater Biology 54: 2212–2224. https://doi.org/10.1111/J.1365-2427.2009.02260.X.

Clapcott, J. E., & L. A. Barmuta, 2010. Forest clearance increases metabolism and organic matter processes in small headwater streams. Journal of the North American Benthological Society 29: 546–561. https://doi.org/10.1899/09-040.1.

Clerici, N., M. L. Paracchini, & J. Maes, 2014. Land-cover change dynamics and insights into ecosystem services in European stream riparian zones. Ecohydrology & Hydrobiology 14: 107–120. https://doi.org/https://doi.org/10.1016/j.ecohyd.2014.01.002.

Climate Atlas, 2012. Climate atlas of the archipelagos of the Canary Islands, Madeira and the Azores. The Meteorological State Agency of Spain and the Institute of Meteorology, Portugal : http://www.meteo.pt/export/sites/default/bin/docs/.

Cole, L. J., J. Stockan, & R. Helliwell, 2020. Managing riparian buffer strips to optimise ecosystem services: A review. Agriculture, Ecosystems & Environment 296: 106891. https://doi.org/https://doi.org/10.1016/j.agee.2020.106891.

Collier, K. J., & M. J. Winterbourn, 1990. Population dynamics and feeding of mayfly larvae in some acid and alkaline New Zealand streams. Freshwater Biology 23: 181–189. https://doi.org/10.1111/J.1365-2427.1990.TB00263.X.

Connor, S. E., J. F. N. van Leeuwen, T. M. Rittenour, W. O. van der Knaap, B. Ammann, & S. Björck, 2012. The ecological impact of oceanic island colonization - a palaeoecological perspective from the Azores. Journal of Biogeography 39: 1007–1023. https://doi.org/10.1111/j.1365-2699.2011.02671.x.

Constância, J. M., 1963. Evolução da paisagem humanizada da Ilha de São Miguel. Boletim Centro Estudos Geográficos 3: 5–60.

Cornut, J., A. Elger, D. Lambrigot, P. Marmonier, & E. Chauvet, 2010. Early stages of leaf decomposition are mediated by aquatic fungi in the hyporheic zone of woodland streams. Freshwater Biology 55: 2541–2556.

Costa, A. C., A. Balibrea, P. M. Raposeiro, S. Santos, M. Souto, & V. Gonçalves, 2021. Non-indigenous and Invasive Freshwater Species on the Atlantic Islands of the Azores Archipelago. Frontiers in Ecology and Evolution 9:. https://doi.org/10.3389/fevo.2021.631214.

Cruz, J. V., 2003. Groundwater and volcanoes: examples from the Azores archipelago. Environmental Geology 44: 343–355. https://doi.org/10.1007/S00254-003-0769-2.

Cruz, J. V., R. M. Coutinho, M. R. Carvalho, N. Oskarsson, & S. R. Gislason, 1999. Chemistry of waters from Furnas volcano, São Miguel, Azores: fluxes of volcanic carbon dioxide and leached material. Journal of Volcanology and Geothermal Research 92: 151–167. https://doi.org/10.1016/S0377-0273(99)00073-6.

Cruz, J. V., & Z. França, 2006. Hydrogeochemistry of thermal and mineral water springs of the Azores archipelago (Portugal). Journal of Volcanology and Geothermal Research 151: 382–398. https://doi.org/10.1016/J.JVOLGEORES.2005.09.001.

Cruz, J. V., C. Melo, D. Medeiros, S. Costa, R. Cymbron, S. Rocha, C. Medeiros, A. Valente, S. Mendes, D. Silva, & F. Martins, 2017. Water management and planning in a small island archipelago: the Azores case study (Portugal) in the context of the Water Framework Directive. Water Policy 19: 1097–1118. https://doi.org/10.2166/wp.2017.187.

Cruz, J. V., & N. Soares, 2018. Groundwater governance in the Azores archipelago (Portugal): Valuing and protecting a strategic resource in small islands. Water 10: 408. https://doi.org/10.3390/w10040408.

Cruz, J. V., D. Pacheco, S. Costa, C. Melo, R. Cymbron, R. Nogueira, & A. G. Brito, 2012. Implementation of the Water Framework Directive in an outermost EU region: The case of Azores archipelago. Open Hydrology Journal 6: 1–14.

Cummins, K. W., R. C. Petersen, F. O. Howard, J. C. Wuycheck, & V. I. Holt, 1973. The utilization of leaf litter by stream detritivores. Ecology 54: 336–345.

Curtis, P. G., C. M. Slay, N. L. Harris, A. Tyukavina, & M. C. Hansen, 2018. Classifying drivers of global forest loss. Science 361: 1108–1111. https://doi.org/10.1126/science.aau3445.

Dang, C. K., M. O. Gessner, & E. Chauvet, 2007. Influence of conidial traits and leaf structure on attachment success of aquatic hyphomycetes on leaf litter. Mycologia 99: 24–32. https://doi.org/10.1080/15572536.2007.11832597.

Dang, C. K., M. Schindler, E. Chauvet, & M. O. Gessner, 2009. Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90: 122–131.

Danger, A. R., & B. J. Robson, 2004. The effects of land use on leaf-litter processing by macro-invertebrates in an Australian temperate coastal stream. Aquatic Sciences 66: 296–304.

Dangles, O., V. Crespo-Pérez, P. Andino, R. Espinosa, R. Calvez, & D. Jacobsen, 2011. Predicting richness effects on ecosystem function in natural communities: insights from high-elevation streams. Ecology 92: 733–734.

Davies, P. J., I. A. Wright, S. J. Findlay, O. J. Jonasson, & S. Burgin, 2010. Impact of urban development on aquatic macroinvertebrates in south eastern Australia: Degradation of in-stream habitats and comparison with non-urban streams. Aquatic Ecology 44: 685–700.

https://doi.org/10.1007/S10452-009-9307-Y/METRICS.

de Fraiture, C., M. Giordano, & Y. Liao, 2008. Biofuels and implications for agricultural water use: blue impacts of green energy. Water Policy 10: 67–81. https://doi.org/10.2166/wp.2008.054.

Delgado, J. D., R. Riera, R. A. Rodríguez, P. González-Moreno, & J. M. Fenrnández-Palacios, 2017. A reappraisal of the role of humans in the biotic disturbance of islands. Environmental Conservation 44: 371–380. https://doi.org/DOI: 10.1017/S0376892917000236.

Descals, E., 2020. Techniques for handling Ingoldian gungi. In Bärlocher, F., M. O. Gessner, & M. A. S. Graça (eds) Methods to study litter decomposition: A practical guide. Springer International Publishing, Cham. pp. 197–209.

Di Sabatino, A., B. Cicolani, F. P. Miccoli, & G. Cristiano, 2020. Plant detritus origin and microbial–detritivore interactions affect leaf litter breakdown in a Central Apennine (Italy) cold spring. Aquatic Ecology 54: 495–504.

Dias, E., R. B. Elias, C. Melo, & C. Mendes, 2007. Biologia e ecologia das florestas das ilhas-Açores. Açores e Madeira: a floresta das Ilhas 6: 51–80.

Ding, D.-X., X.-T. Liu, N. Hu, G.-Y. Li, & Y.-D. Wang, 2012. Removal and recovery of uranium from aqueous solution by tea waste. Journal of Radioanalytical and Nuclear Chemistry 293: 735–741. https://doi.org/https://doi.org/10.1007/s10967-012-1866-z.

Directive Water Framework, 2003. Common implementation strategy for the water framework directive (2000/60/EC). Guidance document 7.

Dolédec, S., & B. Statzner, 2010. Responses of freshwater biota to human disturbances: contribution of J-NABS to developments in ecological integrity assessments. Journal of the North American Benthological Society 29: 286–311. https://doi.org/10.1899/08-090.1.

Don, A., J. Schumacher, & A. Freibauer, 2011. Impact of tropical land-use change on soil organic carbon stocks – a meta-analysis. Global Change Biology 17: 1658–1670.

https://doi.org/https://doi.org/10.1111/j.1365-2486.2010.02336.x.

Dos Santos Fonseca, A. L., I. Bianchini, C. M. M. Pimenta, C. B. P. Soares, & N. Mangiavacchi, 2013. The flow velocity as driving force for decomposition of leaves and twigs. Hydrobiologia 703: 59–67. https://doi.org/10.1007/S10750-012-1342-3/METRICS.

DROTRH-INAG, 2001. Plano regional da água. Relatório técnico. Versão para consulta pública. Ponta Delgada.

DRRF, 2014. Estratégia Florestal Regional. Direção Regional dos Recursos Florestais, Ponta Delgada, Portugal.

Du, J., Y. Zhang, M. Cui, J. Yang, Z. Lin, & H. Zhang, 2017. Evidence for negative effects of ZnO nanoparticles on leaf litter decomposition in freshwater ecosystems. Environmental Science: Nano 4: 2377–2387. https://doi.org/10.1039/c7en00784a.

Du, J., Y. Zhang, Y. Yin, J. Zhang, H. Ma, K. Li, & N. Wan, 2020. Do environmental concentrations of zinc oxide nanoparticle pose ecotoxicological risk to aquatic fungi associated with leaf litter decomposition? Water Research 178: 115840. https://doi.org/https://doi.org/10.1016/j.watres.2020.115840.

Duarte, S., C. Pascoal, A. Alves, A. Correia, & F. Cássio, 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. Freshwater Biology 53: 91–101. https://doi.org/10.1111/j.1365-2427.2007.01869.x.

Duarte, S., C. Pascoal, & F. Cássio, 2004. Effects of zinc on leaf decomposition by fungi in streams: Studies in microcosms. Microbial Ecology 48: 366–374. https://doi.org/10.1007/s00248-003-2032-5.

Ehrman, J. M., F. Bärlocher, R. Wennrich, G.-J. Krauss, & G. Krauss, 2008. Fungi in a heavy metal precipitating stream in the Mansfeld mining district, Germany. Science of The Total Environment 389: 486–496. https://doi.org/https://doi.org/10.1016/j.scitotenv.2007.09.004.

Ellis, E. C., K. Klein Goldewijk, S. Siebert, D. Lightman, & N. Ramankutty, 2010. Anthropogenic transformation of the biomes, 1700 to 2000. Global Ecology and Biogeography 19: 589–606. https://doi.org/https://doi.org/10.1111/j.1466-8238.2010.00540.x.

Elosegi, A., A. Basaguren, & J. Pozo, 2006. A functional approach to the ecology of Atlantic Basque streams. Limnética 25: 123–134.

Encalada, A. C., J. Calles, V. Ferreira, C. M. Canhoto, M. A. S. S. Graça, J. Caller, V. Ferreira, C. M. Canhoto, & M. A. S. S. Graça, 2010. Riparian land use and the relationship between the benthos and litter decomposition in tropical montane streams. Freshwater Biology 55: 1719–1733. https://doi.org/10.1111/j.1365-2427.2010.02406.x.

FAO, 2005. Progress towards sustainable forest management. Forestry Paper : 147.

Feio, M. J., R. M. Hughes, M. Callisto, S. J. Nichols, O. N. Odume, B. R. Quintella, M. Kuemmerlen, F. C. Aguiar, S. F. P. Almeida, P. Alonso-EguíaLis, F. O. Arimoro, F. J. Dyer, J. S. Harding, S. Jang, P. R. Kaufmann, S. Lee, J. Li, D. R. Macedo, A. Mendes, N. Mercado-Silva, W. Monk, K. Nakamura, G. G. Ndiritu, R. Ogden, M. Peat, T. B. Reynoldson, B. Rios-Touma, P. Segurado, & A. G. Yates, 2021. The biological assessment and rehabilitation of the world's rivers: An overview. Water 13:. https://doi.org/10.3390/w13030371.

Feld, C. K., J. P. Sousa, P. M. da Silva, & T. P. Dawson, 2010. Indicators for biodiversity and ecosystem services: towards an improved framework for ecosystems assessment. Biodiversity and Conservation 19: 2895–2919. https://doi.org/10.1007/s10531-010-9875-0.

Fernandes, I., S. Duarte, F. Cássio, & C. Pascoal, 2009. Mixtures of zinc and phosphate affect leaf litter decomposition by aquatic fungi in streams. Science of the Total Environment 407: 4283–4288. https://doi.org/10.1016/j.scitotenv.2009.04.007.

Fernandes, I. R., 2008. Effects of fungal diversity and cadmium on leaf litter decomposition in streams: studies in microcosms. University of Minho, Portugal.

Fernandes, M. R., P. Segurado, E. Jauch, & M. T. Ferreira, 2016. Riparian responses to extreme climate and land-use change scenarios. Science of The Total Environment 569–570: 145–158. https://doi.org/https://doi.org/10.1016/j.scitotenv.2016.06.099.

Fernández-Palacios, J. M., 2010. The islands of Macaronesia. In Terrestrial arthropods of Macaronesia. Biodiversity, Ecology and Evolution, Eds. pp. 1–30.

Fernández-Palacios, J. M., H. Kreft, S. D. H. Irl, S. Norder, C. Ah-Peng, P. A. V Borges, K. C. Burns, L. de Nascimento, J.-Y. Meyer, E. Montes, & D. R. Drake, 2021. Scientists' warning – The outstanding biodiversity of islands is in peril. Global Ecology and Conservation 31: e01847. https://doi.org/https://doi.org/10.1016/j.gecco.2021.e01847.

Ferreira, V., R. Albariño, A. Larrañaga, C. J. LeRoy, F. O. Masese, & M. S. Moretti, 2023a. Ecosystem services provided by small streams: An overview. Hydrobiologia 850: 2501–2535. https://doi.org/10.1007/S10750-022-05095-1.

Ferreira, V., L. M. Bini, M. de los Á. González Sagrario, K. E. Kovalenko, L. Naselli-Flores, A. A. Padial, & J. Padisák, 2023b. Aquatic ecosystem services: An overview of the Special Issue. Hydrobiologia 850: 2473–2483. https://doi.org/10.1007/S10750-023-05235-1/TABLES/2.

Ferreira, V., B. Castagneyrol, J. Koricheva, V. Gulis, E. Chauvet, & M. A. S. Graça, 2015. A meta-analysis of the effects of nutrient enrichment on litter decomposition in streams. Biological Reviews 90: 669–688. https://doi.org/10.1111/brv.12125.

Ferreira, V., J. Castela, P. Rosa, A. M. Tonin, L. Boyero, & M. A. S. Graça, 2016a. Aquatic hyphomycetes, benthic macroinvertebrates and leaf litter decomposition in streams naturally differing in riparian vegetation. Aquatic Ecology 50:. https://doi.org/10.1007/s10452-016-9588-x.

Ferreira, V., & E. Chauvet, 2011a. Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17: 551–564. https://doi.org/10.1111/J.1365-2486.2010.02185.X.

Ferreira, V., & E. Chauvet, 2011b. Future increase in temperature more than decrease in litter quality can affect microbial litter decomposition in streams. Oecologia 167: 279-291.

Ferreira, V., A. Elosegi, S. D. Tiegs, D. Von Schiller, & R. Young, 2020. Organic matter decomposition and ecosystem metabolism as tools to assess the functional integrity of streams and rivers-a systematic review. Water 12: 3523. https://doi.org/10.3390/w12123523.

Ferreira, V., H. Faustino, P. M. Raposeiro, & V. Gonçalves, 2017. Replacement of native forests by conifer plantations affects fungal decomposer community structure but not litter decomposition in Atlantic island streams. Forest Ecology and Management 389: 323–330. https://doi.org/10.1016/J.FORECO.2017.01.004.

Ferreira, V., A. L. Gonçalves, & C. Canhoto, 2012. Aquatic hyphomycete strains from metal-contaminated and reference streams might respond differently to future increase in temperature. Mycologia 104: 613–622. https://doi.org/10.3852/11-154.

Ferreira, V., V. Gulis, & M. A. S. Graça, 2006. Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149: 718–729. https://doi.org/10.1007/S00442-006-0478-0/FIGURES/6.

Ferreira, V., J. Koricheva, S. Duarte, D. K. Niyogi, F. Guérold, V. Onica Ferreira, J. Koricheva, S. Duarte, D. K. Niyogi, & F. Gu Erold, 2016b. Effects of anthropogenic heavy metal contamination on litter decomposition in streams - A meta-analysis. Environmental Pollution 210: 261–270. https://doi.org/10.1016/j.envpol.2015.12.060.

Ferreira, V., J. Koricheva, J. Pozo, & M. A. S. Graça, 2016c. A meta-analysis on the effects of changes in the composition of native forests on litter decomposition in streams. Forest Ecology and Management 364:. https://doi.org/10.1016/j.foreco.2016.01.002.

Ferreira, V., P. M. Raposeiro, A. Pereira, A. M. Cruz, A. C. Costa, M. A. S. Graça, & V. Gonçalves, 2016d. Leaf litter decomposition in remote oceanic island streams is driven by microbes and depends on litter quality and environmental conditions. Freshwater Biology 61: 783–799.

https://doi.org/10.1111/FWB.12749.

Ferreira, V., J. Silva, J. Cornut, O. Sobral, Q. Bachelet, J. Bouquerel, & M. Danger, 2021. Organic-matter decomposition as a bioassessment tool of stream functioning: A comparison of eight decomposition-based indicators exposed to different environmental changes. Environmental Pollution 290: 118111. https://doi.org/10.1016/J.ENVPOL.2021.118111.

Flores, L., J. R. Díez, A. Larrañaga, C. Pascoal, & A. Elosegi, 2013. Effects of retention site on breakdown of organic matter in a mountain stream. Freshwater Biology 58: 1267–1278. https://doi.org/https://doi.org/10.1111/fwb.12125.

Follstad Shah, J. J., J. S. Kominoski, M. Ardón, W. K. Dodds, M. O. Gessner, N. A. Griffiths, C. P. Hawkins, S. L. Johnson, A. Lecerf, C. J. LeRoy, D. W. P. Manning, A. D. Rosemond, R. L. Sinsabaugh, C. M. Swan, J. R. Webster, & L. H. Zeglin, 2017. Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. Global Change Biology 23: 3064–3075. https://doi.org/https://doi.org/10.1111/gcb.13609.

Food and Agriculture Organization (FAO), 2020. Global Forest Resources Assessment 2020.

Food and Agriculture Organization (FAO), 2024. Land statistics 2001–2022. Global, regional and country trends.

Frainer, A., & B. G. McKie, 2015. Chapter seven - Shifts in the diversity and composition of consumer traits constrain the effects of land use on stream ecosystem functioning. In Pawar, S., G. Woodward, & A. I. B. T.-A. in E. R. Dell (eds) Trait-based ecology. From Structure to Function. Academic Press. pp. 169–200.

Freire, P., C. Andrade, R. Coutinho, & J. V. Cruz, 2013. Fluvial geochemistry in São Miguel Island (Azores, Portugal): Source and fluxes of inorganic solutes in an active volcanic environment. Science of The Total Environment 454–455: 154–169. https://doi.org/10.1016/J.SCITOTENV.2013.02.090.

Friberg, Ni., & D. Jacobsen, 1994. Feeding plasticity of two detritivore-shredders. Freshwater Biology 32: 133–142. https://doi.org/10.1111/J.1365-2427.1994.TB00873.X.

Fuchs, S. A., S. G. Hinch, & E. Mellina, 2003. Effects of streamside logging on stream macroinvertebrate communities and habitat in the sub-boreal forests of British Columbia, Canada. Canadian Journal of Forest Research 33: 1408–1415. https://doi.org/10.1139/x03-070.

Funck, J. A., H. Clivot, V. Felten, P. Rousselle, F. Guérold, & M. Danger, 2013a. Phosphorus availability modulates the toxic effect of silver on aquatic fungi and leaf litter decomposition. Aquatic Toxicology 144–145: 199–207. https://doi.org/https://doi.org/10.1016/j.aquatox.2013.10.001.

Funck, J. A., M. Danger, E. Gismondi, C. Cossu-Leguille, F. Guérold, & V. Felten, 2013b. Behavioural and physiological responses of *Gammarus fossarum* (Crustacea Amphipoda) exposed to silver. Aquatic Toxicology 142–143: 73–84. https://doi.org/10.1016/J.AQUATOX.2013.07.012.

Furse, M. T., D. Hering, K. Brabec, A. Buffagni, L. Sandin, & P. F. Verdonschot, 2009. The ecological status of European rivers: evaluation and intercalibration of assessment methods. Springer Science & Business Media.

Furtado, R., J. Baptista, E. Lima, L. Paiva, J. G. Barroso, J. S. Rosa, & L. Oliveira, 2014. Chemical composition and biological activities of *Laurus* essential oils from different Macaronesian Islands. Biochemical Systematics and Ecology 55: 333–341. https://doi.org/10.1016/J.BSE.2014.04.004.

Galic, N., T. Hawkins, & V. E. Forbes, 2019. Adverse impacts of hypoxia on aquatic invertebrates: A meta-analysis. Science of The Total Environment 652: 736–743. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.10.225.

Gaspar, C., P. A. Borges, & K. J. Gaston, 2008. Diversity and distribution of arthropods in native forests of the Azores archipelago. Arquipélago. Life and Marine Sciences 25: 1–30.

Gessner, M. O., & E. Chauvet, 2002. A case for using litter breakdown to assess functional stream integrity. Ecological Applications 12: 498–510. https://doi.org/https://doi.org/10.1890/1051-0761(2002)012[0498:ACFULB]2.0.CO;2.

Gessner, M. O., & F. Peeters, 2020. Determining temperature-normalized decomposition rates. In Methods to Study Litter Decomposition. Springer International Publishing. pp. 553–560.

Giller, P. S., H. Hillebrand, U.-G. Berninger, M. O. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, M. T. Monaghan, P. J. Morin, & G. O'Mullan, 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. Oikos 104: 423–436. https://doi.org/https://doi.org/10.1111/j.0030-1299.2004.13253.x.

Girisha, G. K., L. M. Condron, P. W. Clinton, & M. R. Davis, 2003. Decomposition and nutrient dynamics of green and freshly fallen radiata pine (*Pinus radiata*) needles. Forest Ecology and Management 179: 169–181. https://doi.org/10.1016/S0378-1127(02)00518-2.

Golabi, M. H., S. A. El-Swaify, & C. Iyekar, 2014. Experiment of "No-tillage" farming system on the volcanic soils of tropical islands of Micronesia. International Soil and Water Conservation Research 2: 30–38. https://doi.org/https://doi.org/10.1016/S2095-6339(15)30004-6.

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

Gonçalves, A. L., A. V. Lírio, J. Pratas, & C. Canhoto, 2011. Uranium contaminated water does not affect microbial activity but decreases feeding by the shredder *Sericostoma vittatum*. Fundamental and Applied Limnology 179: 17–25. https://doi.org/10.1127/1863-9135/2011/0179-0017.

Gonçalves, V., H. S. Marques, & P. M. Raposeiro, 2015a. Diatom assemblages and their associated environmental drivers in isolated oceanic island streams (Azores archipelago as case study). Hydrobiologia 751: 89–103. https://doi.org/10.1007/S10750-015-2174-8/METRICS.

Gonçalves, V., P. . Raposeiro, H. Marques, J. Vilaverde, A. Balibrea, J. Camille-Riva, G. Gea, & A.

C. Costa, 2015b. Monitorização das massas de água interiores e de transição da região hidrográfica dos Açores. Relatório da 1ª campanha do ano 1 (R1/Ano 1). Ponta Delgada.

Gonçalves, V., P. M. Raposeiro, & A. C. Costa, 2008. Benthic diatoms and macroinvertebrates in the assessment of the ecological status of Azorean streams. Limnetica 27: 317–328.

Gonçalves, V., P. M. Raposeiro, H. Marques, J. Vilaverde, A. Balibrea, J. Camille-Riva, R. Luz, & A. . Costa, 2016. Monitorização das massas de água interiores e de transição da região hidrográfica dos Açores. Relatório Anual do 1º ano (R5/1º Ano). Ponta Delgada.

Gong, P., J. Wang, L. Yu, Y. Zhao, Y. Zhao, L. Liang, Z. Niu, X. Huang, H. Fu, S. Liu, C. Li, X. Li, W. Fu, C. Liu, Y. Xu, X. Wang, Q. Cheng, L. Hu, W. Yao, H. Zhang, P. Zhu, Z. Zhao, H. Zhang, Y. Zheng, L. Ji, Y. Zhang, H. Chen, A. Yan, J. Guo, L. Yu, L. Wang, X. Liu, T. Shi, M. Zhu, Y. Chen, G. Yang, P. Tang, B. Xu, C. Giri, N. Clinton, Z. Zhu, J. Chen, & J. Chen, 2012. Finer resolution observation and monitoring of global land cover: first mapping results with Landsat TM and ETM+ data. International Journal of Remote Sensing 34: 2607–2654. https://doi.org/10.1080/01431161.2012.748992.

González, E., A. A. Sher, E. Tabacchi, A. Masip, & M. Poulin, 2015. Restoration of riparian vegetation: A global review of implementation and evaluation approaches in the international, peer-reviewed literature. Journal of Environmental Management 158: 85–94. https://doi.org/https://doi.org/10.1016/j.jenvman.2015.04.033.

González, J. M., & M. A. S. Graça, 2003. Conversion of leaf litter to secondary production by a shredding caddis-fly. Freshwater Biology 48: 1578–1592. https://doi.org/10.1046/J.1365-2427.2003.01110.X.

Goodman, K. J., A. E. Hershey, & K. Fortino, 2006. The effect of forest type on benthic macroinvertebrate structure and ecological function in a pine plantation in the North Carolina Piedmont. Hydrobiologia 559: 305–318. https://doi.org/10.1007/s10750-005-0990-y.

Gopalakrishnan, G., M. C. Negri, M. Wang, M. Wu, S. W. Snyder, & L. LaFreniere, 2009.

Biofuels, land, and water: A system approach to sustainability. Environmental Science & Technology 43: 6094–6100. https://doi.org/10.1021/es900801u.

Graça, M. A. S., 2001. The role of invertebrates on leaflLitter decomposition in streams – A review. International Review of Hydrobiology 86: 383–393. https://doi.org/10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D.

Graça, M. A. S., & C. Cressa, 2010. Leaf quality of some tropical and temperate tree species as food resource for stream shredders. International Review of Hydrobiology 95: 27–41. https://doi.org/10.1002/iroh.200911173.

Gregory, S. V, F. J. Swanson, W. A. McKee, & K. W. Cummins, 1991. An ecosystem perspective of riparian Zones. BioScience 41: 540–551. https://doi.org/10.2307/1311607.

Griffiths, N. A., & S. D. Tiegs, 2016. Organic-matter decomposition along a temperature gradient in a forested headwater stream. Freshwater Science 35: 518–533.

Grudzinski, B., K. Fritz, & W. Dodds, 2020. Does riparian fencing protect stream water quality in cattle-grazed lands? Environmental Management 66: 121–135. https://doi.org/10.1007/s00267-020-01297-2.

Gulis, V., V. Ferreira, & M. A. S. Graça, 2006. Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshwater Biology 51: 1655–1669.

Gulis, V., & K. Suberkropp, 2003. Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater Biology 48: 123–134. https://doi.org/10.1046/j.1365-2427.2003.00985.x.

Haggerty, S. M., D. P. Batzer, & C. R. Jackson, 2004. Macroinvertebrate response to logging in coastal headwater streams of Washington, U.S.A. Canadian Journal of Fisheries and Aquatic Sciences 61: 529–537. https://doi.org/10.1139/f04-014.

Harbrow, M. A., 2001. Ecology of streams affected by acid mine drainage near Westport, South Island, New Zealand. University of Canterbury, New Zealand.

Harding, J. S., 2005. Impacts of metals and mining on stream communities. In Moore, TA, Black, A., Centeno, JA, Harding, JS and Trumm, D. (ed) Metal Contaminants in New Zealand. pp. 343–357.

Harrison, L. J., K. A. Pearson, C. J. Wheatley, J. K. Hill, L. Maltby, C. Rivetti, L. Speirs, & P. C. L. White, 2021. Functional measures as potential indicators of down-the-drain chemical stress in freshwater ecological risk assessment. Integrated Environmental Assessment and Management 18: 1135–1147. https://doi.org/10.1002/ieam.4568.

Harrison, P. A., P. M. Berry, G. Simpson, J. R. Haslett, M. Blicharska, M. Bucur, R. Dunford, B. Egoh, M. Garcia-Llorente, N. Geamănă, W. Geertsema, E. Lommelen, L. Meiresonne, & F. Turkelboom, 2014. Linkages between biodiversity attributes and ecosystem services: A systematic review. Ecosystem Services 9: 191–203. https://doi.org/https://doi.org/10.1016/j.ecoser.2014.05.006.

Hedenquist, J. W., M. Aoki, & H. Shinohara, 1994. Flux of volatiles and ore-forming metals from the magmatic-hydrothermal system of Satsuma Iwojima volcano. Geology 22: 585. https://doi.org/10.1130/0091-7613(1994)022<0585:FOVAOF>2.3.CO;2.

Hering, D., A. Borja, J. Carstensen, L. Carvalho, M. Elliott, C. K. Feld, A.-S. Heiskanen, R. K. Johnson, J. Moe, D. Pont, A. L. Solheim, & W. van de Bund, 2010. The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. Science of The Total Environment 408: 4007–4019. https://doi.org/https://doi.org/10.1016/j.scitotenv.2010.05.031.

Hessen, D. O., A. Hindar, & G. Holtan, 1997. The significance of nitrogen runoff for eutrophication of freshwater and marine recipients. Ambio 26: 312–320.

Hieber, M., & M. O. Gessner, 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83: 1026–1038.

Hisabae, M., S. Sone, & M. Inoue, 2011. Breakdown and macroinvertebrate colonization of needle and leaf litter in conifer plantation streams in Shikoku, southwestern Japan. Journal of Forest Research 16: 108–115. https://doi.org/10.1007/S10310-010-0210-0/TABLES/2.

Hladyz, S., K. Åbjörnsson, E. Chauvet, M. Dobson, A. Elosegi, V. Ferreira, T. Fleituch, M. O. Gessner, P. S. Giller, V. Gulis, S. A. Hutton, J. O. Lacoursière, S. Lamothe, A. Lecerf, B. Malmqvist, B. G. McKie, M. Nistorescu, E. Preda, M. P. Riipinen, G. Rîşnoveanu, M. Schindler, S. D. Tiegs, L. B.-M. Vought, & G. Woodward, 2011a. Chapter 4 - Stream Ecosystem eunctioning in an agricultural landscape: The importance of terrestrial–aquatic linkages. In Woodward, G. (ed). Academic Press. pp. 211–276.

Hladyz, S., K. Åbjörnsson, P. S. Giller, & G. Woodward, 2011b. Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. Journal of Applied Ecology 48: 443–452. https://doi.org/https://doi.org/10.1111/j.1365-2664.2010.01924.x.

Hladyz, S., E. Chauvet, M. Dobson, A. Elosegi, N. Ferreira, T. Fleituch, M. O. Gessner, P. S. Giller, V. Gulis, S. A. Hutton, J. O. Lacoursie, S. Lamothe, A. Lecerf, R. Malmqvist, B. G. Mckie, M. Nistorescu, E. Preda, M. P. Riipinen, G. Risnoveanu, M. Schindler, S. D. Tiegs, L. B.-M. Vought, & G. Woodward, 2011c. Stream ecosystem functioning in an agricultural landscape: The importance of terrestrial-aquatic linkages. In Advances in Ecological Research. Academic Press. pp. 211–276.

Hladyz, S., S. D. Tiegs, M. O. Gessner, P. S. Giller, G. Rîsnoveanu, E. Preda, M. Nistorescu, M. Schindler, & G. Woodward, 2010. Leaf-litter breakdown in pasture and deciduous woodland streams: A comparison among three European regions. Freshwater Biology 55: 1916–1929.

Hödl, E., 2018. Chapter 17: Legislative framework for river ecosystem management on international and European level. In Riverine Ecosystem Management. pp. 325–346.

Hogsden, K. L., 2013. Structure and function of food webs in acid mine drainage streams.

University of Canterbury, New Zealand.

Hogsden, K. L., & J. S. Harding, 2012. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution 162: 466–474. https://doi.org/10.1016/j.envpol.2011.10.024.

Hogsden, K. L., & J. S. Harding, 2013. Leaf breakdown, detrital resources, and food webs in streams affected by mine drainage. Hydrobiologia 716: 59–73. https://doi.org/10.1007/s10750-013-1544-3.

Hogsden, K. L., M. J. Winterbourn, J. S. Harding, K. L. Hogsden, M. J. Winterbourn, & J. S. Harding, 2013. Do food quantity and quality affect food webs in streams polluted by acid mine drainage?. Marine and Freshwater Research 64: 1112–1122. https://doi.org/10.1071/MF13016.

Holland, R. A., F. Eigenbrod, P. R. Armsworth, B. J. Anderson, C. D. Thomas, A. Heinemeyer, S. Gillings, D. B. Roy, & K. J. Gaston, 2011. Spatial covariation between freshwater and terrestrial ecosystem services. Ecological Applications 21: 2034–2048. https://doi.org/https://doi.org/10.1890/09-2195.1.

Houghton, R. A., 1994. The worldwide extent of land-use change. BioScience 44: 305–313.

Hughes, S. J., 2005. Application of the Water Framework Directive to Macaronesian freshwater systems. Biology and Environment 105: 185–193. https://doi.org/10.3318/BIOE.2005.105.3.185.

Hughes, S. J., & B. Malmqvist, 2005. Atlantic Island freshwater ecosystems: Challenges and considerations following the EU Water Framework Directive. Hydrobiologia 544: 289–297. https://doi.org/10.1007/s10750-005-1695-y.

Hurwitz, S., W. C. Evans, & J. B. Lowenstern, 2010. River solute fluxes reflecting active hydrothermal chemical weathering of the Yellowstone Plateau Volcanic Field, USA. Chemical Geology 276: 331–343. https://doi.org/10.1016/J.CHEMGEO.2010.07.001.

ICH, 1996. Validation of analytical procedures: Methodology, Q2B (CPMP/ICH/281/96). London.

INAG, I. P., 2008. Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água: Protocolo de amostragem e análise para os macroinvertebrados bentónicos. Ministério do Ambiente, do Ordenamento do Território e do desenvolvimento Regional. Instituto da Água IP, 164.:

Inoue, M., S. ichi Shinotou, Y. Maruo, & Y. Miyake, 2012. Input, retention, and invertebrate colonization of allochthonous litter in streams bordered by deciduous broadleaved forest, a conifer plantation, and a clear-cut site in southwestern Japan. Limnology 13: 207–219. https://doi.org/10.1007/S10201-011-0369-X/METRICS.

IPAC, 2018. Guia para a aplicação da NP EN ISO/IEC 17025:2018.

Irons, J. G., M. W. Oswood, & J. P. Bryant, 1988. Consumption of leaf detritus by a stream shredder: Influence of tree species and nutrient status. Hydrobiologia 1988 160:1 160: 53–61. https://doi.org/10.1007/BF00014278.

Irons, J. G., M. W. Oswood, J. R. Stout, & C. M. Pringle, 1994. Latitudinal patterns in leaf litter breakdown: Is temperature really important? Freshwater Biology 32: 401–411. https://doi.org/10.1111/J.1365-2427.1994.TB01135.X.

Irving, E. C., D. J. Baird, & J. M. Culp, 2003. Ecotoxicological responses of the mayfly *Baetis tricaudatus* to dietary and waterborne cadmium: Implications for toxicity testing. Environmental Toxicology and Chemistry 22: 1058–1064. https://doi.org/10.1897/1551-5028(2003)022<1058:EROTMB>2.0.CO;2.

Iversen, T. M., 1974. Ingestion and growth in *Sericostoma personatum* (Trichoptera) in relation to the nitrogen content of ingested leaves. Oikos 25: 278. https://doi.org/10.2307/3543945.

Jabiol, J., A. Lecerf, S. Lamothe, M. O. Gessner, & E. Chauvet, 2019. Litter quality modulates effects of dissolved nitrogen on leaf decomposition by stream microbial communities. Microbial Ecology 77: 959–966. https://doi.org/10.1007/S00248-019-01353-3/FIGURES/3.

Jain, A., S. Kumar, & S. Seena, 2019. Can low concentrations of metal oxide and Ag loaded metal oxide nanoparticles pose a risk to stream plant litter microbial decomposers? Science of The Total Environment 653: 930–937. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.10.376.

Johnson, D. B., 1998. Microorganisms and the biogeochemical cycling of metals in aquatic environments. In Langston, W. J., & M. J. Bebianno (eds) Metal metabolism in aquatic environments. Springer US, Boston, MA. pp. 31–57.

Johnson, K. S., P. C. Thompson, L. Gromen, & J. Bowman, 2014. Use of leaf litter breakdown and macroinvertebrates to evaluate gradient of recovery in an acid mine impacted stream remediated with an active alkaline doser. Environmental Monitoring and Assessment 186: 4111–4127. https://doi.org/10.1007/s10661-014-3684-y.

Johnson, R. K., & K. Almlöf, 2016. Adapting boreal streams to climate change: effects of riparian vegetation on water temperature and biological assemblages. Freshwater Science 35: 984–997. https://doi.org/10.1086/687837.

Jose, S., D. Walter, & B. Mohan Kumar, 2017. Ecological considerations in sustainable silvopasture design and management. Agroforestry Systems 93: 317–331. https://doi.org/10.1007/s10457-016-0065-2.

Juncos, R., M. Arcagni, A. Rizzo, L. Campbell, M. Arribére, & S. R. Guevara, 2016. Natural origin arsenic in aquatic organisms from a deep oligotrophic lake under the influence of volcanic eruptions. Chemosphere 144: 2277–2289. https://doi.org/https://doi.org/10.1016/j.chemosphere.2015.10.092.

Jupiter, S., S. Mangubhai, & R. T. Kingsford, 2014. Conservation of biodiversity in the Pacific Islands of Oceania: Challenges and opportunities. Pacific Conservation Biology 20: 206–220.

Kandziora, M., B. Burkhard, & F. Müller, 2013. Interactions of ecosystem properties, ecosystem integrity and ecosystem service indicators—A theoretical matrix exercise. Ecological Indicators 28: 54–78. https://doi.org/https://doi.org/10.1016/j.ecolind.2012.09.006.

Kearns, S. G., & F. Bärlocher, 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. Fungal Ecology 1: 13–18. https://doi.org/10.1016/J.FUNECO.2007.07.001.

Kelley, K. D., & T. Hudson, 2007. Natural versus anthropogenic dispersion of metals to the environment in the Wulik River area, western Brooks Range, northern Alaska. Geochemistry: Exploration, Environment, Analysis 7: 87–96. https://doi.org/10.1144/1467-7873/06-121.

Keppel, G., C. Morrison, J. Y. Meyer, & H. J. Boehmer, 2014. Isolated and vulnerable: The history and future of Pacific Island terrestrial biodiversity. Pacific Conservation Biology 20: 136–145.

Khan, N., M. K. Jhariya, A. Banerjee, R. S. Meena, A. Raj, & S. K. Yadav, 2022. Chapter 9 - Riparian conservation and restoration for ecological sustainability. In Jhariya, M. K., R. S. Meena, A. Banerjee, & S. N. Meena (eds) Natural resources conservation and advances for sustainability. Elsevier. pp. 195–216.

Knight, A. W., & R. L. Bottorff, 1984. The importance of riparian vegetation to stream ecosystems. California riparian systems: ecology, conservation and productive management.

Kolya, H., & C.-W. Kang, 2024. Toxicity of metal oxides, dyes, and dissolved organic matter in water: Implications for the environment and human health. Toxics 12:. https://doi.org/10.3390/toxics12020111.

Krauss, G., F. Bärlocher, & G. J. Krauss, 2003. Effects of pollution on aquatic hyphomycetes. Fungal Diversity Research Series 10: 211–230.

Krauss, G. G.-J. J., M. Solé, G. G.-J. J. Krauss, D. Schlosser, D. Wesenberg, & F. Bärlocher, 2011. Fungi in freshwaters: Ecology, physiology and biochemical potential. Microbiology Reviews 35: 620–651. https://doi.org/10.1111/j.1574-6976.2011.00266.x.

Krauss, G., K. R. Sridhar, & F. Bärlocher, 2005. Aquatic hyphomycetes and leaf decomposition in contaminated groundwater wells in Central Germany. Archiv fur Hydrobiologie 162: 417–429.

https://doi.org/10.1127/0003-9136/2005/0162-0417.

Kriska, G., 2013. Freshwater invertebrates in Central Europe: A field guide. Springer.

Lake, P. S., M. A. Palmer, P. Biro, J. Cole, A. P. Covich, C. Dahm, J. Gibert, W. Goedkoop, K. Martens, & J. Verhoeven, 2000. Global change and the biodiversity of freshwater ecosystems: Impacts on linkages between above-sediment and sediment biota: All forms of anthropogenic disturbance—changes in land use, biogeochemical processes, or biotic addition or loss—not only damage th. BioScience 50: 1099–1107. https://doi.org/10.1641/0006-3568(2000)050[1099:GCATBO]2.0.CO;2.

Lambin, E. F., H. J. Geist, & E. Lepers, 2003. Dynamics of land-use and land-cover change in tropical regions. Annual Review of Environment and Resources 28: 205–241. https://doi.org/https://doi.org/10.1146/annurev.energy.28.050302.105459.

Langhans, S. D., S. D. Tiegs, M. O. Gessner, & K. Tockner, 2008. Leaf-decomposition heterogeneity across a riverine floodplain mosaic. Aquatic Sciences 70: 337–346. https://doi.org/10.1007/s00027-008-8062-9.

Larned, S. T., 2000. Dynamics of coarse riparian detritus in a Hawaiian stream ecosystem: A comparison of drought and post-drought conditions. Journal of the North American Benthological Society 19: 215–234. https://doi.org/10.2307/1468066.

Larrañaga, A., A. Martínez, R. Albariño, J. J. Casas, V. Ferreira, & R. Principe, 2021. Effects of exotic tree plantations on plant litter decomposition in streams. In The ecology of plant litter decomposition in stream ecosystems. pp. 297–322.

Leberfinger, K., & I. Bohman, 2010. Grass, mosses, algae, or leaves? Food preference among shredders from open-canopy streams. Aquatic Ecology 44: 195–203. https://doi.org/10.1007/S10452-009-9268-1/TABLES/1.

Lecerf, A., & J. S. Richardson, 2010. Biodiversity-ecosystem function research: Insights gained from streams. River Research and Applications 26: 45–54. https://doi.org/https://doi.org/10.1002/rra.1286.

Lecerf, A., P. Usseglio-Polatera, J.-Y. Charcosset, D. Lambrigot, B. Bracht, & E. Chauvet, 2006. Assessment of functional integrity of eutrophic streams using litter breakdown and benthic macroinvertebrates. Arch. Hydrobiol 165: 105–126. https://doi.org/10.1127/0003-9136/2006/0165-0105.

Lefcort, H., Z. Freedman, S. House, & M. Pendleton, 2008. Hormetic effects of heavy metals in aquatic snails: Is a little bit of pollution good? EcoHealth 5: 10–17. https://doi.org/10.1007/s10393-008-0158-0.

Lehner, B., P. Döll, J. Alcamo, T. Henrichs, & F. Kaspar, 2006. Estimating the impact of global change on flood and drought risks in Europe: A continental, integrated analysis. Climatic Change 75: 273–299. https://doi.org/10.1007/s10584-006-6338-4.

Li, H., F. Santos, K. Butler, & E. Herndon, 2021. A critical review on the multiple roles of manganese in stabilizing and destabilizing soil organic matter. Environmental Science & Technology 55: 12136–12152. https://doi.org/10.1021/acs.est.1c00299.

Li, Y., T. Kasahara, M. Chiwa, & N. Fujimoto, 2020. Effects of dams and reservoirs on organic matter decomposition in forested mountain streams in western Japan. River Research and Applications 36: 1257–1266. https://doi.org/https://doi.org/10.1002/rra.3640.

Little, P., 1970. A study of heavy metal contamination of leaf surfaces. Environmental Pollution 5: 159–172. https://doi.org/10.1016/0013-9327(73)90085-2.

Liu, R., Y. Pan, Y. Fang, L. Pang, J. Shen, & X. Tian, 2021. Effects of heavy metal-mediated intraspecific variation in leaf litter on the feeding preferences of stream detritivores. Science of The Total Environment 763: 144591. https://doi.org/10.1016/J.SCITOTENV.2020.144591.

Liu, X., G. Hu, Y. Chen, X. Li, X. Xu, S. Li, F. Pei, & S. Wang, 2018. High-resolution multitemporal mapping of global urban land using Landsat images based on the Google Earth Engine Platform. Remote Sensing of Environment 209: 227–239. https://doi.org/https://doi.org/10.1016/j.rse.2018.02.055.

Löfgren, S., E. Ring, C. von Brömssen, R. Sørensen, & L. Högbom, 2009. Short-term effects of clear-cutting on the water chemistry of two boreal streams in Northern Sweden: A paired catchment study. A Journal of the Human Environment 38: 347–356. https://doi.org/10.1579/0044-7447-38.7.347.

Lorion, C. M., & B. P. Kennedy, 2009. Relationships between deforestation, riparian forest buffers and benthic macroinvertebrates in neotropical headwater streams. Freshwater Biology 54: 165–180. https://doi.org/10.1111/J.1365-2427.2008.02092.X.

Loureiro, R. C., C. Biasi, & L. U. Hepp, 2024. Effects of copper and cadmium on stream leaf decomposition: evidence from a microcosm study. Environmental Science and Pollution Research 31: 2511–2520. https://doi.org/10.1007/s11356-023-31282-1.

Louvat, P., C. J. Allègre, & C. J. Allegrè, 1998. Riverine erosion rates on Sao Miguel volcanic island, Azores archipelago. Chemical Geology 148: 177–200. https://doi.org/10.1016/S0009-2541(98)00028-X.

Lussier, S. M., S. N. da Silva, M. Charpentier, J. F. Heltshe, S. M. Cormier, D. J. Klemm, M. Chintala, & S. Jayaraman, 2008. The influence of suburban land use on habitat and biotic integrity of coastal Rhode Island streams. Environmental Monitoring and Assessment 139: 119–136. https://doi.org/10.1007/s10661-007-9820-1.

Machado, A. L. F. B., & D. Gonçalves, 2004. Influência do habitat na distribuição da Galinhola (*Scolopax rusticola*) na ilha de S. Miguel (Açores) durante a época de reprodução. International System for Agricultural Science and Technology.

Malmqvist, B., 2002. Aquatic invertebrates in riverine landscapes. Freshwater Biology 47: 679–694. https://doi.org/https://doi.org/10.1046/j.1365-2427.2002.00895.x.

Malmqvist, B., A. N. Nilsson, & M. Baez, 1995. Tenerife’s freshwater macroinvertebrates: Status and threats (Canary Islands, Spain). Aquatic Conservation: Marine and Freshwater Ecosystems 5: 1–24. https://doi.org/10.1002/aqc.3270050103.

Maltby, L., D. M. Forrow, A. B. A. Boxall, P. Calow, & C. I. Betton, 1995. The effects of motorway runoff on freshwater ecosystems: 1. Field study. Environmental Toxicology and Chemistry 14: 1079–1092. https://doi.org/https://doi.org/10.1002/etc.5620140620.

Martínez, A., A. Larrañaga, J. Pérez, E. Descals, A. Basaguren, & J. Pozo, 2013. Effects of pine plantations on structural and functional attributes of forested streams. Forest Ecology and Management 310: 147–155. https://doi.org/10.1016/j.foreco.2013.08.024.

Martínez, A., S. Monroy, J. Pérez, A. Larrañaga, A. Basaguren, J. Molinero, & J. Pozo, 2016. In-stream litter decomposition along an altitudinal gradient: Does substrate quality matter? Hydrobiologia 766: 17–28. https://doi.org/10.1007/s10750-015-2432-9.

Mas-Martí, E., I. Muñoz, F. Oliva, & C. Canhoto, 2015. Effects of increased water temperature on leaf litter quality and detritivore performance: A whole-reach manipulative experiment. Freshwater Biology 60: 184–197.

Mathers, K. L., M. J. Hill, & P. J. Wood, 2017. Benthic and hyporheic macroinvertebrate distribution within the heads and tails of riffles during baseflow conditions. Hydrobiologia 794: 17–30. https://doi.org/10.1007/s10750-017-3092-8.

McKergow, L. A., F. E. Matheson, & J. M. Quinn, 2016. Riparian management: A restoration tool for New Zealand streams. Ecological Management & Restoration 17: 218–227. https://doi.org/https://doi.org/10.1111/emr.12232.

McKie, B. G., & B. Malmqvist, 2009. Assessing ecosystem functioning in streams affected by forest management: Increased leaf decomposition occurs without changes to the composition of benthic assemblages. Freshwater Biology 54: 2086–2100. https://doi.org/10.1111/J.1365-2427.2008.02150.X.

McMillen, H. L., T. Ticktin, A. Friedlander, S. D. Jupiter, R. Thaman, J. Campbell, J. Veitayaki, T. Giambelluca, S. Nihmei, E. Rupeni, L. Apis-Overhoff, W. Aalbersberg, & D. F. Orcherton, 2014. Small islands, valuable insights. Ecology and Society 19:.

Mechaber, W. L., D. B. Marshall, R. A. Mechaber, R. T. Jobe, & F. S. Chew, 1996. Mapping leaf surface landscapes. Proceedings of the National Academy of Sciences 93: 4600–4603. https://doi.org/10.1073/PNAS.93.10.4600.

Medeiros, A., S. Duarte, C. Pascoal, F. Cássio, & M. Graça, 2010. Effects of Zn, Fe and Mn on leaf litter breakdown by aquatic fungi: A microcosm study. International Review of Hydrobiology 95: 12–26. https://doi.org/10.1002/iroh.200911182.

Melo, C. D., C. S. A. M. Maduro Dias, S. Wallon, A. E. S. Borba, J. Madruga, P. A. V. Borges, M. T. Ferreira, & R. B. Elias, 2022. Influence of climate variability and soil fertility on the forage quality and productivity in Azorean pastures. Agriculture 12: 358–376. https://doi.org/10.3390/agriculture12030358.

Menninger, H. L., & M. A. Palmer, 2007. Herbs and grasses as an allochthonous resource in open-canopy headwater streams. Freshwater Biology 52: 1689–1699. https://doi.org/10.1111/J.1365-2427.2007.01797.X.

Metcalfe, J. L., 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: History and present status in Europe. Environmental Pollution 60: 101–139. https://doi.org/10.1016/0269-7491(89)90223-6.

Mieth, A., & H.-R. Bork, 2005. History, origin and extent of soil erosion on Easter Island (Rapa Nui). Catena 63: 244–260. https://doi.org/https://doi.org/10.1016/j.catena.2005.06.011.

Moog, O., S. Schmutz, & I. Schwarzinger, 2018. Chapter 19. Biomonitoring and bioassessment. In Riverine ecosystem management. pp. 371–390.

Moore, O. W., L. Curti, C. Woulds, J. A. Bradley, P. Babakhani, B. J. W. Mills, W. B. Homoky, K.-Q. Xiao, A. W. Bray, B. J. Fisher, M. Kazemian, B. Kaulich, A. W. Dale, & C. L. Peacock, 2023. Long-term organic carbon preservation enhanced by iron and manganese. Nature 621: 312–317. https://doi.org/10.1038/s41586-023-06325-9.

Moore, R., D. L. Spittlehouse, & A. Story, 2005. Riparian microclimate and stream temperature response to fores harvesting: A review. Journal of the American Water Resources Association 41: 813–834. https://doi.org/https://doi.org/10.1111/j.1752-1688.2005.tb03772.x.

Moreirinha, C., S. Duarte, C. Pascoal, & F. Cássio, 2011. Effects of cadmium and phenanthrene mixtures on aquatic fungi and microbially mediated leaf litter decomposition. Archives of Environmental Contamination and Toxicology 61: 211–219. https://doi.org/10.1007/s00244-010-9610-6.

Morton, B., & A. M. de Frias Martins, 2019. Chapter 21 - The Azores. In Sheppard, C. B. T.-W. S. an E. E. (Second E. (ed). Academic Press. pp. 501–530.

Motta-Delgado, P. A., H. E. Ocaña Martínez, E. P. Rojas-Vargas, P. A. Motta-Delgado, H. E. Ocaña Martínez, & E. P. Rojas-Vargas, 2019. Indicators associated to pastures sustainability: A review. Ciencia y Tecnología Agropecuaria 20: 387–430. https://doi.org/10.21930/RCTA.VOL20NUM2ART:1464.

Nabout, J. C., K. B. Machado, A. C. M. David, L. B. G. Mendonça, S. P. da Silva, & P. Carvalho, 2023. Scientific literature on freshwater ecosystem services: trends, biases, and future directions. Hydrobiologia 850: 2485–2499. https://doi.org/10.1007/s10750-022-05012-6.

Naiman, R. J., & H. Décamps, 1997. The ecology of interfaces: Riparian zones. Annual Review of Ecology, Evolution, and Systematics 28: 621–658. https://doi.org/https://doi.org/10.1146/annurev.ecolsys.28.1.621.

Neill, C., L. A. Deegan, S. M. Thomas, & C. C. Cerri, 2001. Deforestation for pastures alters nitrogen and phosphorus in small Amazonian streams. Ecological Applications 11: 1817–1828.

Neumann, M., R. Schulz, K. Schäfer, W. Müller, W. Mannheller, & M. Liess, 2002. The significance of entry routes as point and non-point sources of pesticides in small streams. Water Research 36: 835–842. https://doi.org/https://doi.org/10.1016/S0043-1354(01)00310-4.

Newbold, T., L. N. Hudson, S. L. L. Hill, S. Contu, I. Lysenko, R. A. Senior, L. Börger, D. J. Bennett, A. Choimes, B. Collen, J. Day, A. De Palma, S. Díaz, S. Echeverria-Londoño, M. J. Edgar, A. Feldman, M. Garon, M. L. K. Harrison, T. Alhusseini, D. J. Ingram, Y. Itescu, J. Kattge, V. Kemp, L. Kirkpatrick, M. Kleyer, D. L. P. Correia, C. D. Martin, S. Meiri, M. Novosolov, Y. Pan, H. R. P. Phillips, D. W. Purves, A. Robinson, J. Simpson, S. L. Tuck, E. Weiher, H. J. White, R. M. Ewers, G. M. Mace, J. P. W. Scharlemann, & A. Purvis, 2015. Global effects of land use on local terrestrial biodiversity. Nature 520: 45–50. https://doi.org/10.1038/nature14324.

Newbold, T., L. N. Hudson, H. R. P. Phillips, S. L. L. Hill, S. Contu, I. Lysenko, A. Blandon, S. H. M. Butchart, H. L. Booth, J. Day, A. De Palma, M. L. K. Harrison, L. Kirkpatrick, E. Pynegar, A. Robinson, J. Simpson, G. M. Mace, J. P. W. Scharlemann, & A. Purvis, 2014. A global model of the response of tropical and sub-tropical forest biodiversity to anthropogenic pressures. Proceedings of the Royal Society B: Biological Sciences 281: 20141371. https://doi.org/10.1098/rspb.2014.1371.

Nickus, U., K. Bishop, M. Erlandsson, C. D. Evans, M. Forsius, H. Laudon, D. M. Livingstone, D. Monteith, & H. Thies, 2010. Direct impacts of climate change on freshwater ecosystems. In Climate change impacts on freshwater ecosystems. pp. 38–64.

Nierop, K. G. J. J., B. Jansen, & J. M. Verstraten, 2002. Dissolved organic matter, aluminium and iron interactions: precipitation induced by metal/carbon ratio, pH and competition. Science of The Total Environment 300: 201–211. https://doi.org/https://doi.org/10.1016/S0048-9697(02)00254-1.

Niu, X., H. Wang, T. Wang, P. Zhang, H. Zhang, H. Wang, X. Kong, S. Xie, & J. Xu, 2024. The combination of multiple environmental stressors strongly alters microbial community assembly in aquatic ecosystems. Journal of Environmental Management 350: 119594.

https://doi.org/https://doi.org/10.1016/j.jenvman.2023.119594.

Niyogi, D. K., C. A. Cheatham, W. H. Thomson, & J. M. Christiansen, 2009. Litter breakdown and fungal diversity in a stream affected by mine drainage. Fundamental and Applied Limnology 175: 39–48. https://doi.org/10.1127/1863-9135/2009/0175-0039.

Niyogi, D. K., J. S. Harding, & K. S. Simon, 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.

Niyogi, D. K., W. M. Lewis, D. M. McKnight, K. D. Nyogi, W. M. Lewis, & D. M. McKnight, 2001. Litter breakdown in mountain streams affected by mine drainage: Biotic mediation of abiotic controls. Ecological Applications 11: 506–516. https://doi.org/10.1890/1051-0761(2001)011[0506:LBIMSA]2.0.CO;2.

Niyogi, D. K., D. M. Mcknight, W. Lewis Jr., & W. M. Lewis, 2002a. Effects of mine drainage on breakdown of aspen litter in mountain streams. Water, Air and Soil Pollution: Focus 2: 329–341. https://doi.org/10.1023/A:1020131414738.

Niyogi, D. K., D. M. McKnight, & W. M. Lewis, 2002b. Fungal communities and biomass in mountain streams affected by mine drainage. Archiv fur Hydrobiologie 155: 255–271. https://doi.org/10.1127/archiv-hydrobiol/155/2002/255.

Niyogi, D. K., K. S. Simon, & C. R. Townsend, 2003. Breakdown of tussock grass in streams along a gradient of agricultural development in New Zealand. Freshwater Biology 48: 1698–1708. https://doi.org/10.1046/J.1365-2427.2003.01104.X.

Novotny, V., 1999. Diffuse pollution from agriculture — A worldwide outlook. Water Science and Technology 39: 1–13. https://doi.org/https://doi.org/10.1016/S0273-1223(99)00027-X.

Nsenga Kumwimba, M., J. Huang, M. Dzakpasu, K. De Silva, O. E. Ohore, F. O. Ajibade, X. Li, S. Jingjun, D. K. Muyembe, & H. Kaixuan, 2023. An updated review of the efficacy of buffer zones in warm/temperate and cold climates: Insights into processes and drivers of nutrient retention. Journal of Environmental Management 336: 117646. https://doi.org/https://doi.org/10.1016/j.jenvman.2023.117646.

Nunes, J. ., 1999. A actividade vulcânica na Ilha do Pico do Plistocénio Superior ao Holocénio: mecanismo eruptivo e hazard vulcânico. Universidade dos Açores, Ponta Delgada.

Nyberg, P., & T. Eriksson, 2001. Buffer zones along streams. The Swedish board of fisheries, Gothenburg, Sweden.

Osborne, L. L., & D. A. Kovacic, 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. Freshwater Biology 29: 243–258. https://doi.org/https://doi.org/10.1111/j.1365-2427.1993.tb00761.x.

Ostrofsky, M. L., 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. Journal of the North American Benthological Society 16: 750–759. https://doi.org/10.2307/1468168.

Park, S.-R., S. Kim, & S.-W. Lee, 2021. Evaluating the relationships between riparian land cover characteristics and biological integrity of streams using random forest algorithms. International journal of environmental research and public health 18: 3182. https://doi.org/10.3390/ijerph18063182.

Pascoal, C., & F. Cássio, 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Applied and Environmental Microbiology 70: 5266–5273. https://doi.org/10.1128/AEM.70.9.5266-5273.2004.

Pascoal, C., F. Cássio, & L. Marvanová, 2005. Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. Archiv fur Hydrobiologie 162: 481–496. https://doi.org/10.1127/0003-9136/2005/0162-0481.

Pascoal, C., M. Pinho, F. Cássio, & P. Gomes, 2003. Assessing structural and functional ecosystem condition using leaf breakdown: Studies on a polluted river. Freshwater Biology 48: 2033–2044. https://doi.org/10.1046/j.1365-2427.2003.01130.x.

Pazianoto, L. H. R., A. Solla, & V. Ferreira, 2019. Leaf litter decomposition of sweet chestnut is affected more by oomycte infection of trees than by water temperature. Fungal Ecology 41: 269–278. https://doi.org/https://doi.org/10.1016/j.funeco.2019.07.005.

Pedro, L. G., P. A. G. Santos, J. A. Da Silva, A. C. Figueiredo, J. G. Barroso, S. G. Deans, A. Looman, & J. J. C. Scheffer, 2001. Essential oils from Azorean *Laurus azorica*. Phytochemistry 57: 245–250. https://doi.org/10.1016/S0031-9422(00)00497-0.

Pereira, A., P. Geraldes, E. Lima-Fernandes, I. Fernandes, F. Cássio, & C. Pascoal, 2016. Structural and functional measures of leaf-associated invertebrates and fungi as predictors of stream eutrophication. Ecological Indicators 69: 648–656. https://doi.org/10.1016/j.ecolind.2016.05.017.

Peters, K., M. Bundschuh, & R. B. Schäfer, 2013. Review on the effects of toxicants on freshwater ecosystem functions. Environmental Pollution 180: 324–329. https://doi.org/10.1016/j.envpol.2013.05.025.

Pierri, D., & M. Czop, 2020. Iron index as an organic matter decay intensity indicator in a shallow groundwater system highly contaminated with phenol (case study in northern Poland). Environmental Earth Sciences 79: 287. https://doi.org/10.1007/s12665-020-09042-y.

Piscart, C., R. Genoel, S. Doledec, E. Chauvet, & P. Marmonier, 2009. Effects of intense agricultural practices on heterotrophic processes in streams. Environmental Pollution 157: 1011–1018. https://doi.org/10.1016/J.ENVPOL.2008.10.010.

Porteiro, J., 2000. Lagoas dos Açores: elementos de suporte ao planeamento integrado. University of Azores.

Pozo, J., E. González, J. R. Díez, J. Molinero, & A. Elósegui, 1997. Inputs of particulate organic matter to streams with different riparian vegetation. Journal of the North American Benthological

Society 16: 602–611. https://doi.org/10.2307/1468147.

Prada, S. N., M. O. Silva, & J. V. Cruz, 2005. Groundwater behaviour in Madeira, volcanic island (Portugal). Hydrogeology Journal 13: 800–812. https://doi.org/10.1007/S10040-005-0448-3.

Pradhan, A., S. Seena, C. Pascoal, & F. Cássio, 2011. Can metal nanoparticles be a threat to microbial decomposers of plant litter in streams? Microbial Ecology 62: 58–68. https://doi.org/10.1007/S00248-011-9861-4/FIGURES/5.

Pu, G., J. Tong, A. Su, X. Ma, J. Du, Y. Lv, & X. Tian, 2014. Adaptation of microbial communities to multiple stressors associated with litter decomposition of *Pterocarya stenoptera*. Journal of Environmental Sciences 26: 1001–1013. https://doi.org/https://doi.org/10.1016/S1001-0742(13)60542-2.

Quaternaire-Portugal, 2008. O plano regional de ordenamento do territorio dos Açores. Secretaria Regional do Ambiente e Mar, Ponta Delgada, Portugal.

Quinn, J. M., A. B. Cooper, M. J. Stroud, & G. P. Burrell, 1997. Shade effects on stream periphyton and invertebrates: An experiment in streamside channels. New Zealand Journal of Marine and Freshwater Research 31: 665–683. https://doi.org/10.1080/00288330.1997.9516797.

Quinn, J. M., B. J. Smith, G. P. Burrell, & S. M. Parkyn, 2000. Leaf litter characteristics affect colonisation by stream invertebrates and growth of *Olinga feredayi* (Trichoptera: Conoesucidae). New Zealand Journal of Marine and Freshwater Research 34: 273–287. https://doi.org/10.1080/00288330.2000.9516932.

Quintaneiro, C., J. F. Ranville, & A. J. A. Nogueira, 2016. Physiological effects of essential metals on two detritivores: *Atyaephyra desmarestii* (Millet) and *Echinogammarus meridionalis* (Pinkster). Environmental Toxicology and Chemistry 35: 1442–1448. https://doi.org/10.1002/etc.3284.

Quintela, A., S. F. P. Almeida, D. Terroso, E. Ferreira, D. A. Silva, V. Forjaz, & F. Rocha, 2013. Diatom assemblages of thermal and mineral waters from volcanic environments in São Miguel

Island, Azores. Diatom Research 28: 407–417. https://doi.org/10.1080/0269249X.2013.822833.

Quinton, J. N., & J. A. Catt, 2007. Enrichment of heavy metals in sediment resulting from soil erosion on agricultural fields. Environmental Science & Technology 41: 3495–3500. https://doi.org/10.1021/es062147h.

Ramos, S. M., M. A. S. Graça, & V. Ferreira, 2021. A comparison of decomposition rates and biological colonization of leaf litter from tropical and temperate origins. Aquatic Ecology 55: 925–940. https://doi.org/10.1007/S10452-021-09872-3/FIGURES/5.

Raposeiro, P. M., & A. C. Costa, 2009. Benthic macroinvertebrate based indices for assessing the ecological status of freshwaters on oceanic islands. Arquipélago. Life and Marine Sciences 26: 15–24.

Raposeiro, P. M., A. C. Costa, & V. Gonçalves, 2014a. Qualidade ecológica das ribeiras Açorianas. Boletim do Núcleo Cultural da Horta 23: 95–113.

Raposeiro, P. M., A. C. Costa, S. J. Hughes, A. Cristina Costa, & S. J. Hughes, 2011. Environmental factors-spatial and temporal variation of chironomid communities in oceanic island streams (Azores archipelago). Annales de Limnologie - International Journal of Limnology 47: 325–338. https://doi.org/10.1051/limn/2011048.

Raposeiro, P. M., A. M. Cruz, S. J. Hughes, A. Cristina Costa, & A. C. Costa, 2012. Azorean freshwater invertebrates: Status, threats and biogeographic notes. Limnetica 31: 13–22.

Raposeiro, P. M., V. Ferreira, G. Gea, V. Gonçalves, P. M. Raposeiro, V. Ferreira, G. Gea, & V. Gonçalves, 2018. Contribution of aquatic shredders to leaf litter decomposition in Atlantic island streams depends on shredder density and litter quality. CSIRO. Marine and Freshwater Research 69: 1432–1439. https://doi.org/10.1071/MF18020.

Raposeiro, P. M., A. Hernández, S. Pla-Rabes, V. Gonçalves, R. Bao, A. Sáez, T. Shanahang, M. Benaventec, E. J. Boer, N. Richter, V. Gordon, H. Marques, P. M. Sousa, M. Souto, M. G. Matias,

N. Aguiar, C. Pereira, C. Ritter, M. J. Rubio, M. Salcedo, O. Vázquez-Loureiro, David Margalefd, L. A. Amaral-Zettler, Y. Costa, Ana Cristina Huangi, J. F. N. Van Leeuwen, P. Masqué, R. Prego, A. C. Ruiz-Fernández, J.-A. Sanchez-Cabeza, R. Trigo, & S. Giralt, 2021. Climate change facilitated the early colonization of the Azores Archipelago during medieval times. Proceedings of the National Academy of Sciences 118: e2108236118.

Raposeiro, P. M., S. J. Hughes, & A. C. Costa, 2009. Chironomidae (diptera: Insecta) in Oceanic islands: New records for the Azores and biogeographic notes. Annales de Limnologie 45: 59–67. https://doi.org/10.1051/limn/2009012.

Raposeiro, P. M., S. J. Hughes, & A. C. Costa, 2013. Environmental drivers - Spatial and temporal variation of macroinvertebrate communities in island streams: The case of the Azores Archipelago. Fundamental and Applied Limnology 182: 337–350. https://doi.org/10.1127/1863-9135/2013/0384.

Raposeiro, P. M., G. M. Martins, I. Moniz, A. Cunha, A. C. Costa, & V. Gonçalves, 2014b. Leaf litter decomposition in remote oceanic islands: The role of macroinvertebrates vs. microbial decomposition of native vs. exotic plant species. Limnologica 45: 80–87. https://doi.org/https://doi.org/10.1016/j.limno.2013.10.006.

Rasmussen, J. J., P. Wiberg-Larsen, A. Baattrup-Pedersen, R. J. Monberg, & B. Kronvang, 2012. Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. Science of The Total Environment 416: 148–155. https://doi.org/https://doi.org/10.1016/j.scitotenv.2011.11.057.

Resh, V. H., 2008. Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. Environmental Monitoring and Assessment 138: 131–138. https://doi.org/10.1007/s10661-007-9749-4.

Richardson, J. S., & R. D. D. Moore, 2010. Stream and riparian ecology. In Compendium of forest hydrology and geomorphology in British Columbia. pp. 441–460.

Riipinen, M. P., J. Davy-Bowker, & M. Dobson, 2009. Comparison of structural and functional stream assessment methods to detect changes in riparian vegetation and water pH. Freshwater Biology 54: 2127-2138. https://doi.org/10.1111/J.1365-2427.2008.01964.X.

Riipinen, M. P., T. Fleituch, S. Hladyz, G. Woodward, P. Giller, & M. Dobson, 2010. Invertebrate community structure and ecosystem functioning in European conifer plantation streams. Freshwater Biology 55: 346–359. https://doi.org/10.1111/J.1365-2427.2009.02278.X.

Riis, T., M. Kelly-Quinn, F. C. Aguiar, P. Manolaki, D. Bruno, M. D. Bejarano, N. Clerici, M. R. Fernandes, J. C. Franco, N. Pettit, A. P. Portela, O. Tammeorg, P. Tammeorg, P. M. Rodríguez-González, & S. Dufour, 2020. Global overview of ecosystem services provided by riparian vegetation. BioScience 70: 501–514. https://doi.org/10.1093/biosci/biaa041.

Rosa, J. S., C. Mascarenhas, L. Oliveira, T. Teixeira, M. C. Barreto, & J. Medeiros, 2010. Biological activity of essential oils from seven Azorean plants against *Pseudaletia unipuncta* (Lepidoptera: Noctuidae). Journal of Applied Entomology 134: 346–354. https://doi.org/10.1111/J.1439-0418.2009.01483.X.

Roussel, H., E. Chauvet, & J. M. Bonzom, 2008. Alteration of leaf decomposition in copper-contaminated freshwater mesocosms. Environmental Toxicology and Chemistry 27: 637–644. https://doi.org/10.1897/07-168.1.

Rowe, J., S. Meegan, S. Engstrom, S. Perry, & W. Perry, 1996. Comparison of leaf processing rates under different temperature regimes in three headwater streams. Freshwater Biology 36: 277–288.

Rueda-Delgado, G., K. M. Wantzen, & M. B. Tolosa, 2006. Leaf-litter decomposition in an Amazonian floodplain stream: Effects of seasonal hydrological changes. Journal of the North American Benthological Society 25: 233–249. https://doi.org/10.1899/0887-3593(2006)25[233:LDIAAF]2.0.CO;2.

Rugenski, A. T., G. W. Minshall, & F. R. Hauer, 2017. Chapter 28 - Riparian processes and interactions. In Lamberti, G. A., & F. R. Hauer (eds) Methods in stream ecology (Third edition). Academic Press. pp. 83–111.

Rull, V., A. Lara, M. J. Rubio-Inglés, S. Giralt, V. Gonçalves, P. Raposeiro, A. Hernández, G. Sánchez-López, D. Vázquez-Loureiro, R. Bao, P. Masqué, & A. Sáez, 2017. Vegetation and landscape dynamics under natural and anthropogenic forcing on the Azores Islands: A 700-year pollen record from the São Miguel Island. Quaternary Science Reviews 159: 155–168. https://doi.org/10.1016/J.QUASCIREV.2017.01.021.

Run, L., P. Yueting, C. Siyuan, S. Jiachen, L. Yunchao, Z. Shuiyun, & T. Xingjun, 2022. Effect of metal pollution from mining on litter decomposition in streams. Environmental Pollution 296: 118698. https://doi.org/10.1016/J.ENVPOL.2021.118698.

Russell, J. C., J.-Y. Meyer, N. D. Holmes, & S. Pagad, 2017. Invasive alien species on islands: impacts, distribution, interactions and management. Environmental Conservation 44: 359–370. https://doi.org/10.1017/S0376892917000297.

Sabater, S., & K. Tockner, 2010. Effects of hydrologic alterations on the ecological quality of river ecosystems BT - Water scarcity in the Mediterranean: Perspectives under global change. In Sabater, S., & D. Barceló (eds). Springer Berlin Heidelberg, Berlin, Heidelberg. pp. 15–39.

Sakai, M., Y. Natuhara, K. Fukushima, A. Imanishi, K. Imai, & M. Kato, 2013. Ecological functions of persistent Japanese cedar litter in structuring stream macroinvertebrate assemblages. Journal of Forest Research 18: 190–199. https://doi.org/10.1007/S10310-012-0339-0/METRICS.

Sánchez-Ortiz, K., K. J. M. Taylor, A. De Palma, F. Essl, W. Dawson, H. Kreft, J. Pergl, P. Pyšek, M. van Kleunen, P. Weigelt, & A. Purvis, 2020. Effects of land-use change and related pressures on alien and native subsets of island communities. PLOS ONE 15: 1–19. https://doi.org/10.1371/journal.pone.0227169.

Sandin, L., & A. G. Solimini, 2009. Freshwater ecosystem structure–function relationships: from theory to application. Freshwater Biology 54: 2017–2024. https://doi.org/https://doi.org/10.1111/j.1365-2427.2009.02313.x.

Santos, F. D., M. A. Valente, P. M. Miranda, A. C. Barbosa Aguiar, M. A. Valente, P. M. A.

Miranda, A. Aguiar, E. B. Azevedo, & A. R. Tomé, 2004. Climate change scenarios in the Azores and Madeira. World Resource Review 16: 473–491.

Santos, J. I., T. Vidal, F. J. M. Gonçalves, B. B. Castro, & J. L. Pereira, 2021. Challenges to water quality assessment in Europe – Is there scope for improvement of the current Water Framework Directive bioassessment scheme in rivers? Ecological Indicators 121: 107030. https://doi.org/https://doi.org/10.1016/j.ecolind.2020.107030.

Scarsbrook, M. R., & J. Halliday, 1999. Transition from pasture to native forest land-use along stream continua: Effects on stream ecosystems and implications for restoration. New Zealand Journal of Marine and Freshwater Research 33: 293–310.

Schaller, J., A. Weiske, M. Mkandawire, & E. G. Dudel, 2008. Enrichment of uranium in particulate matter during litter decomposition affected by *Gammarus pulex* L. Environmental Science & Technology 42: 8721–8726. https://doi.org/10.1021/es801456q.

Schlief, J., & M. Mutz, 2006. Palatability of leaves conditioned in streams affected by mine drainage: A feeding experiment with *Gammarus pulex* (L.). Hydrobiologia 2006 563:1 563: 445–452. https://doi.org/10.1007/S10750-006-0028-0.

Schmidt-Kloiber, A., & D. Hering, 2015. www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecological Indicators 53: 271–282.

Schoonover, J. E., K. W. J. Williard, J. J. Zaczek, J. C. Mangun, & A. D. Carver, 2005. Nutrient attenuation in agricultural surface runoff by riparian buffer zones in Southern Illinois, USA. Agroforestry Systems 64: 169–180. https://doi.org/10.1007/s10457-004-0294-7.

Schopka, H. H., & L. A. Derry, 2012. Chemical weathering fluxes from volcanic islands and the importance of groundwater: The Hawaiian example. Earth and Planetary Science Letters 339–340: 67–78. https://doi.org/10.1016/J.EPSL.2012.05.028.

Sedell, J. R., F. J. Triska, & N. S. Triska, 1975. The processing of conifer and hardwood leaves in two coniferous forest streams: Weight loss and associated invertebrates. Internationale Vereinigung Für Theoretische Und Angewandte Limnologie 19: 1617–1627. https://doi.org/10.1080/03680770.1974.11896227.

Shen, K., C. Shen, Y. Lu, X. Tang, C. Zhang, X. Chen, J. Shi, Q. I. Lin, & Y. Chen, 2009. Hormesis response of marine and freshwater luminescent bacteria to metal exposure. Biological Research 42: 183–187.

Silva-Junior, E. F. D., 2016. Land use effects and stream metabolic rates: A review of ecosystem response. Acta Limnologica Brasiliensia 28: 1–10.

Silva, L., & C. W. Smith, 2004. A characterization of the non-indigenous flora of the Azores Archipelago. Biological Invasions 6: 193–204. https://doi.org/10.1023/B:BINV.0000022138.75673.8C/METRICS.

Simões, S., A. L. Gonçalves, J. M. Canhoto, G. Gonçalves, & C. Canhoto, 2021. Eucalyptus spp. leaf traits determine litter processing by fungi and invertebrates. Freshwater Biology 66: 968–977. https://doi.org/10.1111/FWB.13690.

Singh, S., S. Inamdar, M. Mitchell, & P. McHale, 2014. Seasonal pattern of dissolved organic matter (DOM) in watershed sources: influence of hydrologic flow paths and autumn leaf fall. Biogeochemistry 118: 321–337. https://doi.org/10.1007/s10533-013-9934-1.

Singh, S., & L. M. Mosley, 2003. Trace metal levels in drinking water on Viti Levu, Fiji Islands. The South Pacific Journal of Natural Science 21: 31–34.

Sinsabaugh, R. L., T. Weiland, & A. E. Linkins, 1992. Enzymic and molecular analysis of microbial communities associated with lotic particulate organic matter. Freshwater Biology 28: 393–404.

Smith, G. C., A. P. Covich, & A. M. D. Brasher, 2003. An ecological perspective on the biodiversity of Tropical island streams. Oxford Academic. , 1048–1051.

Smith, V. H., G. D. Tilman, & J. C. Nekola, 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100: 179–196. https://doi.org/https://doi.org/10.1016/S0269-7491(99)00091-3.

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

SRAM/DROTRH, 2007. Carta de ocupação do solo da Região Autónoma dos Açores. Secretaria Regional do Ambiente e do Mar, Direção Regional do Ordenamento do Território e dos Recursos Hídricos. Ponta Delgada, Portugal.

Sridhar, K. R., & F. Bärlocher, 2011. Reproduction of aquatic hyphomycetes at low concentrations of Ca2+, Zn2+, Cu2+, and Cd2+. Environmental Toxicology and Chemistry 30: 2868–2873. https://doi.org/https://doi.org/10.1002/etc.697.

Sridhar, K. R., F. Bärlocher, G. J. G. Krauss, & G. J. G. Krauss, 2005. Response of aquatic hyphomycete communities to changes in heavy metal exposure. International Review of Hydrobiology 90: 21–32. https://doi.org/10.1002/iroh.200410736.

Sridhar, K. R., F. Bärlocher, R. Wennrich, G. J. Krauss, & G. Krauss, 2008. Fungal biomass and diversity in sediments and on leaf litter in heavy metal contaminated waters of Central Germany. Fundamental and Applied Limnology 171: 63–74. https://doi.org/10.1127/1863-9135/2008/0171-0063.

Sridhar, K. R., G. Krauss, F. Bärlocher, N. S. Raviraja, R. Wennrich, R. Baumbach, & G. J. Krauss, 2001. Decomposition of alder leaves in two heavy metal-polluted streams in central Germany. Aquatic Microbial Ecology 26: 73–80. https://doi.org/10.3354/AME026073.

Steibl, S., & C. Laforsch, 2019. Disentangling the environmental impact of different human disturbances: A case study on islands. Scientific Reports 9: 13712. https://doi.org/10.1038/s41598-019-49555-6.

Studinski, J. M., K. J. Hartman, J. M. Niles, & P. Keyser, 2012. The effects of riparian forest disturbance on stream temperature, sedimentation, and morphology. Hydrobiologia 686: 107–117.

Stutter, M., B. Kronvang, D. Ó hUallacháin, & J. Rozemeijer, 2019. Current insights into the effectiveness of riparian management, attainment of multiple benefits, and potential technical enhancements. Journal of Environmental Quality 48: 236–247. https://doi.org/https://doi.org/10.2134/jeq2019.01.0020.

Suseela, V., N. Tharayil, B. Xing, & J. S. Dukes, 2014. Warming alters potential enzyme activity but precipitation regulates chemical transformations in grass litter exposed to simulated climatic changes. Soil Biology and Biochemistry 75: 102–112. https://doi.org/https://doi.org/10.1016/j.soilbio.2014.03.022.

Tachet, H. P., P. Richoux, M. Bournaud, & P. Usseglio-Polatera, 2000. Invertébrés d'eau douce: Systématique, biologie, écologie. CNRS Editions, Paris.

Tagliaferro, M., A. M. M. Gonçalves, M. Bergman, O. Sobral, & M. A. S. Graça, 2018. Assessment of metal exposure (uranium and copper) by the response of a set of integrated biomarkers in a stream shredder. Ecological Indicators 95: 991–1000. https://doi.org/10.1016/J.ECOLIND.2017.10.065.

Tank, J. L., & J. R. Webster, 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology 79: 2168–2179.

Tant, C. J., A. D. Rosemond, A. M. Helton, & M. R. First, 2015. Nutrient enrichment alters the magnitude and timing of fungal, bacterial, and detritivore contributions to litter breakdown. Freshwater Science 34: 1259–1271. https://doi.org/10.1086/683255.

Taylor, B. R., & E. E. Chauvet, 2014. Relative influence of shredders and fungi on leaf litter decomposition along a river altitudinal gradient. Hydrobiologia 721: 239–250.

Terroso, D., E. Ferreira da Silva, C. Patinha, F. Rocha, V. Forjaz, & A. Santos, 2006. Hydrogeochemistry of thermal spring and caldera waters in São Miguel Island (Azores, Portugal) and possible applications in Spa treatments. Metal Ions in Biology and Medicine 9: 78–84.

Thompson, M., S. L. R. Ellison, & R. Wood, 2002. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure and Applied Chemistry 74: 835–855. https://doi.org/10.1351/PAC200274050835/MACHINEREADABLECITATION/RIS.

Tolkkinen, M. J., J. Heino, S. H. K. K. Ahonen, K. Lehosmaa, & H. Mykrä, 2020. Streams and riparian forests depend on each other: A review with a special focus on microbes. Forest Ecology and Management 462: 117962. https://doi.org/10.1016/J.FORECO.2020.117962.

Toreti, A., P. Naveau, M. Zampieri, A. Schindler, E. Scoccimarro, E. Xoplaki, H. A. Dijkstra, S. Gualdi, & J. Luterbacher, 2013. Projections of global changes in precipitation extremes from Coupled Model Intercomparison Project Phase 5 models. Geophysical Research Letters 40: 4887–4892. https://doi.org/https://doi.org/10.1002/grl.50940.

Triantis, K. A., P. A. V Borges, R. J. Ladle, J. Hortal, P. Cardoso, C. Gaspar, F. Dinis, E. Mendonça, L. M. A Silveira, R. Gabriel, C. Melo, A. M. C Santos, I. R. Amorim, S. P. Ribeiro, A. R. M Serrano, J. A. Quartau, R. J. Whittaker K A Triantis, O. Preto, B. A. Á R M Serrano, & J. A. Quartau, 2010. Extinction debt on oceanic islands. Ecography 33: 285–294. https://doi.org/10.1111/j.1600-0587.2010.06203.x.

Truchy, A., R. A. Sponseller, F. Ecke, D. G. Angeler, M. Kahlert, M. Bundschuh, R. K. Johnson, & B. G. McKie, 2022. Responses of multiple structural and functional indicators along three contrasting disturbance gradients. Ecological Indicators 135: 108514. https://doi.org/10.1016/J.ECOLIND.2021.108514.

Tubiello, F. N., M. Salvatore, A. F. Ferrara, J. House, S. Federici, S. Rossi, R. Biancalani, R. D. Condor Golec, H. Jacobs, A. Flammini, P. Prosperi, P. Cardenas-Galindo, J. Schmidhuber, M. J.

Sanz Sanchez, N. Srivastava, & P. Smith, 2015. The contribution of agriculture, forestry and other land use activities to global warming, 1990–2012. Global Change Biology 21: 2655–2660. https://doi.org/https://doi.org/10.1111/gcb.12865.

Turner, R. E., & N. N. Rabalais, 2003. Linking landscape and water quality in the Mississippi river basin for 200 Years. BioScience 53: 563–572. https://doi.org/10.1641/0006-3568(2003)053[0563:LLAWQI]2.0.CO;2.

Vadas, R. L., R. M. Hughes, Y. J. Bae, M. J. Baek, O. C. B. Gonzáles, M. Callisto, D. R. de Carvalho, K. Chen, M. T. Ferreira, P. Fierro, J. S. Harding, D. M. Infante, C. J. Kleynhans, D. R. Macedo, I. Martins, N. M. Silva, N. Moya, S. J. Nichols, P. S. Pompeu, R. Ruaro, D. R. O. Silva, R. J. Stevenson, B. de F. Terra, C. Thirion, D. Ticiani, L. Wang, & C. O. Yoder, 2022. Assemblage-based biomonitoring of freshwater ecosystem health via multimetric indices: A critical review and suggestions for improving their applicability. Water Biology and Security 1: 100054. https://doi.org/https://doi.org/10.1016/j.watbs.2022.100054.

Valente-Neto, F., R. Koroiva, A. A. Fonseca-Gessner, & F. D. O. Roque, 2015. The effect of riparian deforestation on macroinvertebrates associated with submerged woody debris. Aquatic Ecology 49: 115–125. https://doi.org/10.1007/s10452-015-9510-y.

Vanderpoorten, A., F. J. Rumsey, & M. A. Carine, 2007. Does Macaronesia exist? Conflicting signal in the bryophyte and pteridophyte floras. American Journal of Botany 94: 625–639. https://doi.org/https://doi.org/10.3732/ajb.94.4.625.

Velastegui-Montoya, A., N. Montalván-Burbano, G. Peña-Villacreses, A. de Lima, & G. Herrera-Franco, 2022. Land use and land cover in Tropical forest: Global research. Forests 13:. https://doi.org/10.3390/f13101709.

Viaroli, S., E. Cuoco, R. Mazza, & D. Tedesco, 2016. Dynamics of natural contamination by aluminium and iron rich colloids in the volcanic aquifers of Central Italy. Environmental Science and Pollution Research 23: 19958–19977. https://doi.org/10.1007/s11356-016-7198-8.

Von Schiller, D., E. Martí, J. L. Riera, M. Ribot, J. . Marks, & F. Sabater, 2018. Influence of land use on stream ecosystem function in a Mediterranean catchment. Freshwater Biology 53: 2600–2612. https://doi.org/10.1111/j.1365-2427.2008.02059.x.

Wagai, R., M. Kajiura, & M. Asano, 2020. Iron and aluminum association with microbially processed organic matter via meso-density aggregate formation across soils: organo-metallic glue hypothesis. Soil 6: 597–627. https://doi.org/10.5194/soil-6-597-2020.

Wallace, J. B., S. L. Eggert, J. L. Meyer, & J. R. Webster, 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277: 102–104. https://doi.org/10.1126/SCIENCE.277.5322.102.

Wang, Z., L. Zhang, J. Zhao, & B. Xing, 2016. Environmental processes and toxicity of metallic nanoparticles in aquatic systems as affected by natural organic matter. Environmental Science: Nano 3: 240–255. https://doi.org/10.1039/C5EN00230C.

Wantzen, K. M., E. Drago, & C. J. Da Silva, 2005. Aquatic habitats of the Upper Paraguay River-Floodplain-System and parts of the Pantanal(Brazil). International Journal of Ecohydrology & Hydrobiology 5: 107–126.

Wantzen, K. M., C. M. Yule, J. M. Mathooko, & C. M. Pringle, 2008. Chapter 3 - Organic matter processing in Tropical streams. In Dudgeon, D. B. T.-T. S. E. (ed) Aquatic Ecology. Academic Press, London. pp. 43–64.

Webster, J. R., & J. L. Meyer, 1997. Stream organic matter budgets: An introduction. Journal of the North American Benthological Society 16: 3–13. https://doi.org/10.2307/1468223.

Whiles, M. R., & & J. B. Wallace, 1997. Leaf litter decomposition and macroinvertebrate communities in headwater streams draining pine and hardwood catchments. Hydrobiologia 353: 107–119.

Whittaker, R. J., & J. M. Fernández-Palacios, 2007. Island biogeography: ecology, evolution, and conservation. Oxford University Press.

Wild, R., B. Gücker, & M. Brauns, 2019. Agricultural land use alters temporal dynamics and the composition of organic matter in temperate headwater streams. Freshwater Science 38: 566–581. https://doi.org/10.1086/704828.

Willis, G. H., & L. L. McDowell, 1982. Pesticides in agricultural runoff and their effects on downstream water quality. Environmental Toxicology and Chemistry 1: 267–279. https://doi.org/https://doi.org/10.1002/etc.5620010402.

Wilson, B., B. Pyatt, & G. Denton, 2009. An evaluation of the bioavailability and bioaccumulation of selected metals occurring in a wetland area on the volcanic island of Guam, Western Pacific Ocean. Journal of Environmental Sciences 21: 1547–1551. https://doi.org/10.1016/S1001-0742(08)62453-5.

Wilson, M. A., & S. R. Carpenter, 1999. Economic valuation of freshwater ecosystem services in the United States: 1971–1997. Ecological Applications 9: 772–783. https://doi.org/https://doi.org/10.1890/1051-0761(1999)009[0772:EVOFES]2.0.CO;2.

Winterbourn, A. M. J., G. Hildrew, & A. Box, 1985. Structure and grazing of stone surface organic layers in some acid streams of southern England. Freshwater Biology 15: 363–374.

Withers, P. J. A., C. Neal, H. P. Jarvie, & D. G. Doody, 2014. Agriculture and eutrophication: Where do we go from here? Sustainability 6: 5853–5875. https://doi.org/10.3390/su6095853.

Woodward, G., M. O. Gessner, P. S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B. G. McKie, S. D. Tiegs, H. Cariss, M. Dobson, A. Elosegi, V. Ferreira, M. A. S. Graça, T. Fleituch, J. O. Lacoursière, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B. M. Vought, & E. Chauvet, 2012. Continental-scale effects of nutrient pollution on stream ecosystem functioning. Science 336: 1438–1440. https://doi.org/10.1126/SCIENCE.1219534/SUPPL_FILE/WOODWARD_ET_AL_DATA_TABLES_S1_AND_S2-SCIENCE.XLSX.

Wurtsbaugh, W. A., H. W. Paerl, W. K. Dodds, W. A. Wuraugh, H. W. Paerl, & W. K. Dodds, 2019. Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. Water 6: e1373. https://doi.org/https://doi.org/10.1002/wat2.1373.

Xenopoulos, M. A., R. T. Barnes, K. S. Boodoo, D. Butman, N. Catalán, S. C. D'Amario, C. Fasching, D. N. Kothawala, O. Pisani, C. T. Solomon, R. G. M. Spencer, C. J. Williams, & H. F. Wilson, 2021. How humans alter dissolved organic matter composition in freshwater: relevance for the Earth's biogeochemistry. Biogeochemistry 154: 323–348. https://doi.org/10.1007/s10533-021-00753-3.

Xu, F.-L., S. Tao, R. W. Dawson, P. Li, & J. Cao, 2001. Lake ecosystem health assessment: Indicators and methods. Water Research 35: 3157–3167. https://doi.org/https://doi.org/10.1016/S0043-1354(01)00040-9.

Xue, S. M., S. Q. Jiang, R. Z. Li, Y. Y. Jiao, Q. Kang, L. Y. Zhao, Z. hua Li, & M. Chen, 2024. The decomposition of algae has a greater impact on heavy metal transformation in freshwater lake sediments than that of macrophytes. Science of The Total Environment 906: 167752. https://doi.org/10.1016/J.SCITOTENV.2023.167752.

Young, R. G., & K. J. Collier, 2009. Contrasting responses to catchment modification among a range of functional and structural indicators of river ecosystem health. Freshwater Biology 54: 2155–2170. https://doi.org/https://doi.org/10.1111/j.1365-2427.2009.02239.x.

Young, R. G., & A. D. Huryn, 1996. Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. Canadian Journal of Fisheries and Aquatic Sciences 53: 2199–2211.

Young, R. G., A. D. Huryn, & C. R. Townsend, 1994. Effects of agricultural development on processing of tussock leaf litter in high country New Zealand streams. Freshwater Biology 32: 413–427. https://doi.org/10.1111/J.1365-2427.1994.TB01136.X.

Young, R. G., C. D. Matthaei, & C. R. Townsend, 2008. Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. Journal of the North American Benthological Society 27: 605–625. https://doi.org/10.1899/07-121.1.

Yue, K., W. Yang, B. Tan, Y. Peng, C. Huang, Z. Xu, X. Ni, Y. Yang, W. Zhou, L. Zhang, & F. Wu, 2019. Immobilization of heavy metals during aquatic and terrestrial litter decomposition in an alpine forest. Chemosphere 216: 419–427. https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.10.169.

Zhang, M., X. Cheng, Q. Geng, Z. Shi, Y. Luo, & X. Xu, 2019. Leaf litter traits predominantly control litter decomposition in streams worldwide. Global Ecology and Biogeography 28: 1469–1486. https://doi.org/10.1111/geb.12966.

Zittis, G., M. Almazroui, P. Alpert, P. Ciais, W. Cramer, Y. Dahdal, M. Fnais, D. Francis, P. Hadjinicolaou, F. Howari, A. Jrrar, D. G. Kaskaoutis, M. Kulmala, G. Lazoglou, N. Mihalopoulos, X. Lin, Y. Rudich, J. Sciare, G. Stenchikov, E. Xoplaki, & J. Lelieveld, 2022. Climate change and weather extremes in the Eastern Mediterranean and Middle East. Reviews of Geophysics 60: e2021RG000762. https://doi.org/https://doi.org/10.1029/2021RG000762.

LIST OF FIGURES

Figure 2.9. Land use cover in the watershed of the selected study streams within a 300-m radius area upstream of the sampling site (red polygon). White coloured polygons correspond to areas of adjacent basins, which were excluded. Purple polygons correspond to native vegetation; green polygons correspond to forested areas (cryptomeria plantations or exotic forest); orange polygons correspond to artificial land uses (urbanization, roads). Pasture areas were estimated by subtracting the area of other land uses and adjacent basins from the total area (red polygon). Images were acquired from Google Earth (accessed in May 2022). Nat1 – Nat3, Native streams; Crypt1 – Crypt3, Cryptomeria streams; Past1 – Past3, Pasture streams.

Figure 2.10. Ash-free dry mass (AFDM) remaining (mean ± SE) of clethra leaves enclosed in coarse- and fine-mesh bags and balsa wood enclosed in fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Figure 2.11. Mean relative contribution (across streams and dates, based on spore production) of aquatic hyphomycete taxa associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Figure 2.12. Taxa richness (A) and sporulation rates (B) of aquatic hyphomycetes (mean ± SE) associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date). Taxa richness and sporulation rates were not determined at day 63 in pasture streams due to low amount of leaf mass remaining.

Figure 2.13. Dispersion of principal coordinates analysis (PCO) done on benthic macroinvertebrate community of native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type), sampled on two dates (d0 and d63), with the main functional feeding groups responsible for

similarities within stream types. Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Figure 2.14. Shredder density (mean ± SE) on clethra leaves enclosed in coarse-mesh bags and incubated in native (Nat), cryptomeria (Crypt) and pasture (Past) streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Figure 3.1. Larvae of *Limnephilus atlanticus* used in the microcosms trials (A); larvae body and case separately, dried larvae body used at the end of the experiments to estimate growth rates (B).

Figure 3.2. Moistened leaves of *Alnus glutinosa* (A); leaf discs of *A. glutinosa* cut with corkborer (B); fine-mesh bags for leaf discs incubation (C).

Figure 3.3. Incubation sites in a stream with low metal concentration (reference stream, A); and in a stream naturally enriched with metals (metal-enriched stream, B).

Figure 3.4. Short-term trials scheme of no-choice treatment and multiple-choice treatment where larvae were exposed to reference and metal-enriched leaves at the same time.

Figure 3.5. Long-term trials scheme where larvae were exposed to reference or metal-enriched leaves of each different leaf species.

Figure 3.6. Relative consumption rates (RCR; mean ± SE, n = 15) of *L. atlanticus* individuals on each leaf species, when given a choice between the three leaf species previously exposed in a stream with low metal concentration (reference leaves; A) and in a stream naturally enriched with metals (metal-enriched leaves; B). Different letters indicate significant differences (Wilcoxon Signed Rank test, $P<0.05$). Adapted from Balibrea et al. (2023).

Figure 3.7. Relative consumption rate (RCR; mean ± SE, n = 15) of L. atlanticus individuals on each leaf species when given a choice between leaves previously exposed in a stream with low metal concentration (reference leaves) or exposed in a stream naturally enriched with metals (metal-

enriched leaves) for *A. glutinosa* (A), *I. perado* (B) and *L. azorica* (C). Different letters indicate significant differences (Wilcoxon Signed Rank test, $P < 0.05$). Adapted from Balibrea et al. (2023).

Figure 3.8. Relative consumption rate (RCR; A) and relative growth rate (RGR; B) (mean ± SE, n = 10) of L. atlanticus individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves). Different letters indicate significant differences (three-way repeated measures ANOVA followed by Fisher's test, $P<0.05$). Adapted from Balibrea et al. (2023).

Figure 3.9. Relative consumption rate (RCR; A) and relative growth rate (RGR; B) (mean ± SE, n = 10) of L. atlanticus individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves). Adapted from Balibrea et al. (2023).

Figure 4.1. Moistened leaves of *Clethra arborea* (A); leaf discs of *C. arborea* cut with corkborer (B); mesh bags for leaf discs incubation (C).

Figure 4.2. Incubation sites in the stream with low metal concentration (reference stream, A) and in a stream naturally enriched with metals (metal-enriched stream, B). Photos were taken on 1st February 2022.

Figure 4.3. Leaf discs incubated in metal- enriched and reference stream after incubation period (A); microcosms consisted of Erlenmeyer flasks with different stream water treatments and leaf discs incubated in metal-enriched and reference streams (B); microcosms deployed in an orbital shaker with continue agitation inside an environmental test chamber (C).

Figure 4.4. Leaf discs deployed in pre-weighed pans at the end of each sampling dates for oven-dried inside and oven (A); leaf discs inside a muffle for ignition process (B); ashes from metal-enriched and reference leaf discs after ignition (C).

Figure 4.5. Conidial suspension from each microcosm fixed with 37% formalin and storage inside graduated tubes (A); system for filtering aliquots of conidial suspension with cellulose nitrate filters (B); filters stained with cotton blue in lactic acid for microscope slides preparation for spores counting and identification under microscope (C).

Figure 4.6. Ash-free dry mass (AFDM) remaining (mean ± SE, n=4) of clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) after 15, 43 and 90 days.

Figure 4.7. Ash-free dry mass (AFDM) remaining (mean ± SE, n=4) of clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) at the end of the experiment (day 90).

Figure 4.8. Sporulation rates and taxa richness of aquatic hyphomycetes (mean ± SE, n=3) associated with clethra leaf discs across two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) after 15, 43 and 90 days.

Figure 4.9. Mean relative contribution (based on spore production, across the three sampling dates) of aquatic hyphomycete taxa associated with clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) across six water treatments (0%, 10%, 25%, 50%, 75% and 100%). Only species contributing at least 2% to total conidial production in at least one treatment are shown; rare species are included within “Others”.

LIST OF TABLES

parameters values are mean ± SE of each stream site and date (n=5). For nutrient concentration samples were only collected at one date. For metal concentration values are mean± SE of each stream site and date (n=2). TDS, total dissolved solids

Table 4.2. Metal concentrations (mean ± SE, n = 3) in leaves before incubation period and after incubation (two weeks) in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves).

SUPPLEMENTARY MATERIAL

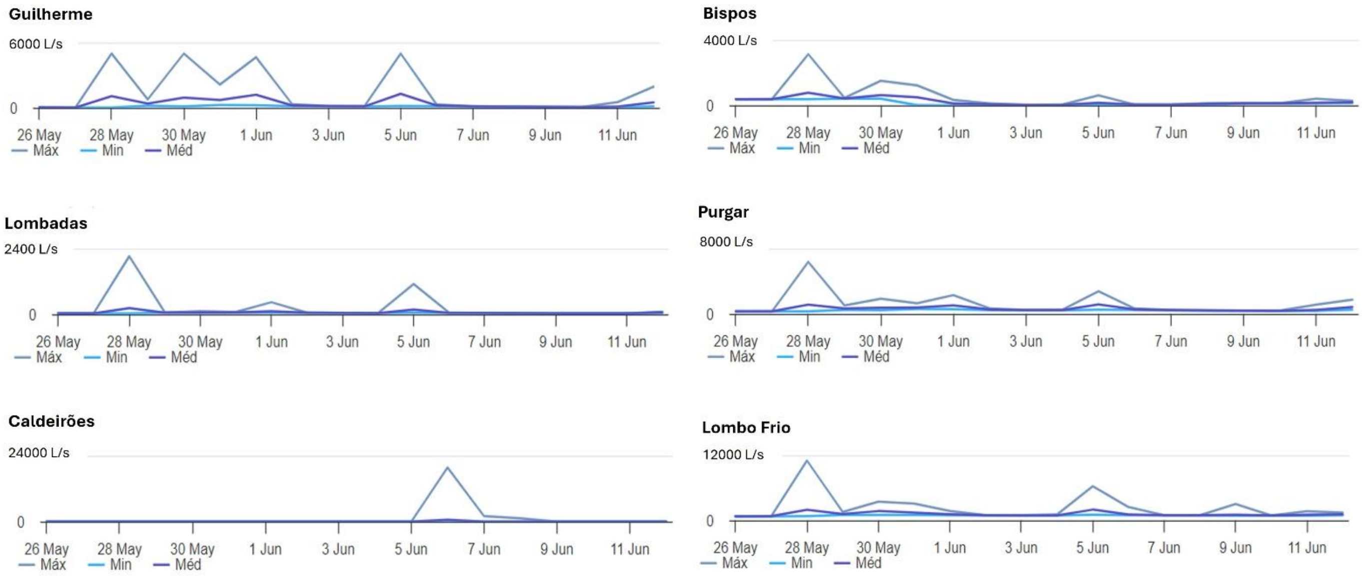

Figure S1. Stream flow (L/s) diagrams obtained from hydrometric stations located in streams in the study area for the period 26th May–11th June, 2022. Guilherme station was located below sites Nat1 and Nat2; Lombadas station was in a tributary of Nat3; Caldeirões station was in middle elevation below Crypt1, Crypt2 and Crypt3 sites; and Bispos, Purgar and Lombo Frio stations were located above sites Past1, Past2 and Past3, respectively.

Figure S2. Detail of *I. perado* cuticle detached from the leaf mesophyll and larvae of *L. atlanticus* feeding directly on the leaf mesophyll.

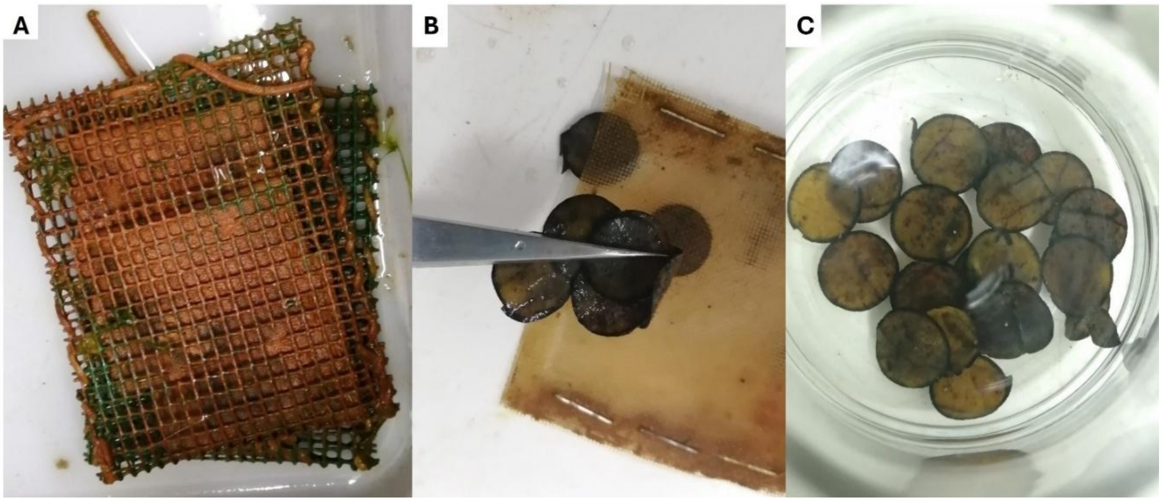

Figure S3. Leaf discs incubated in a metal-enriched stream: bags holding leaf discs after two weeks incubation in the metal-enriched stream (A), metal-enriched leaf discs after two weeks incubation in the stream (B) and metal-enriched leaf discs exposed to 10% water treatment at the middle of the microcosms trial(C).

Table S1. Physical and chemical characteristics of the stream water during the experiment (9th May–12th July, 2022). Values are mean ± SE of each stream (n=5, except for water temperature where n=63). Nat1 – Nat3, native streams; Crypt1 – Crypt3, cryptomeria streams; Past1 – Past3, pasture streams. TDS, total dissolved solids.

Stream	Temp. (°C)	pH	Cond. (µS/cm)	TDS (mg/L)	N-total (µg/L)	NO_3^- (µg/L)	NH_3+NH_4^+ (µg/L)	P_2O_5 (µg/L)
Nat1	14.3 ± 0.2	7.9 ± 0.0	81.7 ± 2.2	40.8 ± 1.0	559.6 ± 105.0	40.2 ± 24.8	26.4 ± 2.9	95.8 ± 3.0
Nat2	15.0 ± 0.5	7.5 ± 0.1	55.5 ± 2.9	27.5 ± 1.5	565.8 ± 104.8	24.8 ± 16.7	23.8 ± 3.4	55.6 ± 21.7
Nat3	14.6 ± 0.6	5.8 ± 0.0	120.2 ± 3.4	60.1 ± 1.6	566.6 ± 103.4	186.0 ± 57.6	20.6 ± 4.2	93.0 ± 7.0
Crypt1	12.7 ± 0.4	7.0 ± 0.1	52.6 ± 2.9	26.2 ± 1.6	567.8 ± 102.6	38.8 ± 7.3	18.0 ± 3.3	31.4 ± 6.3
Crypt2	13.0 ± 0.5	7.5 ± 0.1	56.0 ± 3.1	28.4 ± 1.6	569.8 ± 102.6	15.8 ± 4.1	15.2 ± 5.2	38.2 ± 3.6
Crypt3	13.1 ± 0.6	7.6 ± 0.1	52.8 ± 2.8	26.4 ± 1.4	572.2 ± 101.5	17.8 ± 13.9	22.2 ± 8.6	20.6 ± 3.5
Past1	15.4 ± 0.4	8.0 ± 0.1	136.6 ± 4.4	68.4 ± 2.1	569.3 ± 102.0	256.8 ± 74.4	23.8 ± 8.8	43.7 ± 3.6
Past2	15.7 ± 0.6	8.1 ± 0.1	118.6 ± 4.0	59.2 ± 2.1	560.1 ± 102.5	979.0 ± 177.0	25.3 ± 7.1	147.8 ± 8.7
Past3	15.6 ± 0.4	7.7 ± 0.1	135.1 ± 4.7	67.4 ± 2.4	557.8 ± 98.7	1031.1 ± 423.2	27.7 ± 7.3	190.0 ± 30.0

Table S2. Summary table of two-level nested ANOVA performed on chemical and physical water characteristics of native, cryptomeria and pasture streams (n=3 streams per type).

Water variable	Source	df	MS	F	p
Temperature	Intercept	1	9293.65	8049.65	< 0.001
	Stream (Stream type)	6	0.34	0.30	0.935
	Stream type	2	27.22	23.58	< 0.001
	Error	36	1.15		
pH	Intercept	1	2508.28	109760.76	< 0.001
	Stream (Stream type)	6	2.46	107.47	< 0.001
	Stream type	2	3.11	136.16	< 0.001
	Error	36	0.02		
Conductivity	Intercept	1	363690.45	6051.14	< 0.001
	Stream (Stream type)	6	1937.53	32.24	< 0.001
	Stream type	2	22020.45	366.38	< 0.001
	Error	36	60.10		
TDS	Intercept	1	90855.2	5942.56	< 0.001
	Stream (Stream type)	6	492.75	32.23	< 0.001
	Stream type	2	5466.20	357.53	< 0.001
	Error	36	15.29		
N-total	Intercept	1	14387643.42	273.42	< 0.001
	Stream (Stream type)	6	94.98	0	1.000
	Stream type	2	234.95	0	0.996
	Error	36	52620.45		
NO_3	Intercept	1	3809655.66	31.19	< 0.001
	Stream (Stream type)	6	330920.64	2.71	0.028
	Stream type	2	2446965.92	20.03	< 0.001
	Error	36	122155.30		
NH_3+NH_4^+	Intercept	1	22897.95	125.02	< 0.001
	Stream (Stream type)	6	41.23	0.23	0.966
	Stream type	2	202.96	1.11	0.341
	Error	36	183.16		
P_2O_5	Intercept	1	284840.71	324.76	< 0.001
	Stream (Stream type)	6	10427.75	11.89	< 0.001
	Stream type	2	35392.58	40.35	< 0.001
	Error	36	877.09		

Table S3. Summary table for four-level nested ANOVA performed on the proportion of ash-free dry mass remaining of clethra leaves enclosed in fine- and coarse-mesh bags and balsa wood enclosed in fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Source of variation	df	MS	F	p
Intercept	1	1506610	1503	<0.001
Stream type	2	25156.1	25.1	0.001
Stream (Stream type)	6	1002.5	3.7	0.020
Substrate	2	20082.4	85.6	<0.001
Time	3	15674.6	158.9	<0.001
Stream type x Substrate	4	1477.3	6.3	0.006
Substrate x Stream (Stream type)	12	234.6	3.6	0.001
Stream type x Time	6	1873.2	19	<0.001
Substrate x Time	6	377.3	5.8	<0.001
Stream (Stream type) x Time	18	98.7	1.5	0.137
Stream type x Substrate x Time	12	316.5	4.9	<0.001
Substrate x Stream (Stream type) x Time	36	64.6	2.3	<0.001

Table S4. Decomposition rates (*k*) on a per day (/d) and on a per degree-day basis (/dd) (mean ± SE) of clethra leaves enclosed in coarse- and fine-mesh bags and of balsa wood enclosed in fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) for 63 days (n=3 replicates per stream).

Substrate type	Stream type	*k* (/d)	*k* (/dd)
Clethra coarse-mesh	Native	0.00806 ± 0.00125	0.00057 ± 0.00009
	Cryptomeria	0.02257 ± 0.00402	0.00183 ± 0.00032
	Pasture	0.02673 ± 0.00200	0.00172 ± 0.00013
Clethra fine-mesh	Native	0.00594 ± 0.00057	0.00042 ± 0.00004
	Cryptomeria	0.00541 ± 0.00064	0.00044 ± 0.00005
	Pasture	0.02640 ± 0.00289	0.00170 ± 0.00019
Balsa fine-mesh	Native	0.00361 ± 0.00059	0.00025 ± 0.00004
	Cryptomeria	0.00252 ± 0.00019	0.00020 ± 0.00002
	Pasture	0.01383 ± 0.00086	0.00089 ± 0.00005

Table S5. Summary table for three-level nested ANOVA performed on decomposition rates on a per day and per degree-day basis for clethra leaves enclosed in coarse- and fine-mesh bags and balsa wood enclosed in fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) for 63 days (n=3 replicates per stream).

Source of variation	df	MS	F	p
Decomposition rate on day				
Intercept	1	0.0130000	275.347	<0.001
Stream type	2	0.0020000	41.098	<0.001
Stream (Stream type)	6	0.0000479	0.855	0.553
Substrate	2	0.0010000	19.057	<0.001
Stream type x Substrate	4	0.0000560	4.680	0.016
Substrate x Stream (Stream type)	12	0.0000561	2.268	0.021
Decomposition rate on degree-day				
Intercept	1	0.0000640	242.898	<0.001
Stream type	2	0.0000072	27.319	<0.001
Stream (Stream type)	6	0.0000003	0.774	0.605
Substrate	2	0.0000058	17.125	<0.001
Stream type x Substrate	4	0.0000016	4.828	0.015
Substrate x Stream (Stream type)	12	0.0000003	2.690	0.007

Table S6. Mean relative contribution (%; across sampling streams and dates, based on spore production) of aquatic hyphomycete taxa associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date). Total taxa richness per stream type is also shown.

Aquatic hyphomycetes taxa	Coarse-mesh			Fine-mesh		
	Native	Cryptomeria	Pasture	Native	Cryptomeria	Pasture
Alatospora acuminata Ingold	0.07	0.07	0.03	0.31	0.12	0.36
Alatospora pulchella Marvanová	8.96	1.85	1.27	9.24	1.73	0.58
Anguillospora longissima (Sacc. & P. Syd.) Ingold		0.28			0.26	0.05
Anguillospora rosea Webster & Descals	0.30	0.02		0.28	0.03	
Articulospora tetracladia Ingold	38.75	7.95	0.37	37.24	6.34	0.08
Campylospora chaetocladia Ranzoni	0.01		0.14			0.06
Clavariopsis aquatica de Wild	0.12	0.54	0.77	0.11	0.59	0.31
Clavatospora longibrachiata (Ingold) Marvanová & Sv. Nilsson	0.06	0.16	0.69	0.10	0.34	0.58
Culicidospora aquatica R. H. Petersen			0.02			
Filiform sp.1	0.29				0.01	
Flagellospora curvula Ingold	4.02	10.44	0.01	3.85	10.42	0.06
Fontanospora eccentrica (Petersen) Dyko	0.01	0.02		1.66	0.16	
Fontanospora fusiramosa Marvanová, Peter J. Fisher & Descals		0.01				
Heliscella stellata (Ingold & V. J. Cox) Marvanová	0.07	0.05	0.02	0.30	0.03	
Heliscus lugdunensis Sacc. & Therry	0.65	0.18		3.33	1.65	0.04
Hydrocina chaetocladia Ingold	0.46	1.05	0.15	0.98	0.28	0.11
Lemonniera aquatica de Wild	17.91	40.70	2.06	10.43	37.61	3.30
Lemonniera terrestris Tubaki	0.28	2.85	0.27	0.36	2.77	0.16
Lunulospora curvula Ingold	6.36	2.75	92.81	19.56	4.52	93.48
Other sp.1				0.02		
Other sp.2		0.01				
Other sp.3	0.51	0.35	0.10	0.20	0.12	0.09
Pentarradiate sp.1	0.05	0.17	0.02	0.22	0.34	
Pentarradiate sp.2	0.01			0.08	0.01	
Pentarradiate sp.3	0.01				0.01	
Pleuropedium multiseptatum Marvanová & Descals	0.15	0.22			0.71	
Tetrachaetum elegans Ingold	5.80	26.92	0.04	7.71	30.10	0.13

Tetracladium furcatum Descals				0.01		
Tetracladium marchalianum De Wild	0.02		0.26			0.22
Tetracladium setigerum (Grove) Ingold	0.13	0.31	0.10	1.09	0.11	0.03
Tetracladium sp.	0.01	0.09	0.05	0.21		
Tetrarradiate sp.1	0.20	0.10		0.13	0.06	0.01
Tricellula aquatica Webster	0.18	0.01		0.18	0.01	
Tricladium attenuatum Iqbal	0.02	0.01			0.04	
Tricladium patulum Marvanová & Marvan	0.02			0.03		0.01
Tricladium splendens Ingold	0.24	0.25		0.07	0.11	
Tricladium sp.	1.11				0.01	
Triscelophorus acuminatus Nawawi	13.14	2.48	0.81	2.19	1.43	0.34
Triscelophorus monosporus Ingold	0.03			0.01		
Tripospermum myrti (Lind) S. Hughes				0.06		
Varicosporium elodeae W.Kegel	0.04	0.14		0.04	0.09	
Total taxa richness	34	29	20	30	30	20

Table S7. PERMANOVA test for total abundance data of aquatic hyphomycetes associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date). Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Source of variation	df	MS	Pseudo-F	P (perm)	perms
Stream type	2	54062	6.67	0.002	968
Mesh	1	3044	3.88	0.049	999
Time	3	6139	4.99	0.001	999
Stream (Stream type)	6	8732	14.84	0.001	997
Stream type x Mesh	2	765	0.97	0.494	999
Stream type x Time	6	2005	1.56	0.042	998
Mesh x Time	3	1168	1.25	0.246	996
Stream (Stream type) x Mesh	6	806	1.37	0.068	999
Stream (Stream type) x Time	18	1321	2.25	0.001	999
Stream type x Mesh x Time	5	1052	1.12	0.324	998
Stream (Stream type) x Mesh x Time	16	940	1.60	0.002	999
Residual	128	589			
Total	196				

Table S8. Summary table for four-level nested ANOVA performed on aquatic hyphomycete taxa richness and sporulation rates associated with clethra leaves enclosed in coarse- and fine-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date).

Source of variation	df	MS	F	p
Taxa richness				
Intercept	1	9274	284.826	<0.001
Stream type	2	58	1.685	0.262
Stream (Stream type)	6	36	18.733	0.052
Mesh	1	1	1.493	0.252
Time	3	81	17.617	<0.001
Stream type x Mesh	2	8	10.418	0.004
Mesh x Stream (Stream type)	6	1	0.187	0.976
Stream type x Time	6	4	0.897	0.517
Mesh x Time	3	5	1.503	0.251
Stream (Stream type) x Time	18	5	1.351	0.266
Stream type x Mesh x Time	5	5	1.278	0.320
Mesh x Stream (Stream type) x Time	16	4	1.297	0.209
Sporulation rate				
Intercept	1	42159227	7.602	0.031
Stream type	2	10149989	1.761	0.249
Stream (Stream type)	6	5980940	1.951	0.188
Mesh	1	10048858	4.103	0.087
Time	3	7559983	4.047	0.021
Stream type x Mesh	2	7362195	3.011	0.121
Mesh x Stream (Stream type)	6	2504713	2.022	0.120
Stream type x Time	6	3177018	1.693	0.176
Mesh x Time	3	1901373	1.533	0.243
Stream (Stream type) x Time	18	1900338	1.516	0.189
Stream type x Mesh x Time	5	1423651	1.151	0.374
Mesh x Stream (Stream type) x Time	16	1234386	0.810	0.672

Table S9. Mean relative abundance (%; across streams and dates) of benthic macroinvertebrate taxa collected at the beginning and end of the experiment (day 0 and day 63, respectively) in native, cryptomeria and pasture streams (n=3 streams per type). Total macroinvertebrate abundance, total macroinvertebrate taxa richness, and total shredder abundance are also shown. FFG, functional feeding group.

Order	Taxa	FFG	Native	Cryptomeria	Pasture
Basommatophora	*Galba truncatula* (O.F.Müller, 1774)	Grazer/scraper	0.26		
Basommatophora	*Physella acuta* (Draparnaud, 1805)	Grazer/scraper			0.05
Coleoptera	*Agabus* sp.	Predator		3.81	
Coleoptera	*Dryops* sp.	Predator			0.02
Coleoptera	*Hydroporus* sp.	Predator	0.05		
Crassiclitellata	*Eiseniella tetraedra* (Savigny, 1826)	Gatherer/collector	0.03	0.44	
Cyclopoida		Gatherer/collector		0.14	
Diptera	*Cardiocladius freyi* Storå, 1936	Gatherer/collector	0.01	0.15	0.05
Diptera	Ceratopogonidae Gen. Sp.	Predator	1.55	0.14	0.01
Diptera	*Chaetocladius melaleucus* (Meigen, 1818)	Gatherer/collector	0.21		0.02
Diptera	Clinocerinae Gen sp.	Predator	0.30	2.24	0.24
Diptera	*Cricotopus sylvestris* Fabricius, 1794	Gatherer/collector			0.85
Diptera	*Dicranomyia* sp.	Shredder	5.06	1.98	0.15
Diptera	*Eukefferiella gracei* (Edwards, 1929)	Gatherer/collector			0.24
Diptera	*Limnophyes minimus* (Meigen, 1818)	Gatherer/collector	0.06		
Diptera	Orthocladiinae Gen sp.	Gatherer/collector	22.81	15.72	29.38
Diptera	*Orthocladius fuscimanus* (Kieffer & Thienemann, 1908)	Gatherer/collector	0.95		2.51
Diptera	*Paramerina cingulata* (Walker, 1856)	Predator	0.06		
Diptera	*Parametriocnemus stylatus* (Spärck, 1923)	Gatherer/collector			0.34
Diptera	*Paratanytarsus grimmii* (Schneider, 1885)	Gatherer/collector			0.02
Diptera	*Rheocricotopus atripes* (Kieffer, 1913)	Gatherer/collector	0.62	0.51	0.31
Diptera	*Simulium azorense* (Carlsson, 1963)	Filter feeder	10.15	19.98	7.54
Diptera	*Synorthocladius semivirens* (Kieffer, 1909)	Gatherer/collector			0.10
Diptera	Tanypodinae Gen sp.	Predator	0.29	3.97	
Diptera	Tanytarsini Gen sp.	Gatherer/collector	0.21	1.90	0.04
Diptera	*Thienemanniella clavicornis* (Kieffer, 1911)	Gatherer/collector			0.99
Diptera	*Tipula* sp.	Shredder	0.02		0.02
Diptera	*Zavrelymia nubilus* (Meigen, 1830)	Predator		1.22	
Isopoda	*Jaera nordmanni* subsp. *insulana* Veuille, 1976	Shredder		2.73	
Lumbriculida	*Lumbriculus variegatus* (Müller, 1774)	Gatherer/collector	0.11	15.96	0.05
Monostilifera	*Prostoma* sp.	Gatherer/collector	0.15	0.69	0.09
Sarcoptiformes	*Hydrozetes* sp.	Gatherer/collector	0.88		0.09
Sarcoptiformes	*Platynothrus* sp.	Gatherer/collector		0.24	
Sarcoptiformes	Thrypochtoniidae Gen. sp.	Gatherer/collector			0.09
Sarcoptiformes	Trimalaconothrus sp.	Gatherer/collector	0.77	0.30	0.04
Symphypleona	Sminthurididae Gen. sp.	Gatherer/collector	0.01	0.15	
Trichoptera	*Hydroptila* sp.	Grazer/scraper	2.74	0.15	13.89
Trichoptera	*Limnephilus atlanticus* Nybom, 1948	Shredder		17.04	
Trichoptera	*Oxyethira falcata* Morton, 1893	Grazer/scraper	19.36		3.35
Tricladida	*Dugesia* sp.	Predator	0.40	0.92	1.05

Trombidiformes	*Sperchon brevirostris* Koenike, 1895	Gatherer/collector	1.83	0.57	1.40
Tubificida	*Nais* sp.	Gatherer/collector	30.59	3.46	36.61
Tubificida	*Pristina* sp.	Gatherer/collector			0.43
	Oligochaeta Gen sp.	Gatherer/collector		4.28	
	Ostracoda Gen sp.	Filter feeder	0.37		0.02
	Nematoda sp.	Parasite	0.14	1.31	
Total macroinvertebrate abundance (no. individuals/stream type)			755	101	1708
Total macroinvertebrate taxa richness (no. taxa/stream type)			29	26	31
Total shredder abundance (no. shredders/stream type)			43	21	3

Table S10. PERMANOVA test for total abundance of benthic macroinvertebrates in native, cryptomeria and pasture streams (n=3 streams per type) at the beginning and end of experiment (day 0 and day 63, respectively). Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Source of variation	df	SS	MS	Pseudo-F	P (perm)	perms
Stream type	2	17444.0	8722	6.478	0.007	275
Time	1	419.1	419.1	0.660	0.654	999
Stream (Stream type)	6	8078.5	1346.4	2.121	0.003	999
Stream type x Time	2	2368.9	1184.5	1.866	0.103	998
Residual	6	3808.9	634.8			
Total	17	32119.0				

Table S11. Summary table of one-way ANOVA performed on benthic total shredder density and benthic *L. atlanticus* density in native, cryptomeria and pasture streams (n=3 streams per type, with the two sampling dates averaged per stream).

Source	df	MS	F	p
Benthic total shredder density				
Intercept	1	2301.48	13.52	0.002
Stream type	2	609.31	3.58	0.054
Error	15	170.20		
Benthic *L. atlanticus* density				
Intercept	1	138.89	15.08	0.001
Stream type	2	138.89	15.08	<0.001
Error	15	9.21		

Table S12. Mean relative abundance (%; across streams and dates) and total taxa richness of macroinvertebrates associated with clethra leaves enclosed in coarse-mesh bags over 15,30,45 and 63 days incubation in native, cryptomeria and pasture streams (n=3 streams per type; n=3 replicates per stream and date). FFG, functional feeding groups.

Order	Taxa	FFG	Native	Cryptomeria	Pasture
Basommatophora	*Physella acuta* (Draparnaud, 1805)	Grazer/scraper			0.02
Coleoptera	*Agabus* sp.	Predator		3.33	
Coleoptera	*Dryops* sp.	Predator			0.20
Crassiclitellata	*Eiseniella tetraedra* (Savigny, 1826)	Gatherer/collector	0.15	0.40	0.07
Diptera	*Chaetocladius melaleucus* (Meigen, 1818)	Gatherer/collector	0.19		
Diptera	Chironomini Gen. sp.	Gatherer/collector			0.36
Diptera	Clinocerinae Gen. sp.	Predator	0.66	1.79	2.39
Diptera	*Cricotopus sylvestris* Fabricius, 1794	Gatherer/collector			0.70
Diptera	*Dicranomyia* sp.	Shredder	0.81		0.21
Diptera	*Limnophyes minimus* (Meigen, 1818)	Gatherer/collector			0.05
Diptera	Orthocladiinae Gen. sp.	Gatherer/collector	21.36	15.71	25.86
Diptera	*Orthocladius fuscimanus* (Kieffer & Thienemann, 1908)	Gatherer/collector	0.20		0.80
Diptera	*Parametriocnemus stylatus* (Spärck, 1923)	Gatherer/collector			1.04
Diptera	Psychodidae Gen. sp.	Gatherer/collector			0.05
Diptera	*Rheocricotopus atripes* (Kieffer, 1913)	Gatherer/collector	1.01	0.76	2.43
Diptera	*Simulium azorense* (Carlsson, 1963)	Filter feeder	0.28	0.16	8.19
Diptera	Tanypodinae Gen. sp.	Predator	13.73	11.42	0.10
Diptera	Tanytarsini Gen. sp.	Gatherer/collector		0.76	
Diptera	*Thienemanniella clavicornis* (Kieffer, 1911)	Gatherer/collector			1.57
Diptera	*Tipula* sp.	Shredder			0.01
Lumbriculida	*Lumbriculus variegatus* (Müller, 1774)	Gatherer/collector	1.67	4.35	1.89
Monostilifera	*Prostoma* sp.	Gatherer/collector	0.15	0.25	0.42
Poduromorpha		Gatherer/collector			0.22
Sarcoptiformes	*Hydrozetes* sp.	Gatherer/collector	0.64		0.08
Sarcoptiformes	*Platynothrus* sp.	Gatherer/collector	0.16		0.03
Sarcoptiformes	*Thrypochtoniidae* Gen. sp.	Gatherer/collector			0.01
Sarcoptiformes	*Trimalaconothrus* sp.	Gatherer/collector	0.11		0.05
Trichoptera	*Hydroptila* sp.	Grazer/scraper	0.40		9.67
Trichoptera	*Limnephilus atlanticus* Nybom, 1948	Shredder	0.35	50.41	
Trichoptera	*Oxyethira falcata* Morton, 1893	Grazer/scraper	25.93	0.23	3.51
Tricladida	*Dugesia* sp.	Predator	2.18	1.69	5.67
Tricladida	*Phagocata vitta* (Duges, 1830)	Predator			0.02
Trombidiformes	*Sperchon brevirostris* Koenike, 1895	Gatherer/collector	4.70	0.95	1.38
Tubificida	*Nais* sp.	Gatherer/collector	24.54	7.01	31.94
Tubificida	*Pristina* sp.	Gatherer/collector			0.22
	Nematoda Gen. sp.	Parasite			0.04
	Oligochaeta Gen. sp.	Gatherer/collector	0.40	0.79	0.78
	Ostracoda Gen. sp.	Filter feeder	0.42		
Total taxa richness (no. Taxa/stream type)			22	16	33
Total shredder abundance (no. shredders/stream type)			0	6	0

Table S13. PERMANOVA test for total abundance data of macroinvertebrates associated with clethra leaves enclosed in coarse-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) over 15, 30, 45 and 63 days (n=3 replicates per stream and date). Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Source of variation	df	SS	MS	Pseudo-F	P (perm)	perms
Stream type	2	109300	54648	5.842	0.002	823
Time	3	9744	3248	1.777	0.032	999
Stream (Stream type)	6	56184	9364	8.968	0.001	998
Stream type x Time	6	14834	2472	1.352	0.080	997
Stream (Stream type) x Time	18	32969	1832	1.754	0.001	995
Residual	70	73095	1044			
Total	105	297270				

Table S14**.** Summary table of two-way ANOVA performed on shredder density associated with clethra leaves enclosed in coarse-mesh bags and incubated in native, cryptomeria and pasture streams (n=3 streams per type) at days 15, 30, 45 and 63 (n=3 replicates per stream and date).

Source	df	MS	F	p
Intercept	1	5058.14	33.82	<0.001
Stream type	2	3626.94	24.25	<0.001
Time	3	369.09	2.47	0.067
Stream type x Time	6	290.86	1.94	0.082
Error	94	149.57		

Table S15. Summary table for one-way ANOVAs performed on chemical and physical characteristics of the three leaf species used in the experiments. DM, dry mass.

Source of variation	df	MS	F	P
P (% DM)	2	0.01	23.07	0.002
N (% DM)	2	2.13	172.87	< 0.001
C (% DM)	2	7.61	5.48	0.044
Lignin (% DM)	2	276.18	101.67	< 0.001
Phenols (% DM)	2	58.97	95.53	< 0.001
Toughness (g)	2	30198.86	42.19	< 0.001

Table S16. Summary table for one-way ANOVAs performed on metal concentrations of a stream with low metal concentration (reference stream) and a stream naturally enriched with metals (metal-enriched stream).

Source of variation	df	MS	F	P
Al (µg/L)	1	136831683	10.481	0.084
Fe (µg/L)	1	204339	24.874	0.015
Mn (µg/L)	1	38349	29.028	0.013

Table S17**.** Summary table for two-way ANOVAs performed on metal concentrations in leaves (three species) previously exposed in a stream with low metal concentration and in a stream naturally enriched with metals.

Source of variation		df	MS	F	P
Corrected model	Al	5	423807	4.24	0.043
	Fe	5	10381213	3091.86	<0.001
	Mn	5	4370	453.21	<0.001
Intercept	Al	1	39147698	391.64	<0.001
	Fe	1	56220267	16744.21	<0.001
	Mn	1	79161	8209.15	<0.001
Species	Al	2	88434	0.88	0.454
	Fe	2	925259	275.57	<0.001
	Mn	2	10527	1091.74	<0.001
Stream	Al	1	1694001	16.95	<0.001
	Fe	1	45344712	13505.12	<0.001
	Mn	1	652	67.62	<0.001
Species × Stream	Al	2	153696	1.54	0.280
	Fe	2	400516	119.29	<0.001
	Mn	2	106	11.04	0.007
Error	Al	7	99959		
	Fe	7	3357		
	Mn	7	9		

Table S18**.** Summary table for Friedman's tests performed on feeding preference trials for the two choice situations: (i) choice among the three leaf species incubated either in a stream with low metal concentration (reference leaves) or in a stream naturally enriched with metals (metal-enriched leaves) and (ii) choice between reference and metal-enriched leaves for each leaf species.

Feeding preference trials		N	Chi-Square	df	P
(i)	Reference leaves	9	10.667	2	0.005
	Metal-enriched leaves	11	3.455	2	0.178
(ii)	*A. glutinosa*: Reference vs Metal-enriched	11	11.000	1	<0.001
	I. perado: Reference vs Metal-enriched	14	2.571	1	0.109
	L. azorica: Reference vs Metal-enriched	12	5.333	1	0.021

Table S19**.** Summary table for three-way repeated measures ANOVA performed on relative consumption rates of *L. atlanticus* individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration and in a stream naturally enriched with metals.

Source of variation	df	MS	F	P
Intercept	1	1.673	246.959	<0.001
Species	2	0.226	33.321	<0.001
Stream	1	0.036	5.241	0.028
Species × Stream	2	0.018	2.617	0.086
Error	39	0.007		
Time	2	0.017	4.377	0.016
Time × Species	4	0.016	4.136	0.004
Time × Stream	2	0.003	0.793	0.456
Time × Species × Stream	4	0.005	1.258	0.294
Error	78	0.004		

Table S20. Summary table for three-way repeated measures ANOVA performed on relative growth rate of *L. atlanticus* individuals fed over three weeks with one of the three leaf species previously exposed in a stream with low metal concentration and in a stream naturally enriched with metals.

Source of variation	df	MS	F	P
Intercept	1	47.927	0.426	0.517
Species	2	1238.224	11.010	<0.001
Stream	1	32.545	0.289	0.593
Species × Stream	2	33.693	0.300	0.743
Error	46	112.465		
Time	2	1147.952	10.110	<0.001
Time × Species	4	67.866	0.598	0.665
Time × Stream	2	12.147	0.107	0.899
Time × Species × Stream	4	64.940	0.572	0.684
Error	92	113.547		

Table S21. Summary table for one-way ANOVA performed on water physical and chemical parameters from reference stream and metal-enriched stream) (n=2 replicates per water parameter and stream).

Water parameters	df	MS	F	P
Temperature (ºC)	1	61.92	65.66	<0.001
pH	1	0.94	8.68	0.009
Conductivity (µS/cm)	1	87516.45	28.48	<0.001
TDS (mg/L)	1	21780.00	28.52	<0.001
DO (%)	1	124.50	1.28	0.273
DO (mg/L)	1	5.21	5.59	0.029
Al (µg/L)	1	55860676.00	105.96	0.009
Fe (µg/L)	1	3904576.00	136.37	0.007
Mn (µg/L)	1	49284.00	3.54	0.201

TDS, Total dissolved solids; DO, Dissolved oxygen

Table S22. Summary table for one-way ANOVAs performed on metal concentration (Al, Fe and Mn) on leaves before and after incubation (two weeks) in a stream with low metal concentration (reference leaves) and in a stream naturally enriched with metals (metal-enriched leaves) (n=3 replicates per metal parameter and leaf treatment).

Metals	df	MS	F	P
Al (µg/g)	2	4978245	48.7	<0.001
Fe (µg/g)	2	10104447046	199.9	<0.001
Mn (µg/g)	2	1197751	139.0	<0.001

Table S23**.** Summary table for two-way repeated measures ANOVA performed on AFDM remaining of clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) over time (15, 43 and 90 days) (n=4 replicates per water and leaf treatment and date).

Source of variation	df	MS	F	P
Intercept	1	925715.9	117978.0	<0.001
Leaf	1	6.7	0.9	0.361
Water	5	76.7	9.8	<0.001
Leaf × Water	5	54.2	6.9	<0.001
Error	36	7.8		
Time	2	4214.6	347.7	<0.001
Time × Leaf	2	115.8	9.6	<0.001
Time × Water	10	42.4	3.5	0.001
Time × Leaf× Water	10	18.9	1.6	0.137
Error	72	12.1		

Table S24. Summary table for two-way ANOVA performed on AFDM remaining of clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) at the end of experiment (90 days) (n=4 replicates per water and leaf treatment and date).

Source of variation	df	MS	F	P
Intercept	1	245707.0	15652.1	<0.001
Leaf	1	77.8	5.0	0.032
Water	5	140.3	8.9	<0.001
Leaf × Water	5	62.5	4.0	0.006
Error	36	15.7		

Table S25. Summary table for two-way repeated measures ANOVA performed on aquatic hyphomycete sporulation rates and taxa richness associated with clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) over time (15, 43 and 90 days) (n=4 replicates per water and leaf treatment and date).

Source of variation	df	MS	F	P
Sporulation rate				
Intercept	1	2101875.72	241.18	<0.001
Leaf	1	168741.04	19.36	<0.001
Water	5	85109.55	9.77	<0.001
Leaf × Water	5	2759.51	0.32	0.900
Error	36	8714.82		
Time	2	994393.62	90.19	<0.001
Time × Leaf	2	51744.80	4.69	0.012
Time × Water	10	54857.95	4.98	<0.001
Time × Leaf × Water	10	2347.40	0.21	0.994
Error	72	11025.60		
Taxa richness				
Intercept	1	10421.01	2804.91	<0.001
Leaf	1	7.56	2.04	0.162
Water	5	2.69	0.72	0.610
Leaf × Water	5	2.38	0.64	0.670
Error	36	3.72		
Time	2	25.30	9.02	<0.001
Time × Leaf	2	7.31	2.61	0.081
Time × Water	10	5.58	1.99	0.047
Time × Leaf × Water	10	5.03	1.79	0.077
Error	72	2.81		

Table S26. Mean relative contribution (%; based on spore production) of aquatic hyphomycete taxa associated with clethra leaves for the two leaf treatments (reference and metal-enriched leaves) in the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) along the duration of the experiment (90 days) (n=4 replicates per water and leaf treatment, and date). Total taxa richness per treatment is also shown. (*) Morphology alterations of spores.

Taxa	Reference leaves						Metal-enriched leaves					
	0%	10%	25%	50%	75%	100%	0%	10%	25%	50%	75%	100%
Anguillospora filiformis Greathead									0.07			0.02
Anguillospora longissima (Saccardo & P. Sydow) Ingold	0.02					0.07	0.04	0.03			0.03	0.02
Anguillospora sp.					0.06			0.03				
Alatospora acuminata Ingold	0.75	1.44	0.53	0.77	0.86	0.19			0.04		0.04	
Alatospora pulchella Marvanová	1.89	2.18	2.92	5.70	3.38	2.44	0.58	0.54	0.82	0.60	0.58	0.21
Alatospora pulchella (*5 arms) Marvanová	0.02				0.04			0.07				
Articulospora tetracladia Ingold	7.18	9.88	11.90	6.94	4.15	3.73	1.56	4.28	4.25	4.00	3.54	1.34
Clavariopsis aquatica de Wild		0.03										
Filiform sp.	0.03		0.03					0.02	0.03		0.21	
Flagellospora curvula Ingold	62.36	57.14	62.18	44.90	57.78	44.78	60.25	46.98	55.59	36.53	34.02	69.31
Fontanospora eccentrica (Petersen) Dyko	0.04											
Geniculospora inflata (Ingold) Sv. Nilsson ex Marvanová			0.07							0.03		
Heliscella stellata (Ingold & V. J. Cox) Marvanová			0.07		0.03		0.10			0.06		
Heliscina campanulata Marvanová										0.03		
Neonectria lugdunensis Sacc. & Therry	0.03	0.02	0.03	0.07	0.27	1.33	0.01				0.07	
Lemonniera aquatica de Wild	10.45	12.28	9.60	21.08	15.29	23.43	5.31	15.59	16.49	25.18	25.43	12.65
Lemonniera aquatica (*5 arms) de Wild	0.02	0.10	0.10	0.05	0.03	0.13		0.03	0.07	0.07	0.06	0.04
Lemoniera pseudofloscula Dyko										0.05		
Lemonniera terrestris Tubaki	0.33	0.23	0.78	0.63	0.63	1.37	0.39	1.20	1.44	1.35	1.50	0.33
Lemonniera terrestris (*5 arms) Tubaki			0.10						0.04			
Lemonniera terrestris (*smaller and thinner arms) Tubaki	0.02	0.27	0.07		0.06	0.57	0.16	0.22	0.39	0.25	0.29	0.18
Lunulospora curvula Ingold	13.26	12.02	9.82	16.01	14.56	19.10	15.29	16.66	10.51	15.61	19.45	12.91

Mycocentrospora acerina (Hartig) Deighton							0.03		0.01		0.03	
Mycofaella calcarata Marvanová, Om-Kalth. & J. Webster											0.02	
Pleuropedium multiseptatum Marvanová & Descals					0.02							
Tetrachaetum elegans Ingold	0.03						0.14	0.02	0.01		0.07	
Tetracladium furcatum Descals									0.03			
Tetracladium marchalianum De Wild						0.03						
Tetracladium setigerum (Grove) Ingold		0.03		0.01	0.21	0.26	0.04			0.12	0.33	0.19
Tricellula aquatica J. Webster	0.23	0.31	0.19	0.09	0.13	0.07				0.15		
Tricladium angulatum Ingold		0.03	0.03	0.04		0.04	0.11	0.11	0.08		0.09	0.01
Tricladium attenuatum Iqbal											0.04	
Hydrocina chaetocladia Ingold	0.86	1.39	0.74	2.83	1.41	1.36	14.13	13.19	9.11	11.20	13.11	2.26
Tricladium curvisporum Descals				0.04			0.01	0.10	0.04		0.03	
Tricladium patulum Marvanová & Marvan	0.07				0.02							0.06
Tricladium splendens Ingold	0.03	0.13	0.04	0.04	0.01	0.10	0.15	0.17	0.22	0.43	0.06	0.15
Tricladium sp.						0.03						
Triscelophorus acuminatus Nawawi	2.07	2.03	0.56	0.63	0.61	0.61	0.65	0.18	0.13	2.61	0.30	0.02
Undetermined conidia	0.29	0.50	0.23	0.19	0.46	0.34	1.06	0.58	0.71	1.74	0.66	0.31
Total taxa richness	21	18	20	17	21	20	19	19	21	18	23	17

Table S27. PERMANOVA test for total abundance data of aquatic hyphomycetes associated with clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves) and the six water treatments (0%, 10%, 25%, 50%, 75% and 100%) over time (15, 43 and 90 days) (n=4 replicates per water and leaf treatment and date). Data was log (x + 1) transformed; resemblance was calculated using Bray-Curtis similarity index.

Source of variation	df	MS	Pseudo-F	P(perm)	perms
Leaf	1	9508.30	23.68	0.001	999
Water	5	1461.80	3.64	0.001	998
Time	2	12854.00	32.01	0.001	999
Leaf × Water	5	896.23	2.23	0.002	999
Leaf × Time	2	2693.80	6.71	0.001	999
Water × Time	10	756.12	1.88	0.001	997
Leaf × Water × Time	10	705.75	1.76	0.007	998
Residual	108	401.54			
Total	143				

Table S28. Summary table of average dissimilarity of SIMPER analysis performed on total abundance of aquatic hyphomycetes associated with clethra leaf discs for the two leaf treatments (reference and metal-enriched leaves).

Average dissimilarity = 34.20	Reference	Metal-enriched				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Tricladium chaetocladium	1.19	2.84	4.40	1.59	12.88	12.88
Articulospora tetracladia	2.49	1.73	3.53	1.44	10.31	23.19
Lunulospora curvula	3.02	3.24	3.33	1.14	9.75	32.93
Alatospora pulchella	1.65	0.61	3.32	1.36	9.69	42.63
Flagellospora curvula	4.85	4.53	3.30	1.09	9.64	52.27
Lemonniera aquatica	3.37	3.48	2.50	1.08	7.31	59.58
Lemonniera terrestris	0.59	0.93	2.30	1.25	6.72	66.30
Undetermined conidia	0.40	0.83	2.07	1.14	6.04	72.34
Triscelophorus acuminatus	0.67	0.32	1.94	0.84	5.66	78.00
Alatospora acuminata	0.72	0.03	1.73	0.95	5.07	83.07
Lemonniera terrestris (*smaller)	0.19	0.32	1.02	0.74	2.97	86.04
Tricladium splendens	0.11	0.24	0.73	0.63	2.15	88.19
Tricellula aquatica	0.24	0.04	0.66	0.58	1.94	90.13

UNIVERSIDADE DOS AÇORES

Faculdade de Ciências e Tecnologia

Rua da Mãe de Deus

9500-321 Ponta Delgada

Açores, Portugal