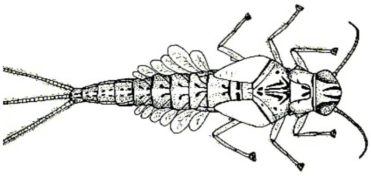

# Estresores ambientales del funcionamiento de arroyos en Uruguay. (Biomonitoreo basado en la estructura taxonómica y funcional de macroinvertebrados y el proceso de descomposición de hojarasca)

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Doctorado en Ciencias Ambientales

Universidad de la República
Facultad de Ciencias
Programa de Doctorado en Ciencias Ambientales

# Environmental stressors of the stream functioning in Uruguay. (Biomonitoring based on macroinvertebrate taxonomic and functional structure and leaf litter decomposition process).

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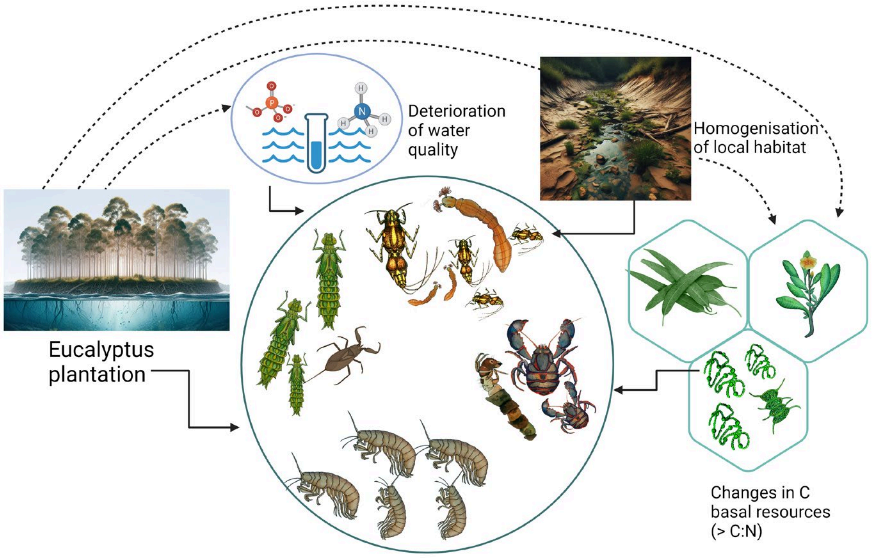

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# Environmental stressors of the stream functioning in Uruguay. Biomonitoring based on macroinvertebrate taxonomic and functional structure and leaf litter decomposition process.

Tesis para la obtención de grado de Doctora en Ciencias Ambientales, Facultad de Ciencias, Universidad de la República.

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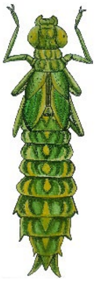
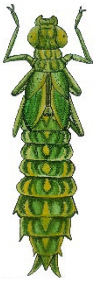


# ABSTRACT

Stream ecosystems are highly dynamic and complex, influenced by climatic, geomorphological, and human factors. These ecosystems are interconnected with terrestrial environments, relying on the reciprocal flow of organisms, matter, and energy. Variations in environmental conditions due to anthropogenic stressors can select macroinvertebrates with specific tolerance capacities or functional traits, impacting ecosystem functioning. In subtropical Uruguayan streams, the effect of stressors such as the removal of riparian forests or the introduction of exotic plantations like *Eucalyptus* spp. on ecological conditions of streams have been poorly assessed. These activities may affect taxonomic and functional community structure through changes in habitat conditions and aquatic community reliance on terrestrial carbon, impacting ecological processes such as leaf litter decomposition. This thesis aims to estimate the effects of these stressors on stream ecosystems' ecological conditions, focusing on subtropical Uruguayan streams. In this case, we analysed the impacts of *Eucalyptus* afforestation and riparian forest loss. In the case of *Eucalyptus* afforestation, water quality and macroinvertebrate metrics were used as bioindicators to identify the impacts of this land use. The study also explored how *Eucalyptus* afforestation may indirectly affect macroinvertebrate functional trait composition and diversity through changes in habitat conditions and basal resource availability. To assess the impact of riparian forest removal, the study monitored leaf litter decomposition in streams and the abiotic and biotic factors influencing this process. The study found adverse effects of *Eucalyptus* afforestation on macroinvertebrate community structure and water quality. However, these effects varied seasonally, particularly higher concentrations $NO_3$-N and $NH_4$-N, and lower proportion (relative abundance) of Ephemeroptera-Plecoptera-Trichoptera families and Zygoptera in summer, while in winter, higher conductivity, total suspended solids, and collector-gatherer proportions. Functional trait-based approaches also reflected the impacts of afforestation increases, mediated by changes in basal resources, especially macrophyte biomass. In this case, we found that submerged and emerged macrophytes played important roles, supporting small macroinvertebrates and attracting predators due to their complex structures. When analysing ecological functioning, the study found no significant effects of riparian cover removal on rates of litter decomposition in streams. For instance, we found that leaf species identity influenced decomposition rates more than stream types, possibly masking the effects of anthropogenic stressors. However, riparian cover buffered the effects of biotic and abiotic drivers on litter decomposition process. The knowledge produced by this thesis contributes to the identification of new research avenues and enhancing the understanding of how aquatic communities and ecological processes respond to environmental deterioration caused by human stressors. These thesis findings have the potential to serve as a tool for developing management practices aimed at preventing or mitigating the effects of the anthropogenic stressors examined in this study, and possibly extending the evaluation of other human stressors.

**Key words**: Bioindication, macrophyte biomass, taxonomical and functional macroinvertebrate diversity, threshold indicator taxa, decomposition of leaf litter, stream ecosystems.



# RESUMEN

Los ecosistemas fluviales son altamente dinámicos y complejos, influenciados por factores climáticos, geomorfológicos y humanos. Estos ecosistemas están interconectados con los ambientes terrestres, dependiendo del flujo recíproco de organismos, materia y energía. Las variaciones en las condiciones ambientales debidas a los estresores antropogénicos pueden seleccionar macroinvertebrados con capacidades de tolerancia específicas o rasgos funcionales, afectando el funcionamiento de los ecosistemas. En los arroyos subtropicales de Uruguay, el efecto de los factores de estrés, como la eliminación de los bosques ribereños o la introducción de plantaciones exóticas como *Eucaliptus* spp. en las condiciones ecológicas de los ríos, ha sido poco evaluado. Estas actividades pueden afectar a las estructuras comunitarias taxonómicas y funcionales a través de cambios en las condiciones del hábitat y la dependencia de las comunidades acuáticas del subsidio terrestre, afectando a procesos ecológicos como la descomposición de hojarasca. Esta tesis tiene como objetivo estimar los efectos de estos estresores (aforestación de *Eucaliptus* y de la pérdida de bosques de los ríos) en la condición ecológica de los ecosistemas fluviales, centrándose en los arroyos subtropicales en Uruguay. En el caso de la aforestación de *Eucaliptus*, la calidad del agua y las métricas de los macroinvertebrados se utilizaron como bioindicadores para identificar los impactos de este uso de la tierra. El estudio también exploró cómo la aforestación puede afectar indirectamente la composición y la diversidad de los rasgos funcionales de los macroinvertebrados a través de cambios en las condiciones del hábitat y la disponibilidad de recursos basales. Para evaluar el impacto de la eliminación de los bosques de las riberas, el estudio se basó en evaluar la descomposición de hojarasca en arroyos y los factores abióticos y bióticos que influyen en este proceso. Mediante el uso de bioindicadores como las métricas relacionadas con la estructura de la comunidad de macroinvertebrados y los parámetros de calidad del agua, el estudio identificó los efectos adversos de la aforestación de *Eucaliptus*. Sin embargo, estos efectos variaron entre las estaciones verano e invierno, especialmente en relación con las altas concentraciones de $NO_3$-N y $NH_4$-N en agua y una menor proporción (abundancia relativa) de las familias Ephemeroptera-Plecoptera-Trichoptera y Zygoptera en verano, mientras que, en invierno, se observó una mayor conductividad, así como mayores concentraciones de sólidos suspendidos y proporción de los colectores-recolectores. Los enfoques basados en los rasgos funcionales también reflejaron los efectos de los aumentos de la aforestación, mediados por cambios en los recursos basales, especialmente la biomasa de macrófitas. En este caso, encontramos que las macrófitas sumergidas y emergentes desempeñan un papel significativo, albergando macroinvertebrados de tallas pequeñas y atrayendo a los depredadores debido a las complejas estructuras de estas macrófitas. Al analizar el funcionamiento ecológico, el estudio no encontró efectos significativos de la eliminación de la cobertura ribereña en las tasas de descomposición de hojarasca. En este caso, encontramos que la identidad de las especies de hojas influyó más en las tasas de descomposición que los tipos de arroyos, posiblemente ocultando los efectos de los estresores antropogénicos. Sin embargo, la cobertura ribereña amortiguó los efectos de los factores abióticos y bióticos en el procesamiento de la hojarasca. El conocimiento producido por esta tesis contribuye a identificar nuevas vías de investigación y mejorar la comprensión de cómo las comunidades acuáticas y los procesos ecológicos responden a la degradación ambiental causada por los estresores humanos. Los hallazgos de la tesis tienen el potencial de servir de herramienta para el desarrollo de prácticas de gestión encaminadas a prevenir o mitigar los efectos de los estresores antropógenos examinados en este estudio, y posiblemente ampliar la evaluación de otros estresores humanos.

**Palabras clave**: Bioindicación, biomasa de macrófitas, diversidad taxonómica y funcional de macroinvertebrados, indicadores de umbrales de taxones, descomposición de hojarasca, ecosistemas fluviales.

# CHAPTER I: GENERAL INTRODUCTION

Stream and river ecosystems are highly dynamic and complex systems influenced by a series of climatic, geomorphological, and human factors (Allan & Castillo, 2007). These factors influence various spatial and temporal habitat configuration patterns that shape the structure of aquatic communities (Frissell et al., 1996; Allan & Castillo, 2007; Thorp et al., 2008; Heino et al., 2015). Likewise, fluvial ecosystems are not isolated systems as they are interconnected with terrestrial ecosystems, and their functioning depends on the reciprocal terrestrial-aquatic flow of organisms, matter, and energy (Polis et al., 1997; Marcarelli et al., 2011; Marks, 2019; González-Bergonzoni et al., 2019).

The main aquatic and terrestrial drivers of the structure and composition of aquatic communities, from the landscape to local scale, include changes in the land use cover and the condition and composition of riparian vegetation (Valle et al., 2013; Ono et al., 2020; Melo et al., 2020; Moi et al., 2023), physico-chemical water variables (Ometo et al., 2000; Cortelezzi et al., 2015), physical structure of local habitat (e.g., substrate type, current velocity, water depth) (Buss et al., 2004), the availability and types of basal resources (Burdett & Watts, 2009, Frainer et al., 2014; Baker et al., 2023), and biotic interactions (Zarnetske et al., 2017; García-Girón et al., 2020). Variations in these conditions, whether due to natural – biogeographic influences or anthropogenic stressors (defined as any anthropogenic intervention than induce a chance in any biotic or abiotic factor; Sabater et al., 2018), can lead to the selection of species with specific physical and/or behavioural traits that enable them to adapt to environmental challenges and maintain their fitness (Spence & Tingley, 2020; Harvey et al., 2022; Paz et al., 2022).

The availability of basal resources for aquatic macroinvertebrate communities depends on the primary productivity of the system in the form of periphyton algae and macrophytes, and by the allochthonous input from terrestrial productivity in the form of leaf litter or coarse particulate organic matter (CPOM > 1 mm, e.g., leaf litter, branches, logs, reproductive parts) (Wallace et al., 1997; Marks, 2019).

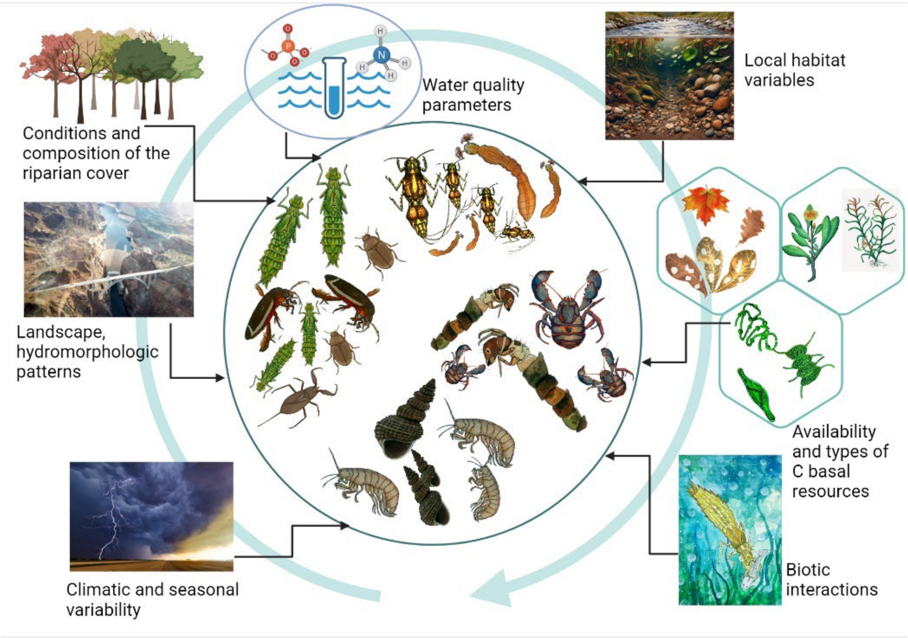


Fig. 1. Spatial arrangement of abiotic and biotic drivers of aquatic macroinvertebrate communities in stream ecosystems. Green arrow in the background represents the arrangement of drivers from larger to finer spatial scale. Illustrations by M. Barrios ensembled with BioRender.com

The river continuum concept, RCC (Vannote et al., 1980; Fig. 2), suggests that material and energy flow vary along the longitudinal axis of the river from its headwaters to its mouth, and this variation determine resource partitioning, taxonomic as well as functional structure of macroinvertebrate groups. In small streams covered with a dense riparian canopy, the entry of solar radiation into the channel is limited, thereby limiting autochthonous primary productivity (PP). Consequently, coarse particulate organic matter (CPOM) may influence both the food webs and the carbon and nutrient cycling in streams (Marks, 2019). In this scenario, CPOM is mainly used by shredder-detritivorous macroinvertebrates (those capable of "shredding" or "cutting" CPOM) and collector-gatherers (those consuming fine benthic detritus or fine particulate organic matter, 1 mm > FPOM > 0.45 µm), which play a significant role in incorporating these resources into the aquatic food web. CPOM processing increases the activity of heterotrophic microorganisms like bacteria and hyphomycete fungi, which leads to a higher respiration rate. This refers to streams with heterotrophic metabolism (PP/ER < 1).

In intermediate order streams where the riparian canopy does not fully shade the channel, there is greater solar radiation input, resulting in increased primary productivity (PP), with productivity exceeding respiration (PP/ER > 1) (Vannote et al., 1980). Herbivorous macroinvertebrate groups, like periphyton scrapers and macrophyte shredders, tend to be more common in these middle-stream areas than detritivorous shredders. In larger river reaches and estuaries, PP decreases due to a reduced photic zone (the depth of water where sunlight penetrates) caused by increased suspended solids. In this scenario, both suspended and benthic coarse particulate organic matter (CPOM), as well as dissolved organic matter (DOM), become more relevant in trophic webs, and the respiration rate is higher (PP/ER < 1). Here, filter-collector macroinvertebrate groups dominate as primary consumers in the food web. Therefore, the presence of forest dictates the type of basal resources and their processing by aquatic organisms, thereby influencing the structure of aquatic trophic webs, particularly those dominated by primary consumer macroinvertebrates (Vannote et al., 1980; Allan & Castillo, 2007; Thorp et al., 2008).

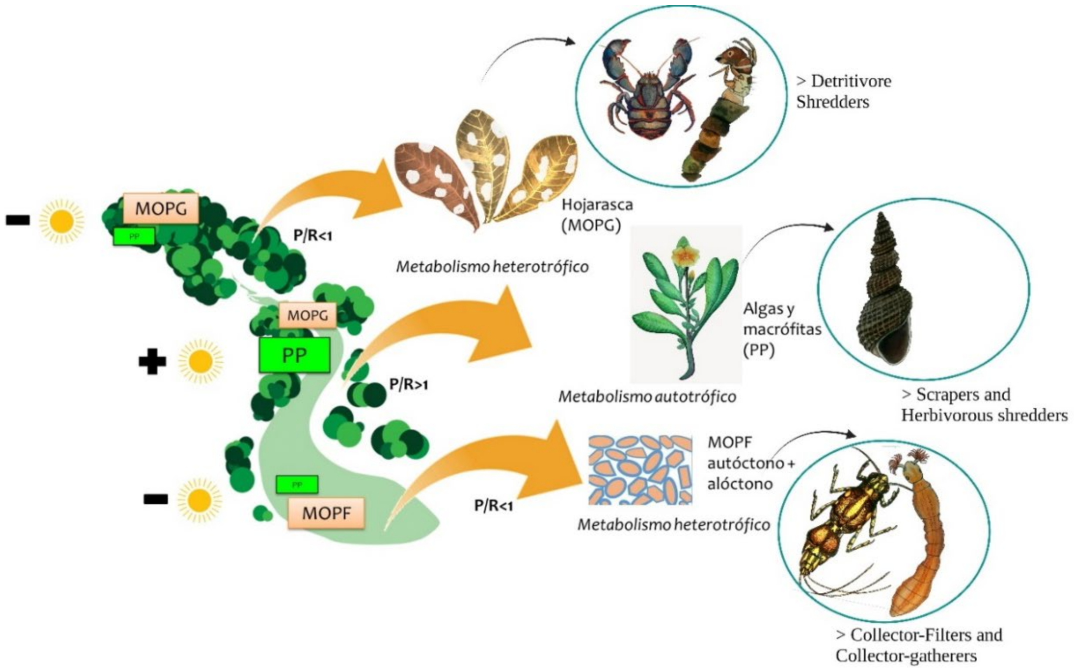


Fig. 2. Spatial arrangement of basal resources and primary consumer macroinvertebrates according to the River Continuum Concept (Vannote et al., 1980). The symbols + and - indicate the amount of solar radiation entering the water body in headwater, midstream, and river mouth segments. CPOM: Coarse Particulate Organic Matter, FPOM: Fine Particulate Organic Matter, PP: Primary Production, R: Respiration. Illustrations by M. Barrios ensembled with BioRender.com

However, the RCC is based only on a model where small streams have riparian forest cover that does not necessarily occur globally, either by the nature of the landscape (e.g., streams in prairie or grassland systems) or by the anthropogenic action that promotes the elimination of riparian cover (Giorgi et al., 2005; Leberfinger et al., 2011; López van Oosteron et al., 2017). In addition, the RCC is based on a model of streams in temperate areas without any kind of disturbance or adverse conditions (Doretto et al. 2020). Therefore, the application of this concept is quite limited to certain types of stream systems that meet habitat characteristics with respect to riparian cover and other geomorphological characteristics.

Contrary to the RCC, there are other concepts that help explain the origin and dynamics of different basal resources in stream ecosystems. For instance, the Flood Pulse Concept (FPC) postulated by Junk et al. (1989) for large rivers in floodplains emphasises the significance of the lateral dimension as the primary factor triggering ecological processes rather than merely a longitudinal gradient as proposed by the RCC. Furthermore, the FPC highlights that fluctuations in discharge or flood pulses facilitate the temporal exchange of carbon and energy between the river channel and its floodplain (Junk et al., 1989). In this context, the production (both primary production and lateral input of coarse particulate organic matter) in the floodplain zone directly or indirectly subsidises the biomass of aquatic trophic webs. The availability of this production to the biota depends on the density and retention of vegetation in the floodplain. However, in streams or rivers with more restricted channels, the FPC is less applicable due to the limited connection between the channel and the terrestrial area. Additionally, the process indicated by the FPC, depends on the frequency on flood events which vary according to the size of the basin, climate, precipitation patterns, geomorphology, and geographic location.

The Riverine Productivity Model (RPM) developed by Thorp & Delong (1994) emphasises the significance of local productivity, encompassing primary production and the local influx of coarse particle organic matter. Additionally, it assigns greater importance to the patterns that occur within the framework of aquatic food webs. According to the RPM, the significance of a specific subsidy for consumer biomass depends on two factors: the amount of the material present in the system (indicated by the RCC) and its nutritional value (partially determined by the C:N ratio, lability, or recalcitrance of carbon molecules; Sterner & Elser, 2002). The nutritional quality of autochthonous resources, which have a low C:N ratio and are labile, is often higher compared to allochthonous resources, which have a high C:N ratio and are recalcitrant (Thorp & Delong, 1994; 2002; Marcarelli et al., 2011).

After that, Humphries et al. (2014) introduced the River Wave Concept (RWC), which combines the RCC, FPC, and RPM while also considering how hydrology and the shape of the channel affect flow waves. These waves collectively regulate the spatial and temporal processes affecting the types of energy subsidies, such as production or input, storage, transformation (fragmentation, incorporation into biomass, excretion), and transport (longitudinal or lateral). Like a wave in a hydrograph, the trough of the wave shows the base flow, where primary production or the input of allochthonous CPOM is most common. This is also where the processing and energy transfer rates in the aquatic food web are highest. The RPM provides the best explanation for this section. With the rising or falling limb of the wave, corresponding to high precipitation events, the transport of allochthonous material from upstream prevails, while storage and transformation are reduced. These processes are better explained by the RCC. High flow moments at the wave's peak directly correlate with the lateral transport of allochthonous material from the floodplain or high primary production, while storage and transformation processes also play a significant role. Thus, the FPC is more suitable for explaining these processes (Humphries et al., 2014). The RWC's proposed integration advances riverine ecosystem ecology by applying the relative roles of the three earlier conceptual models to specific systems based on their hydro-geomorphological characteristics and the impact of specific climatic events (Humphries et al., 2014; Doretto et al., 2020).

After allochthonous leaf litter, or COPM, enters the stream, most of its energy is respired by aquatic microorganisms, but an important part also fuels the stream food web (Marks, 2019). However, aquatic consumers use this resource, and the fate of this carbon (mineralized or sequestered) includes various stages of leaf litter that occur over time and involve several pathways shaped by the interactions of leaf litter, abiotic factors, and organisms (Marks, 2019). These pathways may occur sequentially or simultaneously:

The initial step in the process is leaching, which entails the dissolution of water-soluble chemicals from litter and can result in a significant loss of biomass, around 40% (Tonin et al., 2021). In addition, leachates may be transformed into bacterial biomass or respired as $CO_2$ (Marks, 2019). The rate at which leaching occurs over time depends on the chemical composition of leaf litter.

The presence of soluble substances, such as nutrients, labile and recalcitrant carbon molecules, secondary metabolites, along with the structural characteristics of leaves, including thick cuticles and waxes that enhance their resistance to dissolution, significantly contribute to this process (Gessner et al., 1999; Marks, 2019; Tonin et al., 2021). Moreover, the physico-chemical characteristics of water also impact leaching. Water temperature, pH, water hardness, and mineral content are important factors that determine how certain leaf chemicals dissolve in water (Wantzen et al., 2008; Tonin et al., 2021).

Another significant process that often accompanies or follows leaching is the colonisation by aquatic hyphomycetes and bacteria (Wantzen et al., 2008). These microorganisms contribute to the conditioning of leaf litter, rendering it more palatable by assimilating nutrients dissolved in water (Costello et al., 2022). However, the microbial layer on the leaf litter can be also considered the main food item instead of leaf litter itself as element ratios of microbes (C:N:P) are much lower than leaf litter which meet macroinvertebrate nutritional demands (Cross et al., 2003; Danger et al., 2016; Marks, 2019). Shredder macroinvertebrates can then utilize and consume this conditioned litter, although in some cases, microorganisms may compete with macroinvertebrates for consuming leaf litter and may use toxic compounds that affect macroinvertebrate consumption (Marks, 2019).

Detritivore macroinvertebrates are other key organisms that decompose a large part of the leaf litter (51-64% of litter mass loss; Hieber & Gessner, 2002; Tonin et al., 2021). However, meiofauna are also important consumers of leaf litter and may serve as a trophic connection between leaf-associated microorganisms, leaf litter, and macroinvertebrates (Chambord et al., 2017; Magdi & Traunspurger, 2017; Wang et al., 2020). These organisms play a critical role in incorporating allochthonous basal resources into the aquatic trophic network's biomass by consuming substantial amounts of detritus (Marks, 2019; Jonsson & Sponseller, 2021). Consequently, they generate substantial amounts of fine particulate organic matter, which other organisms like detritivorous collector-gatherers and collector-filters utilize (Graça, 2001; Marks, 2019).

Like the leaching process, microbes colonising and macroinvertebrates feeding on leaf litter are affected by leaf chemical and physical properties. This is especially true when the leaf litter has high C:N:P ratios, carbon molecules that are hard to break down, and secondary compounds like tannins, terpenes, phenols, waxes, lignin, and cellulose that can make it harder for microbes to colonise and for macroinvertebrates to eat (Gessner et al., 2010; Marks, 2019; Swan et al., 2021). Plants use these compounds as defences against terrestrial herbivores, but they remain active after leaf senescence and abscission, affecting detritivorous aquatic consumers (Canhoto & Graça, 1995; Graça, 2001; Wantzen & Wagner, 2006)

Microorganisms and detritivore macroinvertebrates, on the other hand, are influenced by biogeography and phylogeny, as well as abiotic factors such as physical and chemical water variables (e.g., pH and temperature), which may constrain or accelerate their metabolic rates, modifying their physiology and biomass production (Ferreira & Chauvet, 2011; Mas-Martí et al., 2015). Additionally, local habitat variables (e.g., current velocity, water depth and width, sinuosity, and streambed substrate) may influence leaf litter retention and make it available for utilisation by stream biota (Abelho, 2001; Tonin et al., 2017).

The leaf litter decomposition process is also sensitive to changes in abiotic factors (Marks, 2019). For instance, current velocity mediates the physical fragmentation of leaves by small-suspended particles. Although, the presence of thick cuticles and the presence of lignin, cellulose, and hemicellulose may prevent abrasion, especially during the early stages of the decomposition process (Ferreira et al., 2006).

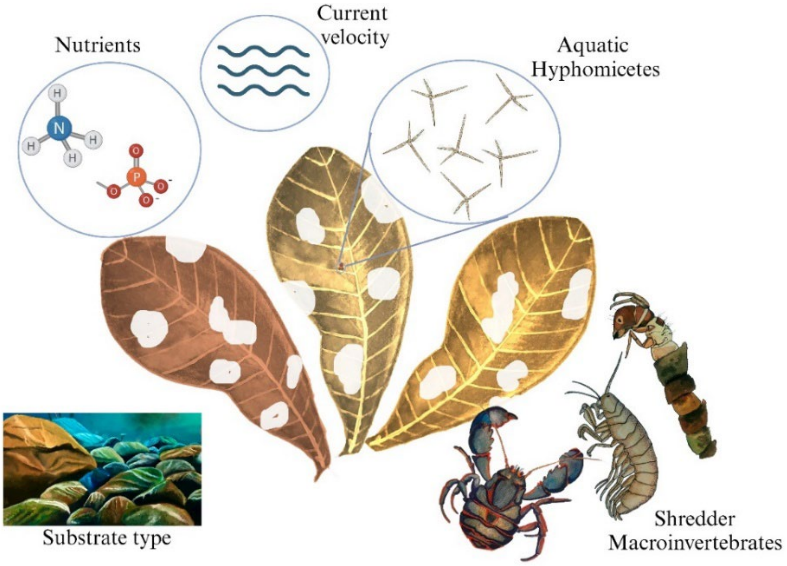


**Fig. 2.** Abiotic (water nutrients, current velocity, substrate type) and biotic factors (aquatic hyphomycetes and shredder macroinvertebrates) related to the decomposition of leaf litter in stream ecosystems. Illustrations by M. Barrios ensembled with BioRender.com

Different studies involving global comparisons of the decomposition process point to multiple pathways in which abiotic and biotic factors affect this process and how they correlate with human-induced stressors (Boyero et al., 2011; 2012a,b; 2021). For instance, microbial decomposition typically decreases as latitude increases, with contrasting temperatures between seasons. This is because microbial decomposers tend to increase their metabolism by accelerating biochemical reactions with an increase in temperature (Boyero et al., 2011; Folstad-Shah et al., 2017). Using a standard cellulose substrate to study how microbial litter decomposition changes with latitude, which affects leaf quality, slows down the rate of decomposition at lower latitudes (Tiegs et al., 2019). However, there are other drivers interacting with temperature that may affect microbial-mediated decomposition in tropical latitudes (Folstad-Shah et al., 2017; Tiegs et al., 2019). Factors such as nutrient concentration in water may determine the immobilisation rates of nutrients by microorganisms, stimulating the decomposition process, especially when litter nutrient concentration is low (Costello et al., 2022). In temperate regions, mean decomposition rates and variability decrease towards the poles and are highly temperature-dependent (Tiegs et al., 2012). Thus, despite global experiments suggesting that climate warming will maintain decomposition rates unchanged, it is expected that an increase in temperature will lead to an increase in decomposition rates, thereby accelerating microbial activity (Boyero et al., 2011).

In the case of shredder-detritivore macroinvertebrates, their density and richness also vary across the world, with a tendency to increase in higher latitudes (Boyero et al., 2009). In temperate regions, a higher density of larger-bodied detritivore macroinvertebrates primarily mediates decomposition, compensating for the lower microbial activity (Boyero et al., 2011; Folstad-Shah et al., 2017). In contrast, in tropic regions, shredder macroinvertebrate diversity tends to be more variable, but with a tendency to decrease in lowland sites due to their sensitivity to higher temperatures, making them more susceptible to global warming (Boyero et al., 2012a; Folstad-Shah et al., 2017). In addition, macroinvertebrates tend to be generalists in the tropics (Boyero et al., 2012b; Boyero et al., 2015).

Multiple factors operating simultaneously at local scales determining the variation in shredder macroinvertebrate diversity in the tropics. For instance, strong competitive interactions among shredder species possible due to limited resource availability in terms of the amount, timing, and nutritional quality of leaf litter (Boyero et al., 2012a, 2017). Another possible factor is the dispersal capacity. In temperate regions, detritivore shredders are usually insects with adult flying states, which allow them to explore longer distances, but in the tropics, this trophic guild, consisting mainly of crustaceans and molluscs with no flying capacity, and thus they are more dispersal-limited (Boyero et al., 2012a).

Variations in of environmental and local habitat conditions, as well as in the availability of basal resources, can lead to the selection of macroinvertebrates with specific physical and/or behavioural traits that enable them to adapt to environmental challenges and maintain their fitness (Spence & Tingley 2020; Harvey et al., 2022; Paz et al., 2022; Baker et al., 2023). For example, the selection of basal resources for food depends on the ability of macroinvertebrate species to search for, feed on, assimilate, and convert them into secondary production (Marczak et al., 2007; Thorp et al., 2015; Doretto et al., 2020; Entrekin et al., 2020; Rüegg et al., 2021). This selection is coupled with the nutritional value of the basal resources (Thorp & Delong, 1994, 2002; Marcarelli et al., 2011).

Furthermore, certain basal resources, such as macrophytes and leaf litter, can also be used as refuges, helping to alleviate predation pressures by minimising interactions between predators and prey (López-Rodríguez et al., 2018; Wolters et al., 2018; Poi et al., 2021; Yofukuji et al., 2021), and may contribute to diminishing the effects of high current flow (Wolters et al., 2018). Using basal resources for shelter also depends on traits, particularly those related to mobility, which reflect their searching and hiding capacity (Christie et al., 2009; Verdonschot et al., 2012). In this sense, macroinvertebrate functional traits indicate taxonomic adaptations and resilience to environmental changes (Forio et al., 2018).

The main threats to ecosystems, as identified by the Millennium Ecosystem Assessment (Reid & Mooney, 2016), are changes in land use and global warming. Human activities introduce various stressors that inhibit the functioning of stream and river ecosystems (Sabater et al., 2018), leading to the deterioration of habitats, the extinction of species, and the subsequent decline of associated ecosystem services (Reid et al., 2018; Sabater et al., 2018; Ríos-Touma & Ramírez, 2018; Sundar et al., 2020; Brauns et al., 2022). Among human stressors, land use changes represent the most intense stressor affecting stream ecosystem causing irreversible effects on the receptors, in this case, the aquatic communities and aquatic ecological processes (Sabater et al., 2018). Land use changes, for instance for agricultural proposes may involve a “cocktail of stressors” through diffuse pollution, siltation, and in-stream habitat degradation affecting aquatic biodiversity (Sabater et al., 2018). Among the stressors affecting stream ecosystem, this thesis emphasises the introduction of exotic plantations like *Eucalyptus* spp. (Seena et al., 2017; Ferreira et al., 2019; Pereira et al., 2024) and the removal of riparian forest (Silva-Araujo et al., 2020). Both stressors will be analysed in separate cases as they represent a threat to the reliance of aquatic communities on terrestrial basal resources (Wang et al., 2021) and they may have different and direct impacts on the taxonomic and functional structure of these communities (Le Provost et al., 2020; Moi et al., 2023; Paz et al., 2022). This, in turn, affects ecological processes such as leaf litter decomposition (Marks, 2019).

### *STRESSOR #1: REPLACEMENT OF GRASSLAND BY EXOTIC TREE PLANTATIONS (EUCALYPTUS SPP. AFFORESTATION).*

*Eucalyptus* spp. is the most common species in tree plantations, occupying over 20 million hectares worldwide (Myburg et al., 2014), and its timber harvesting and other management activities could generate multifaceted impacts on the natural environment (Rodrigues et al., 2019). These impacts include soil acidification and hydrological cycle modifications, heightened surface runoff, and local loss of terrestrial species (Engel et al., 2005; Brazeiro, 2015; Silveira et al., 2016). Moreover, *Eucalyptus* plantations have been associated with the deterioration of streams in watersheds draining areas of tree plantations and their macroinvertebrate communities (Abelho & Graça, 1996; Ferreira et al., 2016, 2019).

Afforestation, which involves replacing natural grasslands with *Eucalyptus* plantations, can have various effects on stream communities (Fig.4). These effects are primarily caused by alterations in water quality (Molinero & Pozo, 2004; Farley et al., 2005) and changes in the physical habitat, such as increased soil siltation resulting in an unstable substrate (Graça et al., 2002). In addition, streams that flow through areas with *Eucalyptus* can also change the availability of carbon-based resources because of the introduction of Eucalyptus leaves into the streams. The leaf litter of *Eucalyptus* spp. contains significant amounts of secondary metabolites and resistant substances, including polyphenols, tannins, cellulose, lignin, and a thick cuticle (Botto et al., 2022; San-Emeterio et al., 2023). These substances have the potential to disrupt the growth of microorganisms and, consequently, hinder the feeding of macroinvertebrates on leaves, ultimately resulting in a decrease in the rate of decomposition (Canhoto & Graça, 1995; Canhoto et al., 2013; Ferreira et al., 2016, 2019; Correa-Araneda et al., 2017).

Additionally, alterations in water quality caused by the release of secondary chemicals during leaf leaching can impact both autochthonous production and macroinvertebrates (Canhoto et al., 2007; 2013; González-Paz et al., 2023). The introduction of *Eucalyptus* leaves can also impact the higher levels of the food chains. The presence of a waxy layer on *Eucalyptus* leaves prevents shredder macroinvertebrates from consuming them and fungal colonisation only occurs beneath this protective layer which shredders cannot access (Graça et al., 2002; Marks, 2019). In this sense, microorganisms are not part of the trophic webs and may act as sinks as they divert carbon and nutrients to sediments or to the atmosphere, depressing the energy flow through higher levels of the trophic web (Gessner et al., 1999; Graça, 2001; Marks, 2019).

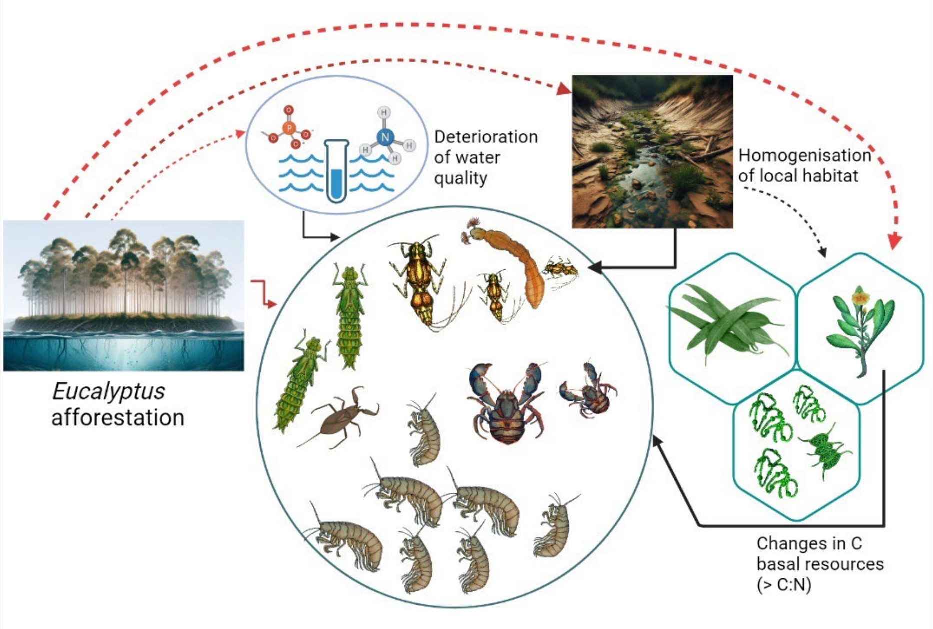


Fig. 4. Effects of Eucalyptus afforestation on stream macroinvertebrate communities mediated by changes in water quality parameters, local habitat conditions, and the availability and nutritional content (stoichiometric C:N ratios) of basal resources. Red dashed arrows indicate indirect negative effects, and black dashed solid arrows indicate indirect and direct effects. Illustrations by M. Barrios ensembled with BioRender.com

Most of the studies focus on the effects of *Eucalyptus* plantations on stream ecosystems, which have reached the temperate and Mediterranean regions and, in less proportion, tropical and subtropical latitudes. Ferreira et al. (2019) conducted a global comparison to assess the impact of stream sites draining *Eucalyptus* plantations on the decomposition process of leaf litter and the organisms associated with it. They found that a general global inhibition in the macroinvertebrate-mediated decomposition rate in stream sites associated with *Eucalyptus* plantations. However, the effects were stronger in some regions, including Uruguay. Therefore, it is imperative to address the impacts of *Eucalyptus* afforestation in the country, especially considering that other human-induced pressures have already been dealt with other studies.

### *STRESSOR #2: REPLACEMENT OF RIPARIAN FOREST BY OTHER LAND USES*

The removal of riparian vegetation, whether through forest conversion to grasslands or agriculture (Iñiguez-Armijos et al., 2018), urban development (Iñiguez-Armijos et al., 2016), forest fires (Pettit & Neiman, 2007; Carvalho et al., 2019), or logging (Richardson & Béraud, 2014), has a significant impact on the environmental state of stream ecosystems. This leads to a decline in aquatic communities and ecological processes, such as the decomposition of leaf litter (Silva-Junior et al. 2014; Silva-Araujo et al. 2020; Ferreira et al. 2020; Sargac et al. 2021; Tonin et al. 2021; Boyero et al. 2021; Tiegs et al., 2024).

The removal of riparian vegetation results in changes in the basal resources of stream ecosystems (Sargac et al., 2021). This occurs because the allochthonous basal resources that come from riparian trees are replaced by autochthonous primary producers as temperatures, nutrients, and light penetration increase and leaf litter inputs from trees decreased (Warren et al., 2016; Collins et al., 2016). In addition, the establishment of grass and herbs in the riparian zone may potentially contribute to allochthonous basal resources in the streams (Menninger & Palmer, 2007; Leberfinger & Bohman, 2010; Biasi et al., 2019). Nevertheless, the nutritional composition, lignin concentrations, and carbon structure of this grass input generally vary from other sources (Hladyz et al., 2009; Biasi et al., 2019).

Changes in the supply and nature of basal resources can lead to modifications in both diversity of macroinvertebrates and composition of their functional feeding groups (Ferreira et al., 2016, 2019; Kominoski et al., 2021). The effects of increasing autochthonous primary production or grass leaf input may depend on the nutrient limitation of the system, and the response of macroinvertebrate consumers can be enhanced in higher nutrient systems or inhibited in lower nutrient availability (Warren et al., 2016). The absence of shredder macroinvertebrates as a consequence of changes in basal resource may result in only microbial mediated decomposition which may act as sink instead of promoting the energy flow through the aquatic trophic web (Marks, 2019).

The removal of riparian forest could also impact macroinvertebrate communities by modifying the inherent characteristics of the stream environment. For instance, alterations in temperature patterns, the homogenisation of substrate composition through increased erosion and the presence of fine sediments, the reduction of in-stream wood, and the decrease in current velocity due to the proliferation of aquatic plants (Bleich et al., 2014; Elosegi et al., 2018; Sargac et al., 2021). This habitat deterioration has implications for macroinvertebrates by filtering only those tolerant species preferring soft-bottom sediments and low oxygen levels (Sargac et al., 2021).

The landscape of subtropical Uruguay is mostly characterised by temperate grassland, with limited natural woodland consisting mainly of the transitional zone between Paranense and Chaquense woody species (Haretche et al., 2012). In this region, the forests are distributed in scattered sections, primarily found near watercourses in hilly regions of the country (Toranza et al., 2024). The occurrence of these riparian patches can be linked to selective wood extraction and changes in land use, including extensive cattle, agricultural, and exotic tree plantations (Gautreau, 2010; Brazeiro et al., 2020).

### ***THIS THESIS: EUCALYPTUS AFFORESTATION AND RIPARIAN COVER REMOVAL EFFECTS ON STREAM ECOSYSTEMS.***

In subtropical Uruguayan streams, these two environmental stressors related to agribusiness, namely, afforestation with *Eucalyptus* and the loss of riparian native forest, have been little studied in terms of their effects on stream ecological conditions (Arocena et al., 2022). In this study, we assessed these two stressors, employing different methods, to determine their impact on environmental conditions, aquatic ecosystems in terms of both taxonomic and functional diversity, and ecological processes such as organic matter decomposition. It is important to remark that the effects of these two stressors on Uruguayan streams were evaluated separately in different areas of the country, and in the case of areas with *Eucalyptus* afforestation, the riparian area is mainly composed by herb and grass species.

First, we evaluated the effects of *Eucalyptus* afforestation on water quality and macroinvertebrate communities using a taxonomic and functional diversity approach. In this case, water quality, macroinvertebrate taxonomical composition, and the tolerance thresholds of macroinvertebrate individuals can be used as bioindicators to identify the potential permanent impacts of *Eucalyptus* plantations on streams (Suriano & Fonseca-Gessner, 2013; Fierro et al., 2017).

Additionally, we explore how *Eucalyptus* afforestation via direct and indirect pathways impact macroinvertebrate communities. For this, we explore how *Eucalyptus* afforestation promotes changes in local habitat conditions and the availability of different basal resources. These changes may alter the macroinvertebrate functional trait composition and functional diversity by filtering those traits that may tolerate or survive the alteration of these habitat and basal resources. These functional aspects of macroinvertebrate communities have been used as approximations of ecological processes. Trait-based approaches reflect important ecological processes such as nutrient and carbon cycling and biomass production performing as a proxy for the ecosystem functioning (Doledec & Statzner, 2010).

To assess the impact of riparian forests removal, we assessed the decomposition of leaf litter in streams, as well as the abiotic factors (e.g., water quality and stream current velocity) and biotic factors (including macroinvertebrate taxonomic composition and functional feeding groups) that influenced this process. Estimating the litter decomposition process and the factors involved provide information on the alterations promoted by human activities (Ferreira et al., 2020). Rapid and only microbial mediated decomposition rates, as a consequences of shredder absence due to more degraded stream conditions, potentially leading to reductions in-stream carbon and nutrient storages or transfers into the aquatic food web, with most of the carbon as $CO_2$ escaping into the atmosphere, contributing to global climate change. However, this increase in microbial-mediated decomposition rates only occurs at moderate levels of stream impairment (e.g., slow increases in water nutrient concentrations and temperature) that favour microbial processing but decrease shredder densities, particularly at low latitudes where their species diversity is low (Boyero et al., 2011, 2016). When human stressors increase, such as hypereutrophic conditions and oxygen depletion, may inhibit microbial composition affecting litter decomposition process (Ferreira et al., 2020). Thus, measuring ecosystem functions may also contribute to biomonitoring practices (Tiegs et al., 2024).

### *GENERAL OBJECTIVE*:

To evaluate the effects of two anthropogenic stressors on stream ecological condition by assessing the responses of water quality parameters and macroinvertebrate taxonomic and functional communities to *Eucalyptus* afforestation and investigating the effects of riparian forest loss on the abiotic and biotic factors involved in the decomposition of organic matter.

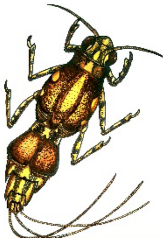
***SPECIFIC OBJECTIVES***:

***Specific Objective 1:*** To evaluate the responses of water quality parameters and macroinvertebrate community indicators to the increase in afforested areas in watersheds with *Eucalyptus* spp.

***Specific Objective 2***: To evaluate the impacts of *Eucalyptus* spp. afforestation on habitat heterogeneity and the availability of basal resources, and how these environmental changes affect functional trait structure and functional diversity of macroinvertebrate communities.

***Specific Objective 3***: To analyse the effects of riparian forest loss on the abiotic (water quality and flow) and biotic (macroinvertebrates) predictors of leaf litter decomposition by comparing open-canopy streams and streams with riparian forest cover.

The following chapters address these objectives by employing adequate methodological approaches. In Chapter II, specifically addressing Specific Objective I, we conducted field sample campaigns to assess the impact of *Eucalyptus* afforestation on water quality indicators and macroinvertebrate community metrics across 30 streams. Results obtained from this chapter were published in *Hydrobiologia* (https://doi.org/10.1007/s10750-023-05248-w). Chapter III, in relation to Specific Objective II, evaluated the indirect effects of *Eucalyptus* spp. afforestation on functional trait structure and functional diversity. This impact was mediated by changes in local habitat variables and the biomass of different basal resources using Chapter II's field sampling approach. This chapter is under review in *Aquatic Science* (submitted on July 22nd, 2024). Chapter IV considers an experimental approach that analyses the importance of riparian forests in the leaf decomposition process and the factors that influence it, both abiotic and biotic. The study compares two types of streams: those with riparian cover, referred to as riparian forest streams (RFS), and those without riparian cover but with grasses, herbaceous vegetation, and some shrubs, referred to as open canopy streams (OCS). Results obtained from this chapter were published in *Aquatic Science* (https://doi.org/10.1007/s00027-022-00886-z).



# *CHAPTER II: Environmental and aquatic macroinvertebrates metrics respond to the Eucalyptus afforestation gradient in subtropical lowland streams.*

This image was created by M. Barrios using Bing Designer by Microsoft.

The content of this chapter is published in the Special Issue "Stream Ecology and Environmental Gradients" in the journal Hydrobiologia and is presented in the original article format.

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Scope: Aquatic Science, Ranking Q1

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STREAM ECOLOGY

# Environmental and aquatic macroinvertebrates metrics respond to the *Eucalyptus* afforestation gradient in subtropical lowland streams

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**Abstract** *Eucalyptus* afforestation may affect stream ecosystems, but the magnitude of these effects on water quality and biota in subtropical lowland streams is little understood. We evaluated the potential effects of *Eucalyptus* afforestation on water quality and macroinvertebrate assemblage. Water quality parameters and macroinvertebrate assemblages were sampled in summer and winter in 30 streams covering an afforestation gradient (from 0.05 to 96% of the catchment area) and tested their relationship. We analyzed the taxa density distribution in afforestation and water parameters using the Thresholds Indicator Taxa Analyses (TITAN). Nutrient concentrations, conductivity, and total dissolved solids showed positive responses to the increase of afforestation, but the responses varied among seasons. Macroinvertebrate metrics showed negative (Ephemeroptera-Trichoptera-Plecoptera (EPT), sensitive families, scrapers), and positive (Chironominae, Oligochaeta) responses to the afforestation. Densities of sensitive taxa (mostly EPT) decrease with an increase in *Eucalyptus* afforestation, which is also related to water quality and possibly to habitat changes attributed to afforestation management. The use of water quality parameters in combination with macroinvertebrates assemblage contributes to managing these streams under catchment afforestation.

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**Keywords** Environmental gradients · Water quality parameters · Bioindicators · Ephemeroptera-Trichoptera-Plecoptera · Thresholds indicator taxa analyses

## Introduction

The growing expansion of the land-use frontier for productive purposes (e.g., forestry, agriculture, and livestock), together with the increase in the demand for water and biological resources, modify natural conditions of the aquatic ecosystem and, consequently, affect biodiversity and ecological processes (Sabater et al., 2019). Stream ecosystems are highly susceptible to anthropogenic stressors at the catchment scale. These stressors include land use changes, soil erosion, and the input of nutrients, contaminants, and sediments, among other factors that can impact water quality, habitat heterogeneity, and aquatic biota (Allan & Castillo, 2007; Sabater et al., 2019). Anthropogenic stressors caused by land use changes in stream conditions can be detected by changes in water quality indicators and biological assemblages (Cairns & Pratt, 1993; Bunn et al., 2010; Liu et al., 2021; Eriksen et al., 2021). Different aspects (metrics) of macroinvertebrate assemblage (e.g., richness, abundance, presence of tolerant taxa) can detect long-term accumulated impacts complementing the analysis of the water physical and chemical parameters (Hughes et al., 1998; Buss et al., 2015). Macroinvertebrates metrics can also respond to the effects of anthropogenic stressors at different temporal and spatial scales from basin to microhabitats (Buss et al., 2015; Roldán-Pérez et al., 2016; Fierro et al., 2019); as well as to the effects of land use changes (Sánchez-Montoya et al., 2010; Clapcott et al., 2012; García et al., 2017; Liu et al., 2021; Suárez et al., 2022). Detecting changes in the macroinvertebrate assemblage along environmental gradients promoted by anthropogenic activities can be a helpful tool for detecting the potential occurrence of thresholds and the upper limits of tolerance of aquatic organisms (Baker & King, 2010). Such thresholds can be useful for detecting the increase of anthropogenic land-use areas and help inform policy-makers and managers in biomonitoring programs and conservation strategies (e.g., Rodrigues et al., 2016; Firmiano et al., 2017; Campos et al., 2021).

Given that more than 20 million hectares of land around the world have been planted with *Eucalyptus* species (Zhang & Wang, 2021), timber harvesting and other management activities could change the ecological status of streams draining *Eucalyptus* plantation areas (Rodrigues et al., 2019).. This could have large direct and indirect effects on aquatic biota due to changes in physical habitat and water quality parameters (Molinero & Pozo, 2004; Farley et al., 2005). Ecological processes, such as the breakdown of organic matter (Ferreira et al., 2015, 2016, and 2018) and secondary production (Canhoto & Laranjeira, 2007), can also be affected by the differences in the amount, quality, and timing of leaf fall between *Eucalyptus* and native leaf species, which can affect the carbon and nutrient cycles (Ferreira et al., 2018). Heterotrophic microorganisms and detritivorous macroinvertebrates that use this allochthonous carbon as a resource and transfer it into the biomass of higher trophic levels are often affected (Gessner et al., 1999; Graça, 2001). Besides, *Eucalyptus* leaves contain a high concentration of potentially toxic secondary chemical compounds that can interfere with microbial colonization and invertebrate leaf-feeding, ultimately slowing down decomposition rates (Canhoto et al., 2013; Ferreira et al., 2015, 2016; Correa-Araneda et al., 2017). Thus, the tolerance thresholds of macroinvertebrate communities can serve as bioindicators for detecting the potential long-lasting effects of *Eucalyptus* plantations on streams (Suriano & Fonseca-Gessner, 2013; Fierro et al., 2017).

Studies on the impact of *Eucalyptus* afforestation on stream quality have mainly focused on water quality parameters, such as nutrient and suspended solids concentrations (Graça et al., 2002; da Silva et al., 2007; Rodrigues et al., 2019), as well as changes in macroinvertebrate assemblages (Graça et al., 2002; Larrañaga et al., 2009; Cordero-Rivera et al., 2017). In addition, researchers have investigated the decomposition rates of *Eucalyptus* and native leaves and the impact on aquatic organisms related to this process (e.g., Basanguren & Pozo, 1994; Canhoto & Graça, 1995; Albelho & Graça, 1996; Pozo et al., 1998; Canhoto & Graça, , 1999; Larrañaga et al., 2006; 2009; Canhoto et al., 2013; Correa-Araneda et al., 2015; Ferreira et al., 2015; 2018). In addition, the effects of *Eucalyptus* afforestation on hydrological regimes and the water balance have also been explored (Farley et al., 2005; Engel et al., 2005; de Paula Lima et al., 2012; Brown et al., 2013; Rodriguez-Suarez et al., 2014; Hawtree et al., 2015; Silveira et al., 2016; Dresel et al., 2018; Ebling et al., 2021).

In the case of Uruguayan subtropical streams, *Eucalyptus* afforestation areas reached more than one million hectares in 2021 (6.2% of the total area of the country) (Dirección General Forestal 2021) compared to the 35,000 hectares in 1990 (0.20% of the total area of the country), mainly replacing grassland ecosystems. Thus, afforestation areas have promoted some impacts on natural conditions such as soil acidification, modification of the hydrological cycle related to the increase in surface runoff, and local loss of terrestrial species characteristic of grasslands (Engel et al., 2005; Brazeiro, 2015; Silveira et al., 2016; Hernández, 2016).

Despite the rapid land conversion to *Eucalyptus* plantations, its impacts on stream structure and functioning in the subtropical regions are incipiently studied (Ferreira et al., 2018), particularly in the Uruguayan streams. Thus, it is necessary to address the effects of *Eucalyptus* afforestation in the country where other anthropogenic stressors have been addressed. In this case, extensive livestock grazing occupies the largest productive land area in the country over native grassland (Bernardi et al., 2016; Jaurena et al., 2021). It is known worldwide that this activity promotes worldwide negative impacts on stream morphology, increased sedimentation, and water nutrient concentration, as well as microbial contamination, and the deterioration of aquatic biota (Belsky et al., 1999; Callaghan et al., 2019; Iteba et al., 2021). However, in the context of Uruguay, extensive livestock grazing produces the lowest impact when compared to agricultural and urban areas (e.g., Chalar et al., 2011; Goyenola et al., 2015, 2020; Benejam et al., 2016; Suárez et al., 2022). For this reason, extensive cattle production (with less than one head per hectare; Estadísticas Agropecuarias, 2020) has been considered the less impacted condition to compare other anthropogenic stressor impacts, as truly pristine areas are practically nonexistent or very scarce (Benejam et al., 2016; Suárez et al., 2022).

It is anticipated that *Eucalyptus* afforestation will continue to rise in Uruguay, as well as in many other areas (Céspedes-Payret et al., 2009; Jobbágy et al., 2012; Pawson et al., 2013); this will increase the need to comprehend ecological changes and to produce knowledge that can guide policy-making and managers (Shah et al., 2022). This study examined the gradient of tree plantation surface cover in lowland subtropical streams, evaluating the response of physical and chemical parameters related to water quality and the macroinvertebrate assemblage, with the ultimate goal of proposing rapid tools that can be used for continuously monitoring the effects of *Eucalytus* afforestation.

## Methods

### Study area

The study was conducted in 30 subtropical lowland streams (< 30 km2 of catchment area; Table 1; Fig. S1) in Uruguay once per season in the summer (February) and winter (August) of 2019. These streams are distributed in the main catchments of Uruguay: rivers Río Negro (18 sites), Río Queguay (3 sites), Arroyo Maldonado (2 sites), Río Santa Lucía (2 sites), and Laguna Merín (5 sites). These streams were distributed within the Uruguayan ecoregions of the Gondwanan Sedimentary Basin, Eastern Sierras, and Western Sedimentary Basin (Brazeiro, 2015). The geomorphology of these micro-basins ranges from flat soils to gentle slopes and hills (Brazeiro, 2015). The range in elevation of the selected streams ranges from 53 to 219 m, and the range of the hill slope is from 0.3 to 5%. The Köppen climate classification describes this subtropical region (Cfa), temperate and humid type "C", rainfall throughout the year type "f", the temperature of the warmest month is higher than 22 °C type "a". Uruguay has a rainy climate, without a dry season, but with high interannual variability (INUMET, 2022). The annual precipitation of the region varies from the north to south of the country between 1582 and 1145 mm, respectively, as well as the temperature in summer (between 25.4 and 21.1 °C) and winter (between 13.7 and 11.1 °C) (INUMET, 2022). All streams are dominated by subtropical mesophyllous grasses such as *Andropogon*, *Panicum*, and other herb species characteristic of the Pampean bioregion, and the littoral vegetation cover is composed mainly of grass, herbaceous plants, and a few shrub species. Native tree cover is practically scarce, typically limited by livestock densities and fire occurrence (Bernardi et al., 2016).

**Table 1** Percentage of *Eucalyptus* afforestation cover and physical and chemical water quality parameters in the streams selected

| Stream | Season | Afforestation (%) | TP | $PO_4$–P | TN | $NO_3$–N | $NH_4$–N | SOM | k | TDS | pH | DO | Temp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DF-NF4 | Summer | 0.05 | 22.4 | 11.1 | 219.5 | 151.1 | 7.3 | 3.3 | 225 | 0.172 | 7.5 | 9.5 | 17.3 |
| | Winter | 0.05 | 20.9 | 4.8 | 241.5 | 222.6 | 6.5 | 2.5 | 102 | 0.093 | 7.2 | 10.8 | 10.1 |
| DF-Monte 3 | Summer | 0.18 | 11.1 | 3.2 | 122.5 | 121.7 | 0 | 0.9 | 523 | 0.382 | 7.6 | 8.6 | 19.2 |
| | Winter | 0.18 | 29.7 | 1.7 | 173.9 | 38.2 | 7.3 | 3 | 100 | 0.086 | 7.6 | 11.4 | 12.2 |
| DF-Monte 2 | Summer | 0.31 | 9.5 | 3.2 | 118.1 | 104.9 | 1 | 1.4 | 391 | 0.275 | 7.7 | 9.5 | 21.1 |
| | Winter | 0.31 | 22.6 | 6.4 | 194.2 | 10.6 | 6.5 | 3.7 | 92 | 0.081 | 7.9 | 11.8 | 11.2 |
| DF-CV | Summer | 0.36 | 16 | 1.7 | 307.7 | 276.5 | 10.8 | 2.2 | 490 | 0.343 | 7.4 | 8.4 | 21.2 |
| | Winter | 0.36 | 43.8 | 3.3 | 471.3 | 75.1 | 4.2 | 1.6 | 422 | 0.356 | 7.4 | 10.1 | 13 |
| DF-NF16 | Summer | 0.46 | 4.7 | 3.2 | 268.1 | 100.7 | 3.4 | 2.4 | 155 | 0.114 | 6.9 | 7.4 | 19.2 |
| | Winter | 0.46 | 26.2 | 6.4 | 288.8 | 139.7 | 4.2 | 0.8 | 80 | 0.074 | 7.1 | 10.4 | 9.3 |
| DF-C9 | Summer | 1.18 | 41.7 | 9.6 | 237.2 | 130.1 | 1 | 6.2 | 86 | 0.055 | 6.8 | 7.6 | 25.9 |
| | Winter | 1.18 | 33.2 | 4.8 | 397 | 204.2 | 5 | 3 | 30 | 0.024 | 7.6 | 10.2 | 14.8 |
| DF-S19 | Summer | 3.34 | 17.6 | 8 | 493.2 | 306.6 | 10.8 | 2.2 | 344 | 0.226 | 7.4 | 8.5 | 24.4 |
| | Winter | 3.34 | 54.4 | 6.4 | 315.9 | 148.9 | 6.5 | 1.7 | 99 | 0.081 | 7.6 | 10.7 | 14.5 |
| DF-NF6 | Summer | 3.8 | 11.1 | 6.4 | 268.1 | 142.7 | 4.2 | 1.4 | 215 | 0.162 | 7.5 | 6.2 | 17.8 |
| | Winter | 3.8 | 26.2 | 6.4 | 336.1 | 241.1 | 15.1 | 2 | 113 | 0.101 | 7.5 | 11 | 10.6 |
| DF-S16 | Summer | 6.19 | 16 | 4.8 | 686.9 | 381.9 | 3.5 | 2.4 | 387 | 0.235 | 8.3 | 11.2 | 28.7 |
| | Winter | 6.19 | 27.9 | 3.3 | 302.3 | 185.8 | 6.5 | 2.3 | 143 | 0.122 | 7.8 | 11.1 | 12.6 |
| DF-2018-4 | Summer | 7.52 | 32.1 | 0.1 | 563.6 | 125.9 | 4.2 | 0.8 | 66 | 0.041 | 7 | 9.6 | 27.9 |
| | Winter | 7.52 | 61.5 | 4.8 | 484.9 | 195 | 6.5 | 3.5 | 30 | 0.028 | 7.6 | 10.6 | 9.2 |
| DF-2018-6 | Summer | 12.76 | 27.2 | 23.8 | 281.3 | 146.9 | 0 | 1.3 | 79 | 0.061 | 6.7 | 7.4 | 16.4 |
| | Winter | 12.76 | 59.8 | 25.4 | 829.6 | 204.2 | 8.1 | 6.5 | 37 | 0.032 | 7.3 | 10.6 | 12 |
| DF-MFC | Summer | 19.57 | 9.5 | 3.2 | 219.5 | 100.7 | 0 | 2.4 | 146 | 0.093 | 6.8 | 8.3 | 25.8 |
| | Winter | 19.57 | 42.1 | 0.9 | 498.4 | 379.4 | 10.4 | 5.5 | 42 | 0.034 | 7.4 | 10.6 | 14.4 |
| DF-2018-2 | Summer | 28.04 | 32.1 | 6.4 | 400.4 | 251.9 | 0 | 7.7 | 101 | 0.066 | 6.5 | 7.4 | 24.5 |
| | Winter | 28.04 | 45.6 | 6.4 | 363.2 | 167.3 | 3.4 | 3.3 | 40 | 0.036 | 7 | 9.9 | 10.8 |
| DF-2018-3 | Summer | 32.88 | 36.9 | 4.8 | 462.2 | 193.1 | 0 | 2.5 | 79 | 0.05 | 6.9 | 4.4 | 26.2 |
| | Winter | 32.88 | 42.1 | 11.2 | 741.7 | 287.2 | 4.2 | 4.5 | 35 | 0.032 | 7 | 9.6 | 9.8 |
| DF-C7F | Summer | 38.56 | 123.7 | 91.8 | 396 | 172.1 | 8.1 | 2.3 | 228 | 0.155 | 6.8 | 3 | 22.6 |
| | Winter | 38.56 | 114.6 | 55.4 | 782.3 | 296.4 | 9.7 | 5 | 81 | 0.069 | 7.6 | 10 | 12.7 |
| DF-2018-8 | Summer | 44.36 | 27.2 | 6.4 | 268.1 | 180.5 | 1.8 | 5.3 | 61 | 0.043 | 7 | 7.1 | 20.8 |
| | Winter | 44.36 | 43.8 | 8 | 606.5 | 195 | 11.2 | 5.5 | 36 | 0.032 | 8.2 | 9.8 | 11.6 |
| DF-Forest 2 | Summer | 45.86 | 6.3 | 3.3 | 307.7 | 253.9 | 4.6 | 1.4 | 154 | 0.117 | 7.4 | 9.1 | 17.3 |
| | Winter | 45.86 | 12 | 9.6 | 302.3 | 139.7 | 20.6 | 3.6 | 188 | 0.182 | 7.9 | 12.5 | 7.9 |
| DF- F8F | Summer | 49.21 | 22.4 | 6.4 | 436.7 | 306.6 | 10.8 | 0.7 | 426 | 0.311 | 7.8 | 8.3 | 19.2 |
| | Winter | 49.21 | 35 | 11.2 | 248.3 | 102.8 | 0 | 1 | 373 | 0.314 | 7.7 | 13.2 | 13.1 |
| DF- F6F | Summer | 50.32 | 16 | 3.3 | 428.7 | 276.5 | 23.4 | 2.4 | 476 | 0.317 | 7.7 | 8.5 | 23.6 |
| | Winter | 50.32 | 31.5 | 1.7 | 349.7 | 185.8 | 0 | 1.1 | 409 | 0.344 | 7.6 | 11.7 | 13.1 |
| DF-DT | Summer | 56.41 | 35.3 | 4.8 | 751.4 | 306.6 | 0 | 3.6 | 533 | 0.383 | 7.1 | 6.7 | 20 |
| | Winter | 56.41 | 104 | 4.8 | 261.8 | 75.1 | 0 | 1.2 | 464 | 0.396 | 7.2 | 11.1 | 12.6 |
| DF-F5 | Summer | 61.39 | 17.6 | 9.6 | 188.7 | 134.3 | 9.7 | 2.8 | 206 | 0.147 | 7.5 | 6.5 | 20.1 |
| | Winter | 61.39 | 70.4 | 9.6 | 701.2 | 241.1 | 10.4 | 2.3 | 99 | 0.085 | 7.2 | 9.5 | 12.6 |
| DF- 2018-19 | Summer | 62.23 | 69 | 17.5 | 945.1 | 404.5 | 5.3 | 5.6 | 128 | 0.078 | 7.8 | 6.8 | 28.7 |
| | Winter | 62.23 | 31.5 | 1.7 | 491.6 | 148.9 | 0.2 | 3 | 184 | 0.163 | 7.4 | 8.2 | 11.3 |
| DF- 2018-20 | Summer | 62.89 | 30.4 | 11.2 | 444.8 | 329.2 | 0 | 1.3 | 277 | 0.179 | 6.9 | 2.3 | 23.3 |
| | Winter | 62.89 | 35 | 3.3 | 376.7 | 47.5 | 1.8 | 2.5 | 473 | 0.429 | 7.3 | 7.5 | 10.2 |

**Table 1** (continued)

| Stream | Season | Afforestation (%) | TP | $PO_4$–P | TN | $NO_3$–N | $NH_4$–N | SOM | k | TDS | pH | DO | Temp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DF-2018-1 | Summer | 67.06 | 28.8 | 3.2 | 343 | 306.5 | 0 | 0.8 | 60 | 0.041 | 6.1 | 6.5 | 22.8 |
| | Winter | 67.06 | 31.5 | 6.4 | 424 | 130.4 | 0 | 2.8 | 40 | 0.036 | 6.9 | 10.2 | 10.5 |
| DF-Forest 3 | Summer | 74.36 | 3.1 | 1.7 | 533.6 | 299.1 | 26.5 | 1.6 | 159 | 0.125 | 7.2 | 7.4 | 16.1 |
| | Winter | 74.36 | 17.3 | 8 | 397 | 93.6 | 8.1 | 4 | 82 | 0.08 | 7.7 | 11 | 7.6 |
| DF- F7 | Summer | 75.75 | 20.8 | 8 | 356.3 | 172.1 | 2.6 | 3.3 | 376 | 0.277 | 7 | 4.6 | 18.7 |
| | Winter | 75.75 | 36.8 | 4.8 | 613.3 | 213.4 | 6.5 | 1.5 | 115 | 0.099 | 6.8 | 9.8 | 12.5 |
| DF-2018-18 | Summer | 79.11 | 31.4 | 7.7 | 410.1 | 204.8 | 4.2 | 2.6 | 53 | 0.035 | 6.9 | 7.3 | 23.3 |
| | Winter | 79.11 | 36.8 | 8 | 363.2 | 130.4 | 1 | 2.5 | 418 | 0.32 | 7.5 | 13 | 17 |
| DF- 2018-13 | Summer | 79.37 | 70.6 | 30.1 | 694.9 | 306.6 | 6.1 | 3 | 100 | 0.061 | 7.2 | 6.3 | 28 |
| | Winter | 79.37 | 40.3 | 1.7 | 471.3 | 75.1 | 0 | 4 | 361 | 0.326 | 6.9 | 4.3 | 10.5 |
| DF- 2018-17 | Summer | 85.8 | 30.4 | 1.7 | 533.6 | 261.5 | 25.7 | 3 | 64 | 0.043 | 7 | 7.6 | 23.3 |
| | Winter | 85.8 | 35 | 0.1 | 424 | 112 | 0.2 | 3.7 | 318 | 0.231 | 7.6 | 10.8 | 19.4 |
| DF-2018-21 | Summer | 96.3 | 46.5 | 31.7 | 630.4 | 299.1 | 17.9 | 1.2 | 224 | 0.149 | 7.1 | 5.1 | 23.7 |
| | Winter | 96.3 | 33.2 | 3.3 | 288.8 | 65.9 | 9.7 | 1.5 | 412 | 0.365 | 7.3 | 8.9 | 11.1 |

*TP*: Total phosphorous, *$PO_4$-P*: Phosphate, *TN*: Total nitrogen, *$NO_3$-N*: Nitrate, *$NH_4$-N*: Ammonium, *SOM*: Suspended organic material, *k*: Conductivity, *TDS*: Total dissolved solids, *DO*: Dissolved oxygen, *Temp*: Temperature

In Uruguay, afforestation has led to the replacement of grassland ecosystems with *Eucalyptus* and *Pinus* plantations. The majority of *Eucalyptus* production is used by pulp mills, which account for 60% of the total production (Dirección General Forestal, 2022). For *Eucalyptus*, the plantation area occupies 1,087,109 effective hectares (6.21% of the total area of the country), and the main species used are *E. dunni, E. globulus, E. grandis, E. saligma, E. maidenii, E. bicostata,* and hybrid species (Dirección General Forestal, 2021). The selected 30 streams are subject to a *Eucalyptus* spp. afforestation gradient from 0.05 to 96% of their catchment area (defined as % afforestation cover) (Table 1). The image classification performed to estimate the afforested area also accounts for inner roads and green firebreaks, which usually occupy up to 30% of the forest-affected area, with the remaining 70% covered by trees. The age of *Eucalyptus* plantations varies greatly among the 30 selected streams, and in some areas, several harvest cycles have already been completed. In addition, sites with *Eucalyptus* plantations in their basins have a buffer area (20 m width) made up of grassland species (Decree N° 405–021, Art. 7 creation of the environmental registry of forest plantations, IMPO, 2021). Based on satellite images, sites with low or no tree cover in the basin area are primarily used for extensive livestock grazing, with less than one head per hectare in large grassland areas. Extensive livestock grazing land-use are among the least affected in the country (Benejam et al., 2016; Suárez et al., 2022). In some areas, cattle can be found inside the afforestation sites, making it difficult to separate the effects of both land uses. The study design, as well as the field and laboratory methods, should be carefully considered in light of these complexities.

Field point sampling campaigns were performed in the summer and winter of 2019 in the 30 streams during normal and homogeneous baseflow conditions and never during storm spate events. Physical and chemical parameters of the water, such as pH, conductivity (µS. $cm^{-1}$), the concentration of total dissolved solids (TDS, mg $l^{-1}$), temperature (°C), and dissolved oxygen ($O_2$, mg $l^{-1}$), were measured in situ once in each stream in both seasons using a YSI multiparameter probe (YSI Inc. V6600). Water samples were also taken for subsequent nutrient concentration analysis: total nitrogen (TN, µg $l^{-1}$), nitrate ($NO_3$–N, µg $l^{-1}$), ammonium ($NH_4$-N, µg $l^{-1}$) were analyzed according to the method developed by Müller & Wiedemann, (1955), and total phosphorous (TP, µg $l^{-1}$) and phosphate ($PO_4$–P, µg $l^{-1}$) according to Valderrama, (1981). A sub-sample of the integrated water sample (500 mL or until filter saturation) was filtered through GF/C glass microfiber filters (1.2 µm pore size and 47 mm filter diameter), and suspended organic material (SOM, mg $l^{-1}$) and total suspended solids (TSS, mg $l^{-1}$) concentrations were determined as the difference in dry (110 °C during 24 h) and ashed (500 °C during 15 min) filter weights (Bridgewater et al., 2017).

We collected three macroinvertebrate samples with a modified Surber net (25 cm width, 50 cm length, 50 cm height, 500 µm mesh size), consisting of a closed structure allowing sampling in low current waters and with high macrophyte cover. We tried to cover dominant microhabitats that followed these characteristics: those with high macrophyte cover, those without macrophytes, and those dominated by rocks and sand. These microhabitats are located in reaches with low slopes and little current, which homogenized the flow throughout the stream length and eliminated any riffles. The material collected was stored in plastic jars with ethanol (70%) and placed into cool boxes. In the laboratory, the material collected was rinsed under tap water using a 500 µm mesh sieve to separate the macroinvertebrates and then identified to genus level, except for some groups that were classified to subclass (Oligochaeta, Copepoda, and Collembola), suborder (Cladocera), family (Hirudinea and some Diptera and Coleoptera), and subfamily level (Chironomidae). Acari was identified as Hydracarina, the level used for water mites. Organisms were later classified into functional feeding groups (FFGs) using literature (Ramírez & Gutierrez-Fonseca, 2014).

Different standard macroinvertebrate metrics were selected as bioindicators of the potential effects of *Eucalyptus* spp. afforestation on the ecological conditions of stream ecosystems, including taxa richness, density as the number of individuals per area (no. ind. $m^{-2}$), diversity of taxa as the exponential of Shannon index, which gives us the result of the equally common species, which is the 2nd Hill number (Jost 2006; Chao et al., 2014), and the total biomass (mg dry mass. $m^{-2}$). The biomass was obtained from exponential or power regressions of length-dry mass, where the estimators (intercept and slope) of the models were obtained from references (Benke et al., 1999; Miserendino et al., 2001; Morley et al., 2020; Cummins et al., 2022). We also estimate the effects of *Eucalyptus* afforestarion on the proportion of sensitive and tolerant families based on the categorization of the different macroinvertebrate families to their response to anthropogenic disturbance in the region (e.g., Rodríguez-Capítulo et al., 2001; 2003; Ocón & Rodríguez-Capítulo, 2004; Strieder et al., 2006 Ocón & Rodríguez-Capítulo, 2012; Cortelezzi et al., 2019). We also used the EPT taxa richness and the EPT:Chironomidae index, which is the proportion of the taxa belonging to the sensitive orders Ephemeroptera, Plecoptera, and Trichoptera versus the proportion of tolerant families (Plafkin et al., 1989). Index values close to or equal to 1 suggest a good ecological condition; while, values under 0.5 indicate a poor ecological stream condition (Segnini 2003). Other bioindicators used were the proportion of specific tolerant groups such as Oligochaeta, Anisoptera, Zygoptera, and Chironomidae (Brand & Miserendino, 2015; Calvão et al., 2016). In the case of Chironomidae, subfamilies were used because they respond differently to changes in water quality and organic pollution (Rosa et al., 2014). We also used the proportion of different functional feeding groups, as they relate some ecosystem functionality to the effects of land-use changes because environmental degradation can influence food availability (Schmera et al., 2017; Ruaro et al., 2019).

## Statistical analyses

To evaluate the effects of the *Eucalyptus* afforestation gradient (% of the forested area) and its interaction with the season (summer and winter) on the physical an chemical water variables and macroinvertebrate metrics, general linear models were performed (GLM). The distributions of the physical and-chemical and macroinvertebrate metrics as response variables were previously tested using the ’fitdisplus’ package (Delignette-Muller & Dutang, 2015) to select the corresponding exponential families to use in the GLMs (Zuur et al., 2009). As a result of this, the Gamma family (log-link) was used for TN, $NH_4$–N, TP, $PO_4$–P, and SOM., Whereas the Gaussian family (identity-link) was used for $NO_3$–N, pH, and $O_2$; and the Gaussian family (log-link) was used for TDS, and conductivity. In the case of macroinvertebrate metrics, the Gaussian family (identity-link) was used for taxa richness, the Gaussian family (log-link) was used for the density of macroinvertebrates, and the Gamma family (log-link) was used for the Shannon diversity index. The Beta family (logit-link) was used for the proportions of the rest of the taxa and FFGs selected. All models included the season as a covariable with the afforestation gradient (measured as percentage of the total cover). For the selection of the final models, the ‘step-wise’ function was used with the analyses of the Akaike information criteria (AIC) and the visual inspection of the residuals (Zuur et al., 2009). We used the Cleveland dot-plot and boxplot for data cleaning criteria for outliers to identify and remove the values higher than 99.5% percentile (Zuur et al., 2010). Models were constructed using the R software (R Core Team, 2022), and the packages ‘betareg’ (Cribari-Net & Zeileis, 2010), and for the construction of the graphics, we used the predict function and the ‘ggplot’ package (Wickham, 2016).

We used nonmetric multidimensional scaling (NMDS) ordinations to look at the effects of afforestation gradient and water parameters on the structure of macroinvertebrate assemblages. For this, we first constructed a Bray–Curtis dissimilarity matrix describing the distribution of macroinvertebrate taxa between summer and winter, followed by a one-way factorial permutational multivariate analysis of variance (PERMANOVA, $\alpha = 0.05$) (Anderson, 2011). Then, we fit afforestation cover and water variables to the ordination (after checking for highly correlated variables; Fig. S2) using 999 permutations. The results are shown in a plot with the environmental variables as vectors scaled by their correlation (Oksanen et al., 2013). Indicator species analyses were used to detect the macroinvertebrate taxa that separated each group. For this, 9999 permutations were used. NMDS and PERMANOVAs were done with the functions ‘metaMDS’ and ‘adonis’, respectively, and the environmental variables were fitted with the ‘envfit’ function in the “vegan” R package ‘vegan’ (Oksanen et al., 2013). For indicator species analyses, the ‘indicspecies’ R package was used with the ‘multipatt’ command and the Monte Carlo test with 9999 permutations (de Caceres et al., 2010).We estimated the thresholds of macroinvertebrate metrics for afforestation and water parameter gradients using the Thresholds Indicator Taxa Analysis (TITAN, Baker & King, 2010). TITAN can detect the change point (King & Richardson, 2003) and the indicator value (Z-value) (Dufrêne & Legendre, 1997) in the frequency of occurrence of the macroinvertebrate metrics along the gradients of environmental variables. Positive (Z+) values indicate a positive response at the change point, which can be related to a tolerant response to the gradients. On the contrary, negative (-Z) values indicate a negative response to the change point and indicate that the macroinvertebrate metric is sensitive to environmental gradients. To account for the quality of the response (Z-values) and confirm the thresholds of each macroinvertebrate metric, the purity and reliability were obtained by resampling using the bootstrap method (500 resamples with replacement). Purity corresponds to the proportion of change points (if Z + or Z−) along the resampling that matches with the observed values, and reliability corresponds to the proportion of the resampling resulting in an indicator value accompanied by a *p*-value of significance (Baker & King, 2010). Taxa densities were $\log(x + 1)$ transformed to reduce the influence of highly variable taxa on indicator score calculations in each data set. Taxa with the occurrence of < 5% of the samples were deleted to remove outliers and minimize the effect of a potential operator bias (Baker & King, 2010). TITAN analyses were performed using the TITAN2 packages (Baker et al., 2015). All analyses were performed in R version 4.2.1 (R Development Core Team, 2022).

## Results

### Water quality

Some water quality parameters measured at some sites in this study (Table 1) presented values outside those allowed by national standards for water quality established by DINACEA (National Bureau of Environment; Decree 253–79, Water Code law). For instance, the values of total phosphorus (TP) ranged from 3.03 to 123.7 µg $l^{-1}$ and the higher values usually corresponded to the streams with medium and high afforestation cover (> 50%) usually exceeding the maximum allowed limit (25 µg $l^{-1}$ in both seasons). In sites with less afforestation cover, this maximum allowed limit was reached in winter. In the case of nitrogen fraction concentrations, $NO_3$–N values ranged from 10.6 to 404.5 µg $l^{-1}$ and $NH_4$-N ranged from 0 to 26.5 µg $l^{-1}$, and in both cases concentrations were also higher than the maximum allowed limit (20 µg $l^{-1}$ for $NH_4$–N and 1000 µg $l^{-1}$ for $NO_3$–N), especially in summer (Table 1).

Total nitrogen concentrations ranged from 118.1 to 945.1 µg $l^{-1}$ with a tendency to increase with afforestation cover, but no seasonal pattern was detected. For

$PO_4$-P concentrations, values ranged between 0.08 and 91.8 µg $l^{-1}$ and higher values corresponded to sites with medium to high afforestation cover, occurring in most cases in summer (Table 1). The pH values (ranging from 6.1 to 8.3) were within the limits accepted by the national standards (6.5–8.5) with no pattern according to season or afforestation gradient. The dissolved oxygen concentrations in water (ranging from 2.3 to 13.2 mg $l^{-1}$) in some streams with high afforestation showed lower values than those established by the National Standard values (minimum acceptable limit = 5 mg $l^{-1}$), but the lowest values corresponded with sites with high afforestation cover, especially in summer.

Values of suspended organic material ranged from 0.71 to 7.7 mg $l^{-1}$ with no pattern according to the afforestation gradient, but with a tendency to increase in winter. Regarding total dissolved solids and conductivity, values ranged from 0.02 to 0.43 mg $l^{-1}$ and from 30 to 533 µS. $l^{-1}$, respectively. In both cases, values tended to increase with the afforestation gradient in winter. Range temperature values were 16.1–28.7 in summer and 11.9 °C 7.6–19.4 in winter (Table 1).

Selected models indicated that physical and chemical water variables showed significant responses to the afforestation gradient, which accounted for between 11 and 61% of the explained variance (Table S2). ncreased afforestation cover increased nutrient concentrations such as TN ($R^2 = 0.11$; *p-value* = 0.02), $NO_3$–N ($R^2 = 0.26$; *p-value* = 0.001), $NH_4$–N ($R^2 = 0.18$; *p-value* = 0.009), TP ($R^2 = 0.18$; *p-value* = 0.01), and $PO_4$–P ($R^2 = 0.12$; *p-value* = 0.04); while dissolved oxygen concentration decreased ($R^2 = 0.61$; *p-value* = 0.001) (Fig. 1; Table S2). The afforestation cover had no significant effects on the total suspended solids, nor did its interaction with the season ($R^2 = 0.01$; *p-value* = 0.84; Fig. 1; Table S2). For most of the water variables, the direction of the responses depended on the season: for $NO_3$–N, $NH_4$–N, and $PO_4$–P, concentration showed a tendency to increase with the afforestation gradient in the summer, but in the winter, the values tended to decrease with increasing afforestation. Contrarily, the conductivity, and total suspended solids, showed a tendency to increase in winter, but their values decreased in summer (Fig. 1). Values of dissolved oxygen decrease in both seasons with the increase of afforestation, but values were significantly lower in the summer. pH ($R^2 = 0.13$; *p-value* = 0.02), SOM ($R^2 = 0.13$; *p-value* = 0.02), and TSS ($R^2 = 0.01$; *p-value* = 0.84). did not show a significant change along the afforestation gradient, but in the case of pH and suspended organic material, there was a significant increase in their values in winter (Fig. 1; Table S2).

## Biological indicators

The best models selected indicated that most macroinvertebrate metrics showed significant relationships with the afforestation gradient except for taxa richness ($R^2 = 0.04$; *p-value* = 0.28), density ($R^2 = 0.000$; *p-value* = 1), total biomass ($R^2 = 0.003$; *p-value* = 0.34), and Orthocladiinae ($R^2 = 0.01$; *p-value* = 0.84) which showed no significant response. Some metric only showed significant variations among seasons such as Anisoptera ($R^2 = 0.15$; *p-value* = 0.01), Tanypodinae ($R^2 = 0.30$; *p-value* = 0.001), collectors-filters ($R^2 = 0.14$; *p-value* = 0.03) and predators ($R^2 = 0.29$; *p-value* = 0.001) (Fig. 2; Table S4). The metrics with a significant response to the afforestation gradient usually showed similar tendencies among both winter and summer seasons (Fig. 2). The proportion of sensitive families (Table S3) ($R^2 = 0.19$; *p-value* = 0.002), and the EPT:Chironomidae index ($R^2 = 0.21$; *p-value* = 0.001), showed a negative response to the increase in afforestation gradient (Fig. 2). The EPT index ($R^2 = 0.15$; *p-value* = 0.02) only showed a negative response to afforestation in summer. The proportion of tolerant families (Table S3) ($R^2 = 0.12$; *p-value* = 0.02), Chironominae ($R^2 = 0.12$; *p-value* = 0.03), and Oligochaeta ($R^2 = 0.19$; *p-value* = 0.001) showed a positive response to the increase in afforestation in both seasons (Fig. 2). The response of Zygoptera ($R^2 = 0.26$; *p-value* = 0.002) showed different responses between both seasons and a positive response to the afforestation gradient was evident in summer, but in winter, a contrary response was obtained (Fig. 2; Table S4). The scrapers ($R^2 = 0.19$; *p-value* = 0.002) showed a negative response to the afforestation in both seasons, the shredders ($R^2 = 0.29$; *p-value* = 0.001) showed a positive response to the afforestation cover, but in the case of collectors-gatherers ($R^2 = 0.07$; *p-value* = 0.21), this response was evident in winter (Fig. 2; Table S4).

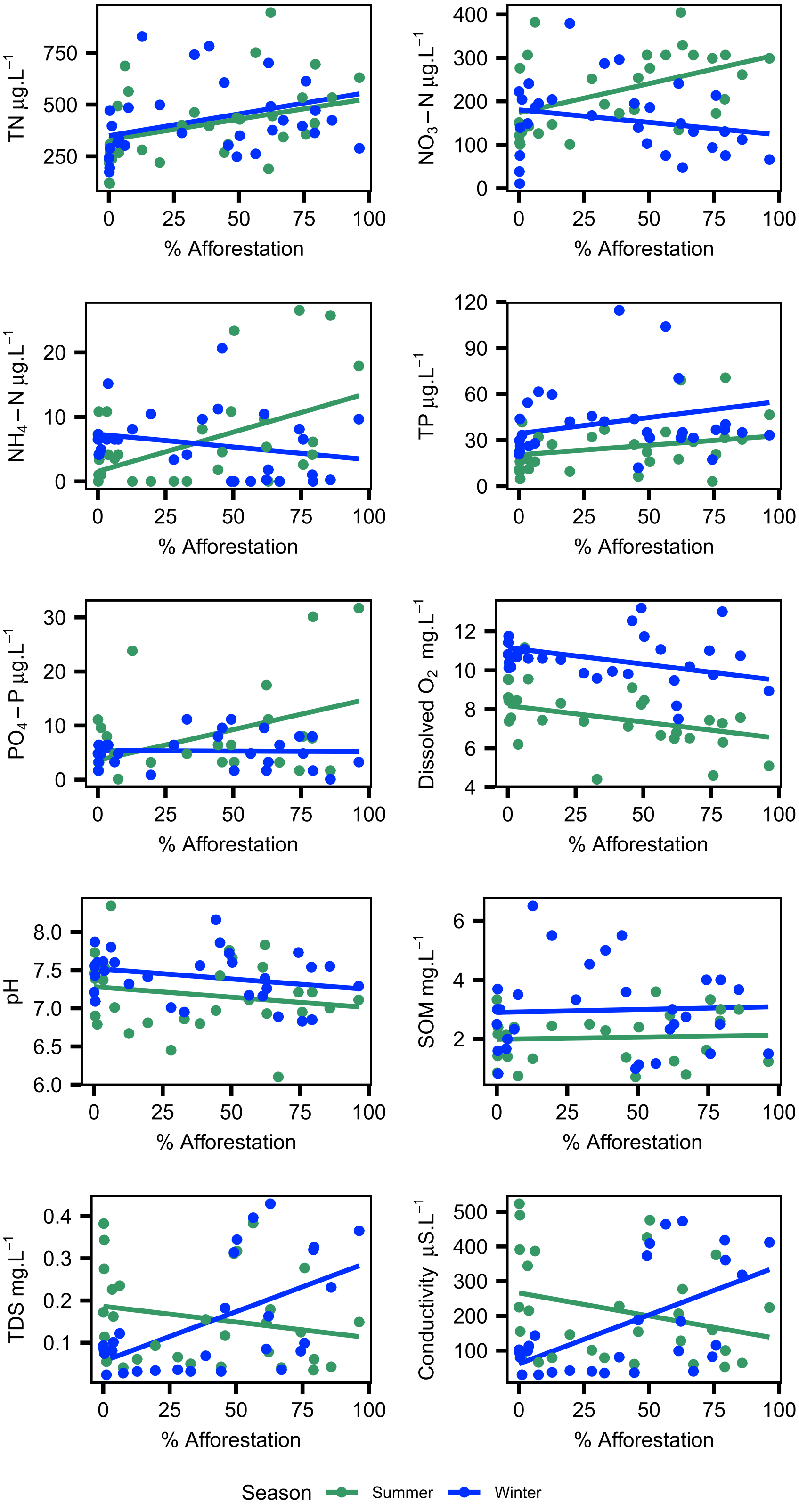
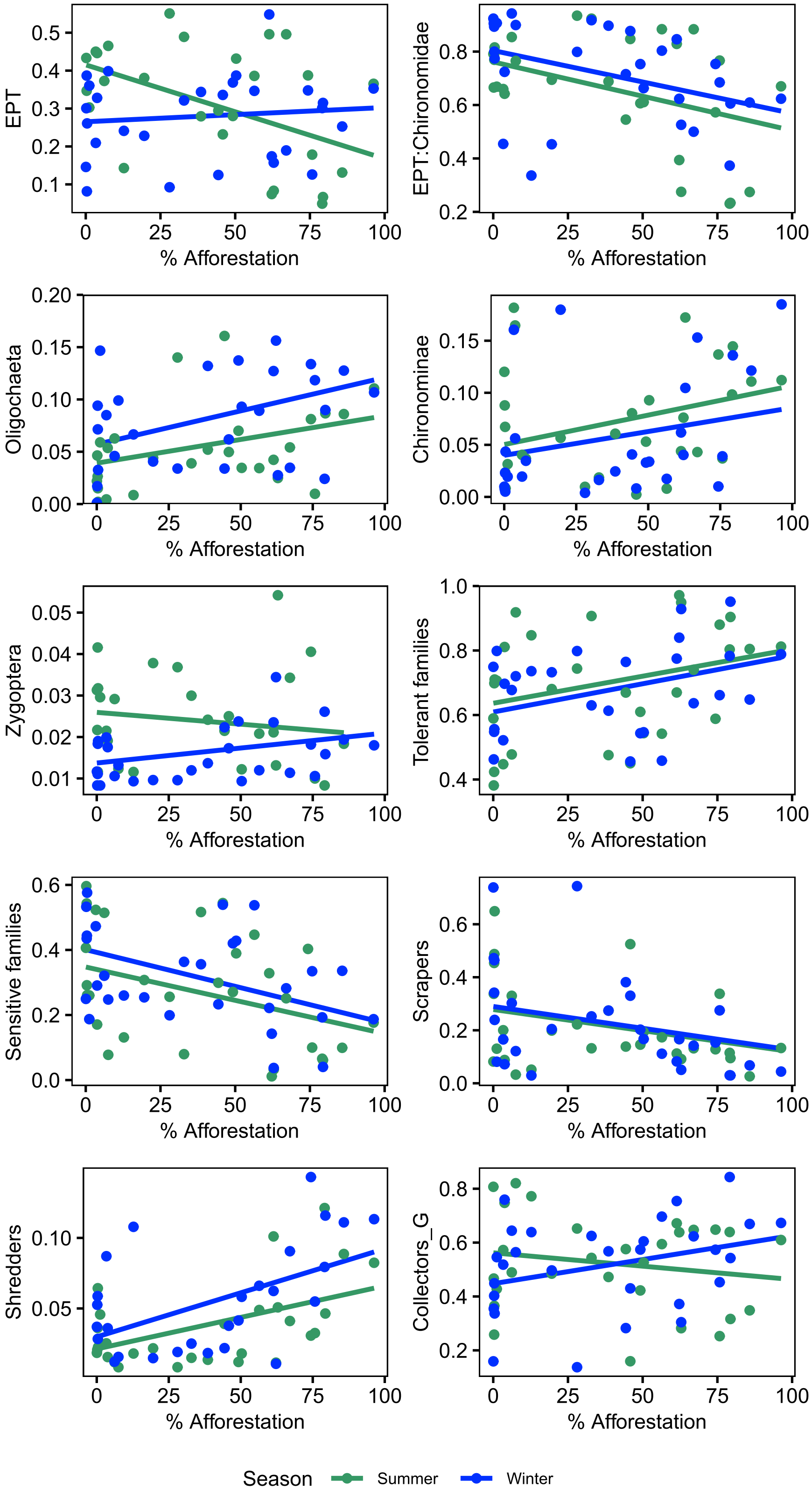


**Fig. 1** Prediction of best models for the responses of physical and chemical water variables to the *Eucalyptus* afforestation gradient (% Afforestation) and season. Total nitrogen (TN), nitrate ($NO_3$–N), ammonium ($NH_4$–N), total phosphorous (TP) and phosphate ($PO_4$–P), dissolved oxygen ($O_2$), suspended organic material (SOM), total dissolved solids (TDS). The mean of the response variables were back transformed using the exponential of the model parameters

**Fig. 2** Prediction of best models for the responses of macroinvertebrate metrics to the *Eucalyptus* afforestation gradient (% Afforestation) and season



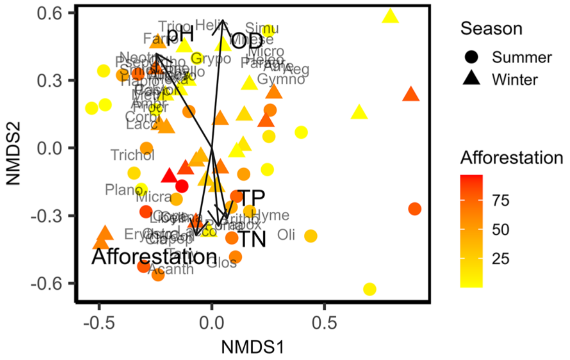


**Fig. 3** Non-metric Multidimensional Scaling (NMDS) ordination of de distribution of macroinvertebrate assemblage separated by summer and winter season and the fitted vectors for significant ($p < 0.01$) *Eucalyptus* afforestation gradient and water quality variables (black arrows). Stronger relationships are represented by longer vectors. Abbreviations: *OD*: Dissolved oxygen, *NT*: Total nitrogen. Stress: 0.18. Strong predictors showed longer vectors and their direction cosines in the ordination space indicating which environmental vector has maximal correlation with the ordination gradient. List of full names of macroinvertebrate taxa are in Appendix S1—Supplementary material

## Macroinvertebrate assemblage ordination

The ordination of macroinvertebrate assemblages did not show a visual split of the streams into groups according to differences in macroinvertebrate assemblage densities between summer and winter (Stress = 0.19; Fig. 3). However, the PERMANOVA test showed these differences were highly significant (PERMANOVA; $F = 3.73$; $p < 0.001$). When the environmental variables were fitted in the NMDS plot (Table 2), the *Eucalyptus* afforestation and the TN concentration variables were related to the taxa that reported higher densities in summers, such as Tanypodinae (Chironomidae), *Oxyethira* (Hydroptilidae), *Paraponyx* (Crambidae), Orthoptera, Glossiphonidae (Hirudinea), *Laccophilus* (Dytiscidae), *Acanthagrion* (Coenagrionidae), *Castoraeshna* (Aeshnidae), *Campsurus* (Polymitarcyidae), and *Aphylla* (Gomphidae). In comparison, the concentration of dissolved oxygen and pH were related to taxa with higher densities in winters, such as *Gripopteryx* and *Paragripopteryx*, (Gripopterygidae), *Americabaetis* (Baetidae), Dugesiidae, *Simulium* (Simuliidae), Elmidae, *Belostoma* (Belostomatidae), *Hyalella* (Hyalellidae), *Physa* (Physidae), and Planorbiidae. (Table 2).

**Table 2** Environmental explanatory variables (afforestation gradient and water quality parameters) that had maximal correlation with the ordination gradient ($p$ value < 0.05)

| Environmental variables | NMDS1 | NMDS2 | $p$ value | |
|---|---|---|---|---|
| Afforestation | − 0.070 | − 0.387 | 0.007 | ** |
| TP | 0.068 | − 0.312 | 0.060 | |
| $PO_4$–P | − 0.042 | − 0.161 | 0.449 | |
| TN | 0.030 | − 0.345 | 0.036 | * |
| $NO_3$–N | − 0.057 | − 0.195 | 0.279 | |
| $NH_4$–N | − 0.136 | 0.232 | 0.120 | |
| SOM | − 0.011 | − 0.187 | 0.367 | |
| Conductivity | − 0.179 | 0.111 | 0.271 | |
| pH | − 0.247 | 0.419 | 0.001 | *** |
| OD | 0.049 | 0.567 | 0.002 | *** |

*TP*: Total phosphorous, *$PO_4$-P*: Phosphate, *TN*: Total nitrogen, *$NO_3$-N*: Nitrate, *$NH_4$-N*: Ammonium, *SOM*: Suspended organic material, *DO*: Dissolved oxygen

## The TITAN analysis

The TITAN revealed changes in macroinvertebrate assemblages along afforestation and water quality parameters gradients. The assemblage threshold of the *Eucalyptus* afforestation occurred at 0.34% for sensitive (Z−), and 79.36% for tolerant (Z+) taxa (Table 3). 14 macroinvertebrate taxa were identified as robust bioindicators of *Eucalyptus* afforestation (purity and reliability ≥ 0.95; Fig. 4; Table S5). Among these taxa, 10 were negative indicators (sensitive taxa, Z−) and 4 were positive indicators (tolerant taxa, Z+) of the increase of the afforestation gradient (Fig. 4; Table S5): The densities of the most sensitive taxa such as *Phylloicus* (Calamoceratidae), *Helycopsyche* (Helycopsychidae), *Tricorythopsys* (Leptohyphidae), *Mneserate* (Calopterygidae), had a narrow response in the increase of afforestation gradient (5th–95th percentile = 0.24–16.2%). Other taxa classified as sensitive but with broader intervals upper change-point limit (95th percentile) were *Farrodes* (Leptophlebiidae), *Neotrichia* (Hydroptilidae), *Psephenus* (Psephenidae) (5th–95th percentile = 0.3–75.1%). Other less sensitive taxa such as *Polycentropus* (Polycentropodidae), *Thricorythodes* (Leptohyphidae), and *Heleobia* (Cochliopidae) had broad quantile intervals spanning most of the range of the afforestation gradient (5th–95th percentile = 3.6–79%; Fig. 4; Table S5). Tolerant taxa such

**Table 3** Assemblage-level thresholds estimated from macroinvertebrate assemblage responses to afforestation gradient and water quality parameters selected

| Gradients | Sum(Z) | cp | 5% | 10% | 50% | 90% | 95% | N taxa | % Total taxa |
|---|---|---|---|---|---|---|---|---|---|
| *Eucalyptus* afforestation (%) | Z- | 0.3 | 0.3 | 0.3 | 0.3 | 6.9 | 6.9 | 10 | 11.1 |
| | Z+ | 79.4 | 35.7 | 45.1 | 62.6 | 82.5 | 82.6 | 4 | 4.4 |
| TN (µg $L^{-1}$) | Z- | 206.8 | 173.1 | 181.3 | 254.8 | 322.0 | 328.7 | 12 | 13.3 |
| | Z+ | 690.9 | 369.9 | 412.0 | 621.8 | 721.5 | 726.3 | 3 | 3.3 |
| DO (mg $L^{-1}$) | Z- | 10.6 | 11.2 | 11.1 | 10.7 | 10.6 | 10.2 | 15 | 16.7 |
| | Z+ | 9.3 | 9.5 | 9.5 | 8.2 | 4.5 | 4.3 | 10 | 11.1 |
| pH | Z- | 7.55 | 7.83 | 7.69 | 7.60 | 7.54 | 7.52 | 10 | 11.1 |
| | Z+ | 6.82 | 7.40 | 7.39 | 6.83 | 6.81 | 6.81 | 2 | 2.2 |

Sum(z) associated with decrease (Z−) or increase (Z+) along the gradient; "cp" is the assemblage change point; 5%, 10%, 50%, 90% and 95% are bootstrap quantile intervals capturing true thresholds. *TN*: Total nitrogen, *DO*: Dissolved oxygen

as Corixidae, Collembola, *Laccophilus* (Dytiscidae), and Copepoda had broad quantile intervals spanning most of the range of afforestation gradient (5th–95th percentile = 1.2–79.4%; Fig. 4; Table S5).

The assemblage threshold of TN concentration occurred at 206.8 µg $l^{-1}$ for sensitive taxa (Z−), and at 690.9 µg $l^{-1}$ for tolerant taxa (Z+) (Table 3). Among the 13 taxa detected as robust bioindicators of TN, 12 were sensitive to the increase of TN concentrations. In this case, *Helycopsyche, Tricorythodes, Farrodes, Phylloicus, Polycentropus, Psephenus, Neotrichia*, and *Smicridea*, showed a narrow threshold with the increase of TN (5th–95th percentile = 215 – 396 µg $l^{-1}$; Fig. 4; Table S5) but other less sensitive taxa had broad intervals upper change-point limits (95%) such as *Tricorythodes, Polycentropus, Helycopsyche*, and *Psephenus* (5th–95th percentile = 258.2–609.9 µg $l^{-1}$; Fig. 4; Table S5). Only 3 taxa such as Copepoda, *Aphylla*, and *Tipula* were identified as strong tolerant predictors (Z+) with broad quantile intervals spanning most of the range of TN water concentration (5th–95th percentile = 2285.1– 721.5 µg $l^{-1}$; Fig. 4; Table S5).

The assemblage threshold for concentration of dissolved oxygen occurred at 10.6 mg $l^{-1}$ for sensitive taxa, and at 9.3 mg $l^{-1}$ for tolerant taxa (Table 3). Among the 25 robust indicator taxa, 15 of them were considered sensitive to the decrease of the concentration of DO. Some sensitive taxa had a narrow response to the decrease of DO (5th–95th percentile = 11.7–6.4%) such as *Neotrichia, Hydroptila, Anacroneuria, Alisotrichia, Tricorythopsys, Smicridea, Simulium, Paragripopteryx, Microcylloepus, Farrodes, Tricorythodes, Gripopteryx, Haplohyphes*, and Dugesiidae (Fig. 4; Table S5). The densities of the most tolerant taxa such as *Cladocera, Pomacea, Paraponyx, Laccophilus, Callibaetis,* Forcipomyiinae (Ceratopogonidae), Collembola, *Acanthagrion,* Tanypodinae, and *Aphylla,* had a variable response to the decrease of DO concentration (5th–95th percentile = 11.3–6.5%), but Cladocera had a broader quantile interval (5th–95th percentile = 9.5–5.1%) (Fig. 4; Table S5).

In the case of the response to the decrease of pH, the assemblage threshold occurred at 7.55 and 6.82 for sensitive and tolerant taxa respectively (Table 3). Among robust sensitive taxa (9), *Tricorythopsys, Mortoniella, Macrelmis, Anacroneuria,* and *Enocrus,* had higher densities when pH is higher (5th–95th percentile = 7.86–7.56; Fig. 4; Table S5), but other less sensitive *Smicridea, Hydroptila, Argia, Tricorythodes,* and Orthocladiinae increased their densities when pH is lower (5th–95th percentile = 7.55–6.81; Fig. 4; Table S5). Tolerant robust taxa (2) densities showed higher values at lower pH (5th–95th percentile = 7.48–6.82; Fig. 4; Table S5).

## Discussion

Our results showed a significant negative relationship between the increase in *Eucalyptus* afforestation in the catchments and most of the selected water quality parameters and biotic metrics, as compared to streams with areas dedicated to extensive livestock grazing and less afforestation.

We found a consistent response pattern in the 30 models across a wide range of afforestation in

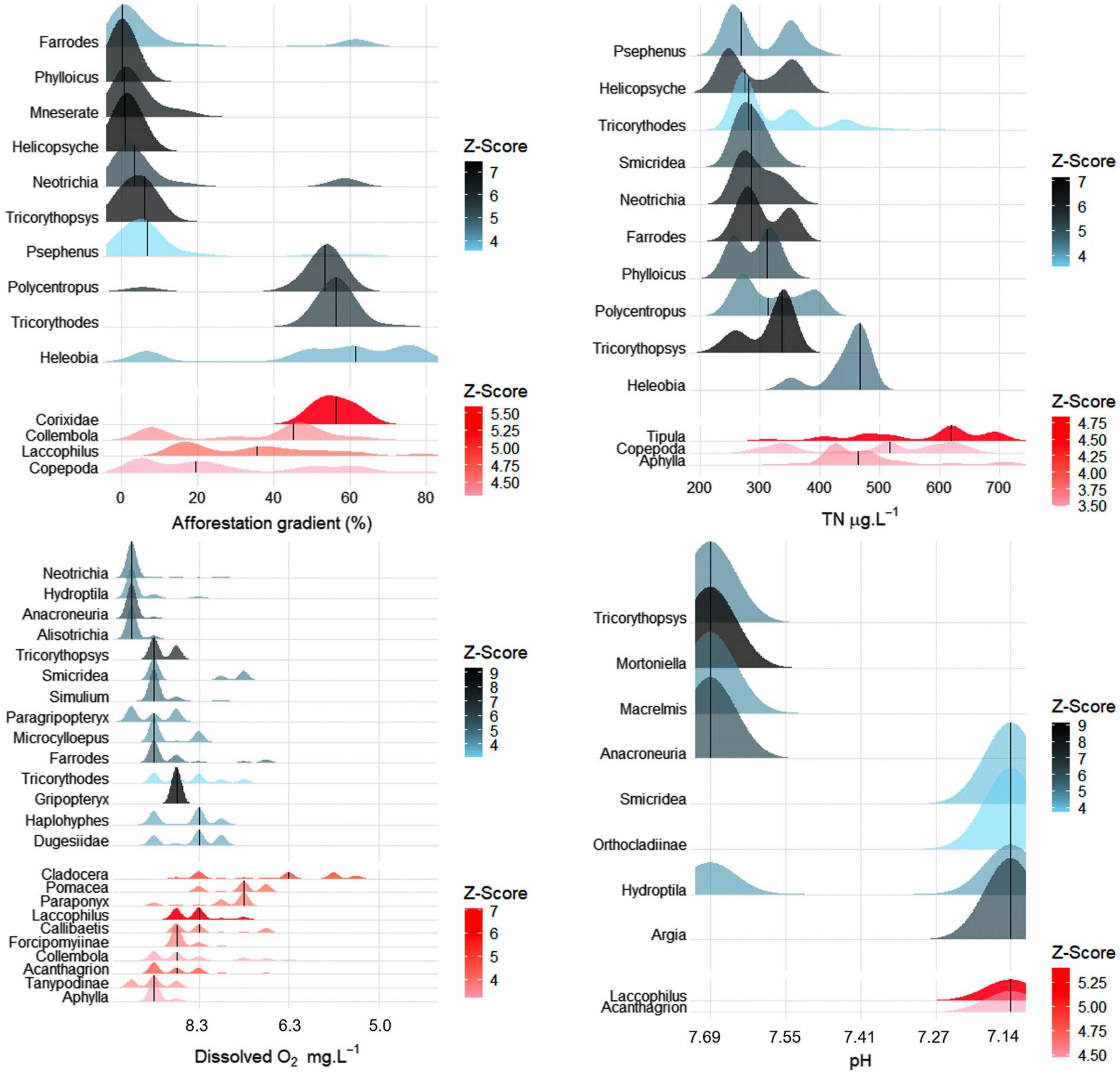


**Fig. 4** Threshold indicator analysis (TITAN) of macroinvertebrate assemblage response to *Eucalyptus* afforestation gradient and water quality parameters showing significant response ($p < 0.05$) indicator taxa. Z− taxa decreased and Z+ taxa increased with the environmental variables represented in blue and red scales, respectively, and the color intensity is proportional to the magnitude of the response. The color area under the curve represents the 5th and 95th percentiles among 500 bootstraps and black vertical bars indicate the change point of each taxon

all of Uruguay's major forested areas, in different geomorphological zones, and with different forest management activities like fertilizing and harvesting (e.g., fertilization and harvesting). Water quality parameters showed significant responses to the afforestation gradient, and in some cases, the response varied according to the season (Fig. 1). In this case, nutrient concentrations showed a tendency to increase with the afforestation gradient in summer. However, in winter, the values tended to decrease (Fig. 1). Results obtained for N concentrations from other streams in Uruguay, showed a contrary response to the season, which may be related to higher temperatures in summer promoting denitrification processes and a higher N assimilation by aquatic macrophytes and periphyton (Goyenola et al., 2021). In summer, biomass production of autochthonous primary producers is expected to be higher (Giorgi et al. 2005); however, in our streams, the biomass of macrophytes was similar between seasons but periphyton biomass was higher in winter (Barrios, *unpublished data*).

Another explanation related to the increase of nutrient concentration in warmer seasons is probably related to the leaf decomposition rates (Silsbee & Larson, 1982). In temperate latitudes where leaf fall peaks occur in autumn, nutrients present in the leaf initially are used by heterotrophic microorganisms (such as bacteria and hyphomycete fungi) accompanied by nutrient release via leaching, and due to less nutrient uptake by primary producers, nutrients are accumulated in water in winter (Silsbee & Larson, 1982; Biasi et al. 2017). In our case, leaf fall usually does not follow the same period as temperate zones; instead, it occurs at different moments between the species (Heretche et al., 2012). In addition, in lowland prairie streams with low or no cover or riparian trees, the input of allochthonous vegetation (grass and herbs) and *Eucalyptus* leaf litter occurs with the lateral movement of material promoted by the wind or by stochastics flood pulses (Burwood et al., 2021; Barrios et al., 2022). Thus, the interaction of the season with the afforestation may be explained by the higher leaf abscission of *Eucalyptus* leaf in summer (Canhoto et al. 2013), which may enter together with the organic material that comes from the buffer zones increasing the nutrient concentration in water (Canhoto et al., 2013). The increase in nutrient concentrations observed in the stream may be explained by the inputs of leaves, which promote higher microbial respiration rates and faster leaf decomposition rates, particularly under conditions of higher temperatures and lower dissolved oxygen concentrations (Manning et al., 2021).

Other basin scale variables like hydrology or instream characteristics may also operate to influence our results (Mulholland & Hill 1997). In this case, seasonal changes could be related to hydrological and biogeochemical mechanisms determining the mobilization or retention of nutrients, sediments, and *Eucalyptus* leaf litter from the soil, groundwater, and finally into the streams (Allen & Chapman, 2001; Farley et al., 2008; Rodrigues et al., 2019; de Barros Ferraz et al., 2019). The decrease of streamflow in catchments under afforestation is explained by the increase in evapotranspiration and groundwater removal by the *Eucalyptus* deeper root system (Calder, 1992; Allen & Chapman, 2001; Jobbágy et al., 2012; de Barros Ferraz et al., 2019). This could be reflected by a decrease in water volume and an increase in nutrient mineralization, together with an increase in organic matter decomposition, which may generate a major concentration of water nutrients in the summer (Manning et al., 2021). However, additional complementary studies are needed to better understand the combined effects of afforestation and seasonality on hydrology and ecological processes, such as decomposition. Other potential variables that should be considered include stream flow, groundwater recharge, and water runoff. Measuring these environmental factors may provide a more comprehensive understanding of the impact of afforestation on stream ecosystems.

Mobilization or retention of nutrients varies according to the tree-growing stage and the moment of forest management practices, and this may influence the diffuse entry of nutrients into the stream (Shah et al., 2022). In this case, nitrogen transport into the stream can be lower when nutrient demands by *Eucalyptus* trees are high, especially in the young growth stages of trees, and nutrients are retained and converted into biomass (Allen & Chapman, 2001). Contrarily, higher nutrient concentration, organic matter transport, and siltation in stream water can occur after clearing due to nitrification in soil water accompanied by higher soil erosion and run-off (Rodrigues et al., 2019; Shah et al., 2022). In our case, we do not account for the dates of forest management practices and tree-growing stages at the moment of sampling, but these variables may also influence the response of water parameters to the afforestation gradient in our study. Therefore, it is necessary to consider forest management factors in our models to explain seasonal changes in water quality.

Despite the significant impacts of *Eucalyptus* afforestation on water quality parameters found in our study, these impacts are lower than those of urban and intensive agriculture. For instance, urban and intensive agricultural land use are considered to promote eutrophication and higher pollution levels in Uruguayan streams (e.g.,Goyenola et al., 2015; Benejam et al., 2016; Chalar et al., 2017; Griffero et al., 2019; Soutullo et al., 2020; Alvareda et al., 2020; Goyenola et al., 2021; Alcantara et al., 2022; Suárez et al., 2022; Barrios & Teixeira de Mello, 2022). The presence of a buffer area in streams with afforestation cover in our study may also explain why the increase in nutrients and suspended soils is low (Shah et al., 2022). However, in our study, other forest management practices such as the combined use of afforestation and the presence of livestock, which in most cases have access to buffer zones and streams, may contribute to a greater input of nutrients and organic matter.

In the case of the macroinvertebrate assemblage, the sampled methods were appropriate and allowed for obtaining a high richness and abundance of organisms effectively. The results obtained from the models using different macroinvertebrate metrics and the use of TITAN were efficient in accounting for the response of the aquatic organisms to the increase in *Eucalyptus* afforestation. In addition, the use of TITAN represents an important contribution to understanding at what point of change, whether small or abrupt, in the *Eucalyptus* afforestation gradient or water quality parameters, the macroinvertebrate assemblage responds with changes in their densities (Baker & King, 2010). Identifying these thresholds may have important implications for managing ecological tools in our region (Rodrigues et al., 2016; Campos et al., 2021).

We found no significant association between diversity and density of macroinvertebrate assemblages with the increase in *Eucalyptus* afforestation, in contrast to other research where this land use has significant negative effects on these macroinvertebrate attributes (e.g., Larrañaga et al., 2009; Fierro et al., 2016; 2017). In those cases, the negative effects of *Eucalyptus* afforestation have been related to the input of *Eucalyptus* leaves into the streams, affecting the microbial colonization and consequently the increase of palatability of leaves as food resources for macroinvertebrates (Graça et al., 2002). However, in other regions, the potential effects of *Eucalyptus* afforestation on macroinvertebrates have been inconsistent, which could be related to differences in the hydrological regime and the presence of a buffer area of native vegetation that limits the input of *Eucalyptus* leaves (Graça et al., 2002). In our case, the studied streams with afforestation areas have a buffer area ($\geq$20 m) with natural grassland vegetation, which may explain the lack of response in terms of diversity and density of the macroinvertebrate assemblage. Other potential effects of *Eucalyptus* afforestation on macroinvertebrate assemblages could be related to the changes in physical and chemical water quality parameters (Fig. 3). For instance, the increase in conductivity, concentrations of nutrients, and suspended sediments in streams have been related to changes in the macroinvertebrate assemblage composition (Fierro et al., 2017). Besides, other in-stream microhabitat variables (e.g., substrate heterogeneity and macrophyte cover) may be important in shaping these organisms' richness and abundance (Muñoz-Mas et al., 2019; Ferreira et al., 2021).

In our study, the proportion of sensitive families, the EPT, and EPT:Chironomidae indexes, and the proportion of scrapers responded negatively to the afforestation gradient; however, the EPT index showed a significant response only in the summer. This seasonal response of the EPT index to the afforestation gradient may be related to the seasonal variation of different taxa in this group (Callanan et al., 2008). Another explanation of the response of the EPT index is that some families, such as Caenidae, Baetidae, and Hydropsychidae may have a higher tolerance range (Chang et al., 2014; Masese & Raburu, 2017) and the variation of their abundance may influence the response of the index. TITAN showed that a low increase in the *Eucalyptus* afforestation was sufficient to trigger a significant decrease in the densities of sensitive taxa, mostly EPT. These taxa usually respond negatively to water nutrient enrichment and low oxygenated waters (Wang et al., 2007; Miserendino et al., 2011; Wagenhoff et al., 2017; Horak et al., 2020) which were the conditions that prevailed in streams with high afforestation, and thus, the densities of EPT decrease under high concentrations of TN and low dissolved oxygen (Fig. 4). Among taxa classified as scrapers, some of them were represented in the TITAN as sensitive (e.g., *Farrodes*, *Neotrichia*, *Helycopsyche, Alisotrichia, Hydroptila*, *Psephenus*) which may explain their negative response of this FFG to the afforestation increase. Trophic enrichment, low oxygenated waters, and low pH may affect the grazer activity of these organisms, with consequences for bloom algae production (Woodward et al., 2012).

Our results showed that the proportion of tolerant families, Chironominae, Oligochaeta, and the proportion of shredders and collectors-gatherers showed a positive response to the increase in *Eucalyptus* afforestation in both seasons, but Orthocladiinae and Tanypodinae did not show significant responses. Chironominae (*Chironomus* genus), and Oligochaeta can tolerate lower water quality conditions, especially low dissolved oxygen concentrations, and due to their feeding behavior of organic material in the sediments, they can tolerate high organic pollution (Marques et al., 1999; Rosa et al., 2014). In contrast, Orthocladiinae and Tanypodinae can be more sensitive and have been associated with a higher concentration of dissolved oxygen (Marques et al., 1999; Rosa et al., 2014). Contrarily, the threshold responses of these taxa in our study, showed that Tanypodinae had a robust response tolerating low concentrations of dissolved oxygen, but Orthocladiinae showed a sensitive response to the decrease in pH (Fig. 4). This suggests that the classification of Chironomidae to subfamily and Oligochaeta at order sub-class levels may be insufficient to account for changes in the water quality parameters and afforestation gradient. For instance, among Oligochaeta taxa, *Pristina* and *Aulodrilus* may show differences among different levels of organic and metal pollution, and some species are considered sensitive (Capurro et al., 2021). Concerning FFGs, shredders, and collectors-gatherers feed on organic matter in the sediments (da Silva et al., 2007; Rodrigues et al., 2019; Larrañaga et al., 2021), which may be associated with soil erosion due to harvesting practices (Shah et al., 2022). Several studies have shown that shredders may be more affected by *Eucalyptus* plantations due to the poor-quality leaf litter (Ferreira et al., 2016 and references therein), but in our case, the response was the opposite. However, the proportion of shredders is very low (maximum reported was 1% of the total FFGs reported), and many of them have other complementary diets based on fine detritus and periphyton (e.g., *Hyallela, Aegla*, and several genera of Leptoceridae; Barrios et al., 2022).

Zygoptera had a positive response to *Eucalyptus* afforestation in winter but a negative response in summer, whereas the proportion of Anisoptera did not vary. Odonata species are particularly effective bioindicators for detecting anthropogenic stressors (Miguel et al., 2017; Schröder et al., 2020), but different tolerant responses may exist among both odonatan suborders or even among species due to differences in their ecophysiology and local habitat requirements (Corbet, 1999; de Marco Junior et al., 2015; Miguel et al., 2017). For instance, a lower tolerance response is expected from Zygoptera due to their low dispersal capacity in the terrestrial and larval stages, which is attributed to their thermal sensitivity (Silva et al., 2021). Thus, highly connected habitat patches and lower water temperatures are required for their larval stages (Crumrine et al., 2008; Guillermo-Ferreira & Del-Claro, 2011; Silva et al., 2021). On the other hand, odonatan larvae develop in the winter season, which could explain the increase in their proportion during this season (Villalobos-Jiménez et al., 2016). In the case of the threshold responses of Zygoptera taxa, we detected that *Mneserate* had a sensitive response to the increase in the afforestation gradient, but *Acanthagrion* showed a tolerant response to lower DO concentration and pH (Fig. 4), suggesting some difference in the tolerance response among Zygoptera species (Villalobos-Jiménez et al., 2016; Rodrigues et al., 2016).

## Conclusion

The results obtained from this research give empirical support to the notion that *Eucalyptus* afforestation in subtropical lowland streams has negative effects on water quality and macroinvertebrate assemblages. However, despite these clear indications, *Eucalyptus* afforestation impacts seem lower than those of other productive land uses (e.g., crop agriculture). These responses could be related to the presence of buffer areas in streams with afforestation cover, which would explain the low increase in nutrients and suspended soils. However, in our study, other forest management practices, such as the combined use of afforestation and the presence of livestock, which in most cases have access to buffer zones and streams, may contribute to a greater input of nutrients and organic matter. In this sense, it is recommended to experimentally evaluate the effect of excluding livestock from the streams with high afforestation in their watersheds and confirm if there is any positive effect on water quality parameters and macroinvertebrate assemblage. One important issue in this study is that we only detect the effects of *Eucalyptus* afforestation in the physical and chemical water parameters and macroinvertebrate metrics. However, other effects that we did not measure may be co-occurring, such as changes in the input of low-quality leaf litter that alter the decomposition process, the alteration of stream hydrological regimes, tree-growing stages, and forest management practices.

The water quality and macroinvertebrate metrics models and the detection of threshold responses of macroinvertebrate assemblages to afforestation and water quality parameters provide a possible approach to improving monitoring and management rapid tools in subtropical lowland stream ecosystems. The knowledge about the thresholds using higher taxonomic resolution (most of them classified to genus level) will serve as a basis for constructing our own sensitive/tolerance classifications, improving the bioindication analyses in our streams. However, applying these tools for evaluating other land uses such as agriculture and urbanization is necessary to obtain a better background in the macroinvertebrate responses and adjust monitoring programs that could benefit from these results.

EPT taxa were the best at showing how Eucalyptus afforestation changed water quality parameters. However, seasonal sampling campaigns covering a continuum over all seasons and years are necessary to understand not only the variations in their sensitive response, and changes in the EPT families that may be tolerant in our systems (e.g., Baetidae, Caenidae, and Hydropsychidae), but also to account for phenological aspects, as well as their preference for microhabitat conditions, allowing for improved sampling and the local adaptation of this index to the subtropical Uruguayan streams.

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**Data availability** The datasets generated and analyzed during the current study are available in the supplementary material. Additional information may be acquired from the corresponding authors upon a reasonable request.

**Code availability** Upon a reasonable request, the code used to analyze the data that supported our results is available from the corresponding authors.

**Declarations**

**Conflicts of interest** The authors declare no conflict of interest.

**Ethical approval** No approval from ethics committees was required to accomplish the goals of this study because research work was conducted with unregulated invertebrate taxa.

**Consent to participate** All authors consent to publish this research.

**Consent for publication** No applicable.

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## Supplementary information

## Journal: Hydrobiologia

# Environmental and aquatic macroinvertebrates metrics respond to the *Eucalyptus* afforestation gradient in Subtropical lowland streams.

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**Table S1**. Coordinates and basin area (km$^2$) of the stream sites selected.

| **Basin** | **Stream site** | **Latitiud Y** | **Longitud X** | **Basin area (Km$^2$)** | **Afforestation area (Km$^2$)** | **Afforestation (%)** |
|---|---|---|---|---|---|---|
| Laguna Merín | DF-NF4 | -34.172 | -54.364 | 6.39 | 0.003 | 0.05 |
| A° Maldonado | DF-Monte 3 | -34.651 | -54.999 | 3.9 | 0.007 | 0.18 |
| A° Maldonado | DF- Monte 2 | -34.637 | -54.990 | 10.18 | 0.032 | 0.31 |
| Río Queguay | DF-CV | -32.270 | -57.601 | 1.96 | 0.007 | 0.36 |
| Laguna Merín | DF-NF16 | -34.138 | -54.359 | 8.79 | 0.040 | 0.46 |
| Río Negro | DF-C9 | -32.213 | -55.580 | 7.14 | 0.084 | 1.18 |
| Río Negro | DF-S19 | -32.473 | -56.405 | 0.33 | 0.011 | 3.34 |
| Laguna Merín | DF-NF6 | -34.176 | -54.355 | 5.39 | 0.205 | 3.8 |
| Río Negro | DF-S16 | -32.554 | -56.459 | 22.51 | 1.393 | 6.19 |
| Río Negro | DF-2018-4 | -32.044 | -55.816 | 8.21 | 0.617 | 7.52 |
| Río Negro | DF-2018-6 | -31.815 | -55.957 | 4.1 | 0.523 | 12.76 |
| Río Negro | DF-MFC | -32.203 | -55.595 | 19.34 | 3.785 | 19.57 |
| Río Negro | DF-2018-2 | -32.085 | -55.822 | 6.61 | 1.853 | 28.04 |
| Río Negro | DF-2018-3 | -32.071 | -55.822 | 7.82 | 2.571 | 32.88 |
| Río Negro | DF-C7F | -32.196 | -55.612 | 14.33 | 5.526 | 38.56 |
| Río Negro | DF-2018-8 | -31.834 | -55.933 | 4.53 | 2.010 | 44.36 |
| Río Santa Lucía | DF-Forest 2 | -34.198 | -55.269 | 3.92 | 1.798 | 45.86 |
| Río Negro | DF- F8F | -32.838 | -56.962 | 14.39 | 7.081 | 49.21 |
| Río Negro | DF- F6F | -32.767 | -57.142 | 29.76 | 14.975 | 50.32 |
| Río Queguay | DF-DT | -32.249 | -57.647 | 2.17 | 1.224 | 56.41 |
| Laguna Merín | DF-F5 | -34.155 | -54.322 | 2.47 | 1.516 | 61.39 |
| Río Queguay | DF- 2018-19 | -32.353 | -57.512 | 5.79 | 3.603 | 62.23 |
| Río Negro | DF- 2018-20 | -32.527 | -57.342 | 3.49 | 2.195 | 62.89 |
| Río Negro | DF-2018-1 | -32.084 | -55.823 | 5.27 | 3.534 | 67.06 |
| Río Santa Lucía | DF-Forest 3 | -34.177 | -55.235 | 5.45 | 4.053 | 74.36 |
| Laguna Merín | DF- F7 | -34.136 | -54.313 | 2.01 | 1.523 | 75.75 |
| Río Negro | DF-2018-18 | -32.475 | -57.303 | 6.99 | 5.530 | 79.11 |
| Río Negro | DF- 2018-13 | -32.490 | -57.346 | 1.19 | 0.945 | 79.37 |
| Río Negro | DF- 2018-17 | -32.475 | -57.298 | 4.35 | 3.732 | 85.8 |
| Río Negro | DF-2018-21 | -32.520 | -57.330 | 3.75 | 3.611 | 96.3 |

**Table S2**. Best models selected to detect physical and chemical water variables' responses to the afforestation gradient (%) and season. Total nitrogen (TN), nitrate ($NO_3$-N), ammonium ($NH_4$-N), total phosphorous (TP) and phosphate ($PO_4$-P), dissolved oxygen ($O_2$), suspended organic material (SOM), total dissolved solids (TDS), total suspended solids (TSS).

| **Metric** | **Factors** | **Estimate** | **Std. Error** | **t value** | ***p-value*** |
|---|---|---|---|---|---|
| **log(TN)** | Intercept | 5.8 | 0.1 | 55.3 | <0.001 *** |
| $R^2$=0.11 | Season (Winter) | 0.1 | 0.1 | 0.5 | 0.616 |
| *p-value= 0.02** | Afforestation | 0.0 | 0.0 | 2.7 | 0.009 ** |
| **$NO_3$-N** | Intercept | 173.5 | 23.6 | 7.4 | <0.001 *** |
| $R^2$=0.26 | Season (Winter) | 6.8 | 33.3 | 0.2 | 0.840 |
| *p-value <0.001**** | Afforestation | 1.3 | 0.5 | 2.8 | 0.007 ** |
| | Season (Winter)*Afforestation | -1.9 | 0.7 | -2.8 | 0.006 ** |
| **log($NH_4$-N)** | Intercept | 1.1 | 0.3 | 4.3 | <0.001 *** |
| $R^2$=0.18 | Season (Winter) | 0.8 | 0.4 | 1.9 | 0.068 . |
| *p-value= 0.009** | Afforestation | 0.0 | 0.0 | 2.2 | 0.029 * |
| | Season (Winter)*Afforestation | -0.02 | 0.0 | -2.5 | 0.016 * |
| **log(TP)** | Intercept | 3.0 | 0.1 | 21.9 | <0.001 *** |
| $R^2$=0.18 | Season (Winter) | 0.5 | 0.1 | 3.5 | 0.001 *** |
| *p-value= 0.01*** | Afforestation | 0.0 | 0.0 | 2.1 | 0.040 * |
| **log($PO_4$-P)** | Intercept | 1.7 | 0.2 | 7.8 | <0.001 *** |
| $R^2$=0.12 | Season (Winter) | -0.02 | 0.3 | -0.1 | 0.963 |
| *p-value= 0.04** | Afforestation | 0.0 | 0.0 | 2.0 | 0.046 * |
| | Season (Winter)*Afforestation | -0.01 | 0.0 | -1.5 | 0.135 |
| **$O_2$ dissolved** | Intercept | 8.19 | 0.32 | 25.5 | <0.001 *** |
| $R^2$= 0.61 | Season (Winter) | 2.97 | 0.35 | 8.6 | <0.001 *** |
| *p-value< 0.001**** | Afforestation | -0.02 | 0.01 | -3.0 | 0.004 ** |
| **pH** | Intercept | 7.28 | 0.10 | 76.23 | <0.001 *** |
| $R^2$= 0.13 | Season (Winter) | 0.24 | 0.10 | 2.34 | 0.023 * |
| *p-value= 0.02** | Afforestation | -0.003 | 0.00 | -1.67 | 0.100 |
| **log(SOM)** | Intercept | 0.69 | 0.12 | 5.80 | <0.001 *** |
| $R^2$= 0.13 | Season (Winter) | 0.38 | 0.12 | 3.02 | 0.004 ** |
| *p-value= 0.02** | Afforestation | 0.00 | 0.00 | 0.33 | 0.741 |
| **log(TDS)** | Intercept | -1.67 | 0.17 | -9.57 | <0.001 *** |
| $R^2$=0.22 | Season (Winter) | -1.00 | 0.39 | -2.54 | 0.014 * |
| *p-value= 0.002** | Afforestation | 0.00 | 0.00 | -1.14 | 0.259 |
| | Season (Winter)*Afforestation | 0.02 | 0.01 | 3.23 | 0.002 ** |
| **log(Conductivity)** | Intercept | 5.6 | 0.2 | 36.5 | <0.001 *** |
| $R^2$=0.24 | Season (Winter) | -1.2 | 0.4 | -3.0 | 0.004 ** |
| *p-value= 0.001*** | Afforestation | 0.0 | 0.0 | -1.6 | 0.118 |
| | Season (Winter)*Afforestation | 0.0 | 0.0 | 3.5 | 0.001 ** |
| **log(TSS)** | Intercept | 4.5 | 0.6 | 7.7 | <0.001 *** |
| $R^2$=0.01 | Season (Winter) | -0.1 | 0.6 | -0.1 | 0.905 |
| *p-value= 0.844* | Afforestation | 0.0 | 0.0 | -0.6 | 0.582 |

**Table S3**. Classification of macroinvertebrate families according to their tolerance or sensitive response to environmental degradation.

| Classification | Family | Classification | Family | Classification | Family |
|---|---|---|---|---|---|
| Sensitive | Elmidae | Tolerant | Cochliopidae | Non-classificated | Ceratopogonidae |
| Sensitive | Baetidae | Tolerant | Sphaeriidae | Non-classificated | Crambidae |
| Sensitive | Leptohyphidae | Tolerant | Glossiphoniidae | Non-classificated | Heteroceridae |
| Sensitive | Hydroptilidae | Tolerant | Planorbidae | Non-classificated | Stratiomyidae |
| Sensitive | Hydropsychidae | Tolerant | Hyalellidae | Non-classificated | Naucoridae |
| Sensitive | Psephenidae | Tolerant | Coenagrionidae | Non-classificated | Noteridae |
| Sensitive | Gripopterygidae | Tolerant | Copepoda | Non-classificated | Tateidae |
| Sensitive | Leptophlebiidae | Tolerant | Ostracoda | Non-classificated | Orthoptera |
| Sensitive | Leptoceridae | Tolerant | Hydrophilidae | Non-classificated | Veliidae |
| Sensitive | Simuliidae | Tolerant | Hydrachnidia | Non-classificated | Corixidae |
| Sensitive | Helicopsychidae | Tolerant | Cladocera | Non-classificated | Pleidae |
| Sensitive | Aeglidae | Tolerant | Corbiculidae | Non-classificated | Hydrometridae |
| Sensitive | Polymitarcyidae | Tolerant | Nematoda | Non-classificated | Haliplidae |
| Sensitive | Polycentropodidae | Tolerant | Libellulidae | Non-classificated | Ephydridae |
| Sensitive | Calopterygidae | Tolerant | Ampullariidae | Non-classificated | Gerridae |
| Sensitive | Perlidae | Tolerant | Collembola | Non-classificated | Dolichopodidae |
| Sensitive | Gomphidae | Tolerant | Dytiscidae | Non-classificated | Mycetopodidae |
| Sensitive | Tipulidae | Tolerant | Dugesiidae | Non-classificated | Scirtidae |
| Sensitive | Philopotamidae | Tolerant | Tabanidae | Non-classificated | Notonectidae |
| Sensitive | Psychodidae | Tolerant | Physidae | Non-classificated | Sciomyzidae |
| Sensitive | Glossosomatidae | Tolerant | Curculionidae | Non-classificated | Hydrochidae |
| Sensitive | Belostomatidae | Tolerant | Temnocephalidae | Non-classificated | Erebidae |
| Sensitive | Aeshnidae | Tolerant | Limoniidae | Non-classificated | Sphaeriusidae |
| Sensitive | Calamoceratidae | Tolerant | Macromiidae | Non-classificated | Helotrephidae |
| Sensitive | Odontoceridae | Tolerant | Culicidae | Non-classificated | Pyralidae |
| Sensitive | Ptilodactylidae | Tolerant | Chaoboridae | Non-classificated | Dryopidae |
| Sensitive | Trichodactylidae | Tolerant | Palaemonidae | Non-classificated | Torridincolidae |
| Sensitive | Corydalidae | Tolerant | Staphylinidae | Non-classificated | Chilinidae |
| | | Tolerant | Gyrinidae | Non-classificated | Isopoda |
| | | Tolerant | Syrphidae | Non-classificated | Hebridae |
| | | | | Non-classificated | Lymnaeidae |
| | | | | Non-classificated | Empididae |

**Table S4.** Best models selected to detect the responses of macroinvertebrate metrics to the *Eucalyptus* afforestation gradient (%) and season.

| Metric | Factors | Estimate | Std. Error | t value | *p-value* | |
|---|---|---|---|---|---|---|
| **Taxa Richness** | Intercept | 18.61 | 1.24 | 14.98 | <0.001 | *** |
| $R^2$= 0.04 | Season (Winter) | 0.07 | 1.33 | 0.05 | 0.96 | |
| *p-value= 0.276 ns* | Afforestation | 0.03 | 0.02 | 1.59 | 0.118 | |
| **log(Density )** | Intercept | 9.39 | 0.18 | 52.74 | <0.001 | *** |
| $R^2$= 0 | Season (Winter) | 0.07 | 0.19 | 0.36 | 0.720 | |
| *p-value = 1* | Afforestation | 0.00 | 0.00 | 0.01 | 0.995 | |
| **log(Total Biomass )** | Intercept | 8.34 | 0.20 | 40.70 | <0.001 | *** |
| $R^2$= 0.03 | Season (Winter) | -0.08 | 0.21 | -0.38 | 0.705 | |
| *p-value = 0.342* | Afforestation | 0.00 | 0.00 | 1.32 | 0.194 | |
| **Mean size** | Intercept | 1.01 | 0.07 | 14.42 | <0.001 | *** |
| $R^2$= 0.12 | Season (Winter) | -0.14 | 0.08 | -1.88 | 0.066 | . |
| *p-value= 0.07 .* | Afforestation | 0.00 | 0.00 | 1.09 | 0.280 | |
| **EPT proportion** | Intercept | -0.320 | 0.190 | -1.710 | 0.088 | . |
| $R^2$= 0.15 | Season (Winter) | -0.700 | 0.27 | -2.640 | 0.008 | ** |
| *p-value= 0.02** | Afforestation | -0.011 | 0.003 | -3.050 | 0.002 | ** |
| | Season (Winter)*Afforestation | 0.013 | 0.01 | 2.580 | 0.010 | ** |
| **EPT:Chironomidae** | Intercept | 1.143 | 0.192 | 5.950 | <0.001 | *** |
| $R^2$= 0.21 | Season (Winter) | 0.244 | 0.195 | 1.246 | 0.213 | |
| *p-value <0.001**** | Afforestation | -0.012 | 0.003 | -3.678 | <0.002 | *** |
| **Oligochaeta** | Intercept | -3.17 | 0.19 | -16.36 | <0.001 | *** |
| $R^2$= 0.19 | Season (Winter) | 0.40 | 0.18 | 2.20 | 0.028 | * |
| *P-value= 0.001*** | Afforestation | 0.01 | 0.00 | 2.95 | 0.003 | ** |
| **Chironominae** | Intercept | -2.91 | 0.21 | -14.14 | <0.001 | *** |
| $R^2$= 0.12 | Season (Winter) | -0.24 | 0.20 | -1.21 | 0.226 | |
| *P-value= 0.03** | Afforestation | 0.01 | 0.00 | 2.65 | 0.008 | ** |
| **Orthocladiinae.sc** | Intercept | -3.71 | 0.16 | -23.27 | <0.001 | *** |
| $R^2$= 0.01 | Season (Winter) | 0.07 | 0.17 | 0.44 | 0.444 | |
| *P-value= 0.836* | Afforestation | 0.00 | 0.00 | -0.34 | -0.339 | |
| **Tanypodinae.sc** | Intercept | -3.00 | 0.14 | -21.31 | <0.001 | *** |
| $R^2$= 0.30 | Season (Winter) | -0.63 | 0.15 | -4.11 | <0.001 | *** |
| *P-value< 0.001**** | Afforestation | 0.00 | 0.00 | 1.78 | 0.076 | . |
| **Zygoptera.sc** | Intercept | -3.624 | 0.107 | -33.731 | <0.001 | *** |
| $R^2$= 0.26 | Season (Winter) | -0.642 | 0.172 | -3.735 | <0.001 | *** |
| *P-value= 0.002*** | Afforestation | -0.002 | 0.002 | -1.000 | 0.317 | *** |
| | Season (Winter)*Afforestation | 0.007 | 0.004 | 1.925 | 0.054 | . |
| **Anisoptera.sc** | Intercept | -4.26 | 0.080 | -51.630 | <0.001 | *** |
| $R^2$= 0.15 | Season (Winter) | -0.25 | 0.090 | -2.760 | 0.006 | ** |
| *P-value= 0.014** | Afforestation | 0.00 | 0 | 1.430 | 0.152 | |
| **Tolerant** | Intercept | 0.552 | 0.164 | 3.370 | <0.001 | *** |
| $R^2$= 0.12 | Season (Winter) | -0.120 | 0.179 | -0.645 | 0.519 | |
| *P-value< 0.019** | Afforestation | 0.008 | 0.003 | 2.775 | 0.006 | ** |
| **Sensible** | Intercept | -0.615 | 0.172 | -3.570 | <0.001 | *** |
| $R^2$= 0.19 | Season (Winter) | 0.225 | 0.188 | 1.201 | 0.229 | ** |
| *P-value= 0.002*** | Afforestation | -0.011 | 0.003 | -3.470 | <0.001 | |
| **Scrapers** | Intercept | -0.939 | 0.190 | -4.938 | <0.001 | *** |
| $R^2$= 0.19 | Season (Winter) | 0.056 | 0.206 | 0.272 | 0.785 | |
| *P-value= 0.002*** | Afforestation | -0.010 | 0.003 | -2.856 | 0.004 | ** |
| **Filters** | Intercept | -3.162 | 0.236 | -13.391 | <0.001 | *** |
| $R^2$= 0.14 | Season (Winter) | 0.532 | 0.223 | 2.384 | 0.017 | ** |
| *P-value= 0.030** | Afforestation | 0.004 | 0.004 | 1.225 | 0.221 | |
| **Predators** | Intercept | -1.613 | 0.124 | -13.046 | <0.001 | *** |
| $R^2$= 0.27 | Season (Winter) | -0.615 | 0.139 | -4.441 | <0.001 | *** |
| *P-value< 0.001**** | Afforestation | 0.002 | 0.002 | 0.844 | 0.399 | |
| **Shredders.sc** | Intercept | -3.757 | 0.169 | -22.193 | <0.001 | *** |
| $R^2$= 0.29 | Season (Winter) | 0.359 | 0.152 | 2.355 | 0.019 | * |
| *P-value< 0.001**** | Afforestation | 0.012 | 0.002 | 4.947 | <0.001 | *** |
| **Collectors-Gatherers** | Intercept | 0.249 | 0.198 | 1.258 | 0.208 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| $R^2$= 0.07 | Season (Winter) | -0.460 | 0.274 | -1.667 | 0.094 | . |
| *P-value= 0.221 ns* | Afforestation | -0.004 | 0.004 | -1.008 | 0.313 | |
| | Season (Winter)*Afforestation | 0.011 | 0.006 | 2.024 | 0.043 | * |

**Table S5**. Assemblage-level thresholds estimated from the most robust macroinvertebrate taxa (purity and reliability ≥ 0.95) responses to afforestation gradient and water quality parameters selected. Sum(z) associated with decrease (Z−) or increase (Z+) along the gradient; 5$^{th}$-95$^{th}$ bootstrap quantile intervals (5, 10, 50, 90, 95%) capturing true thresholds.

| Order | Family | Taxa | TITAN classification | Eucalyptus afforestation (%) | | | | | TN (µg. L-1) | | | | | DO (mg. L-1) | | | | | pH | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 5% | 10% | 50% | 90% | 95% | 5% | 10% | 50% | 90% | 95% | 5% | 10% | 50% | 90% | 95% | 5% | 10% | 50% | 90% | 95% |
| Coleoptera | Hydrophilidae | *Enocrus* | Z- | | | | | | | | | | | | | | | | 7.66 | 7.60 | 7.58 | 7.55 | 7.55 |
| Coleoptera | Elmidae | *Macrelmis* | Z- | | | | | | | | | | | | | | | | 7.78 | 7.74 | 7.68 | 7.21 | 7.18 |
| Coleoptera | Elmidae | *Microcylloepus* | Z- | | | | | | | | | | | 10.6 | 10.1 | 9.7 | 8.3 | 8.2 | | | | | |
| Coleoptera | Psephenidae | *Psephenus* | Z- | 0.3 | 0.3 | 6.9 | 67.1 | 75.1 | 241.5 | 244.9 | 268.1 | 396.5 | 397.0 | | | | | | | | | | |
| Diptera | Chironominae | Orthocladiinae | Z- | | | | | | | | | | | | | | | | 7.60 | 7.58 | 7.55 | 6.85 | 6.79 |
| Diptera | Simuliidae | *Simulium* | Z- | | | | | | | | | | | 10.6 | 10.3 | 9.7 | 8.8 | 7.3 | | | | | |
| Ephemeroptera | Leptophlebiidae | *Farrodes* | Z- | 0.3 | 0.3 | 0.4 | 67.9 | 75.1 | 228.4 | 264.9 | 285.1 | 353.1 | 359.7 | 10.9 | 10.8 | 10.8 | 8.8 | 6.4 | | | | | |
| Ephemeroptera | Leptohyphidae | *Haplohyphes* | Z- | | | | | | | | | | | 11.1 | 11.1 | 10.6 | 8.2 | 7.4 | 7.60 | 7.60 | 7.54 | 7.26 | 7.21 |
| Ephemeroptera | Leptohyphidae | *Tricorythodes* | Z- | 49.8 | 50.3 | 56.4 | 61.8 | 74.4 | 258.2 | 264.9 | 281.3 | 533.6 | 609.9 | 11.0 | 10.8 | 9.5 | 7.3 | 6.9 | 7.55 | 7.54 | 7.43 | 6.83 | 6.82 |
| Ephemeroptera | Leptohyphidae | *Tricorythopsys* | Z- | 0.3 | 0.3 | 6.2 | 9.5 | 10.1 | 219.5 | 248.3 | 336.1 | 353.0 | 371.3 | | | | | | 7.69 | 7.69 | 7.54 | 7.19 | 7.18 |
| Mesogastropoda | Cochliopidae | *Heleobia* | Z- | 6.2 | 6.9 | 61.4 | 79.1 | 79.2 | 339.6 | 353.0 | 466.8 | 478.1 | 481.5 | | | | | | | | | | |
| Odonata | Coenagrionidae | *Argia* | Z- | | | | | | | | | | | | | | | | 7.69 | 7.60 | 7.10 | 7.03 | 7.01 |
| Odonata | Calopterygidae67 | *Mneserate* | Z- | 0.4 | 0.5 | 0.8 | 16.2 | 16.2 | | | | | | | | | | | | | | | |
| Plecoptera | Perlidae | *Anacroneuria* | Z- | | | | | | | | | | | | | | | | 7.73 | 7.69 | 7.60 | 7.55 | 7.54 |
| Plecoptera | Gripopterygidae | *Gripopteryx* | Z- | | | | | | | | | | | 9.8 | 9.7 | 9.5 | 8.7 | 8.5 | | | | | |
| Trichoptera | Helycopsychidae | *Helicopsyche* | Z- | 0.3 | 0.3 | 1.2 | 5.0 | 5.7 | 215.2 | 240.6 | 278.5 | 366.5 | 397.0 | | | | | | | | | | |
| Trichoptera | Glossossomatidae | *Mortoniella* | Z- | | | | | | | | | | | | | | | | 7.86 | 7.84 | 7.72 | 7.55 | 7.55 |
| Trichoptera | Hydroptilidae | *Neotrichia* | Z- | 0.3 | 0.3 | 3.6 | 58.9 | 59.3 | 254.8 | 261.8 | 285.1 | 353.0 | 356.4 | | | | | | | | | | |
| Trichoptera | Calamoceratidae | *Phylloicus* | Z- | 0.2 | 0.3 | 0.4 | 0.8 | 2.3 | 228.4 | 251.6 | 311.8 | 329.8 | 356.6 | | | | | | | | | | |
| Trichoptera | Polycentropodidae | *Polycentropus* | Z- | 3.6 | 9.3 | 53.4 | 56.8 | 58.9 | 239.4 | 252.3 | 307.7 | 397.0 | 432.7 | | | | | | | | | | |
| Trichoptera | Hydropsychidae | *Smicridea* | Z- | | | | | | 255.0 | 258.2 | 285.1 | 326.0 | 346.4 | 11.0 | 10.9 | 10.6 | 7.4 | 7.0 | 7.63 | 7.61 | 7.58 | 7.06 | 6.96 |
| Tricladida | Dugesiidae | Dugesiidae | Z- | | | | | | | | | | | 10.8 | 10.6 | 8.5 | 7.3 | 7.3 | | | | | |
| Architaenioglossa | Ampullariidae | *Pomacea* | Z+ | | | | | | | | | | | 8.8 | 8.5 | 7.0 | 6.4 | 6.4 | | | | | |
| Coleoptera | Dytiscidae | *Laccophilus* | Z+ | 12.8 | 16.2 | 35.7 | 77.7 | 79.4 | | | | | | 9.5 | 9.5 | 8.4 | 7.2 | 6.7 | 7.48 | 7.46 | 7.34 | 6.82 | 6.82 |
| Collembola | | Collembola | Z+ | 6.9 | 6.9 | 45.1 | 63.0 | 79.2 | | | | | | | | | | | | | | | |
| Diptera | Ceratopogonidae | Forcipomyiinae | Z+ | | | | | | | | | | | 9.7 | 9.5 | 9.0 | 8.2 | 6.7 | | | | | |
| Diptera | Chironominae | Tanypodinae | Z+ | | | | | | | | | | | 11.3 | 11.3 | 9.8 | 8.7 | 8.5 | | | | | |
| Diptera | Tipulidae | *Tipula* | Z+ | | | | | | 307.1 | 403.5 | 618.5 | 721.5 | 721.5 | | | | | | | | | | |
| Ephemeroptera | Baetidae | *Callibaetis* | Z+ | | | | | | | | | | | 9.7 | 9.7 | 8.4 | 6.7 | 4.4 | | | | | |
| Hemiptera | Corixidae | Corixidae | Z+ | 49.8 | 50.3 | 56.4 | 62.2 | 62.6 | | | | | | | | | | | | | | | |
| Lepidoptera | Crambidae | *Paraponyx* | Z+ | | | | | | | | | | | 11.1 | 11.1 | 7.5 | 7.1 | 6.4 | | | | | |
| Odonata | Coenagrionidae | *Acanthagrion* | Z+ | | | | | | | | | | | 10.2 | 10.0 | 9.2 | 6.6 | 6.3 | 7.41 | 7.39 | 6.94 | 6.82 | 6.81 |
| Odonata | Gomphidae | *Aphylla* | Z+ | | | | | | 305.0 | 414.6 | 471.3 | 718.3 | 721.5 | 10.6 | 10.6 | 9.9 | 8.4 | 8.4 | | | | | |
| | | Cladocera | Z+ | | | | | | | | | | | 9.5 | 9.0 | 6.4 | 5.3 | 5.2 | | | | | |
| | | Copepoda | Z+ | 1.2 | 5.0 | 22.1 | 79.2 | 79.2 | 285.1 | 302.3 | 516.0 | 650.1 | 650.1 | 10.6 | 9.9 | 9.0 | 6.5 | 5.6 | | | | | |

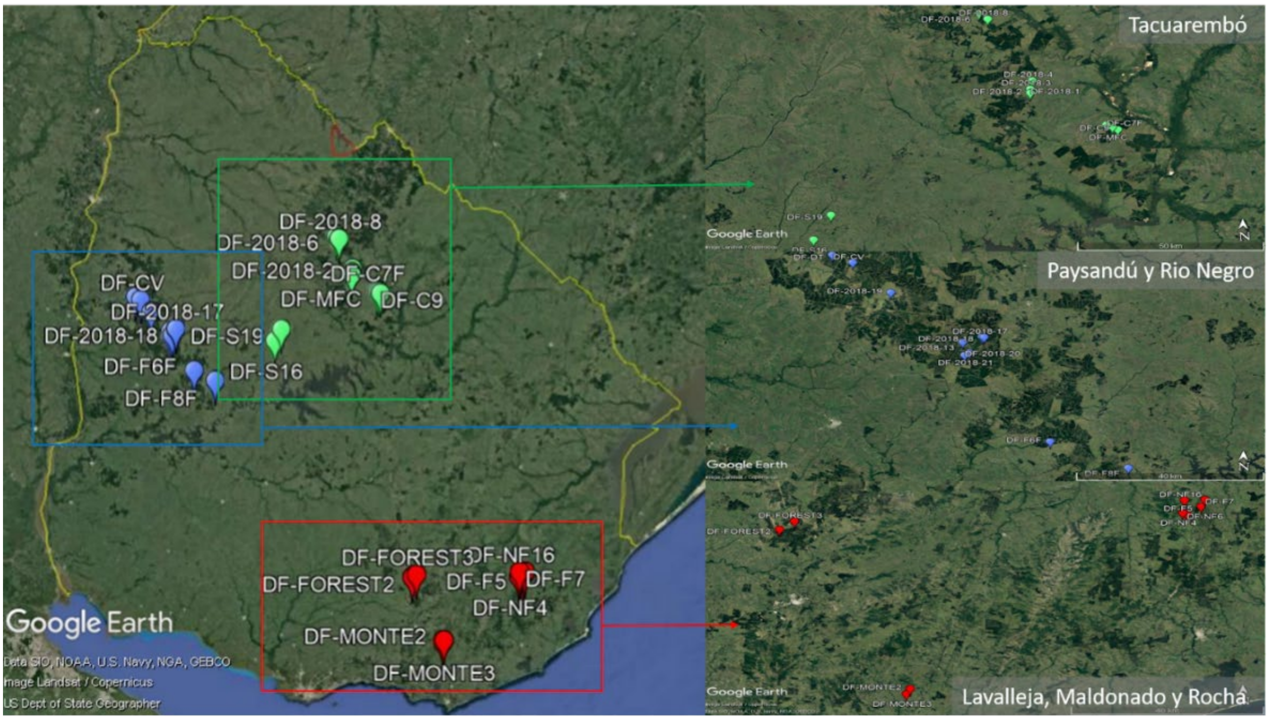


**Fig. S1**. Location of sampling sites (n=30) in micro watersheds covering a wide gradient of forested area. They were located in three large study areas: green: Tacuarembó, blue: Paysandú-Río Negro, red: Lavalleja-Maldonado-Rocha.

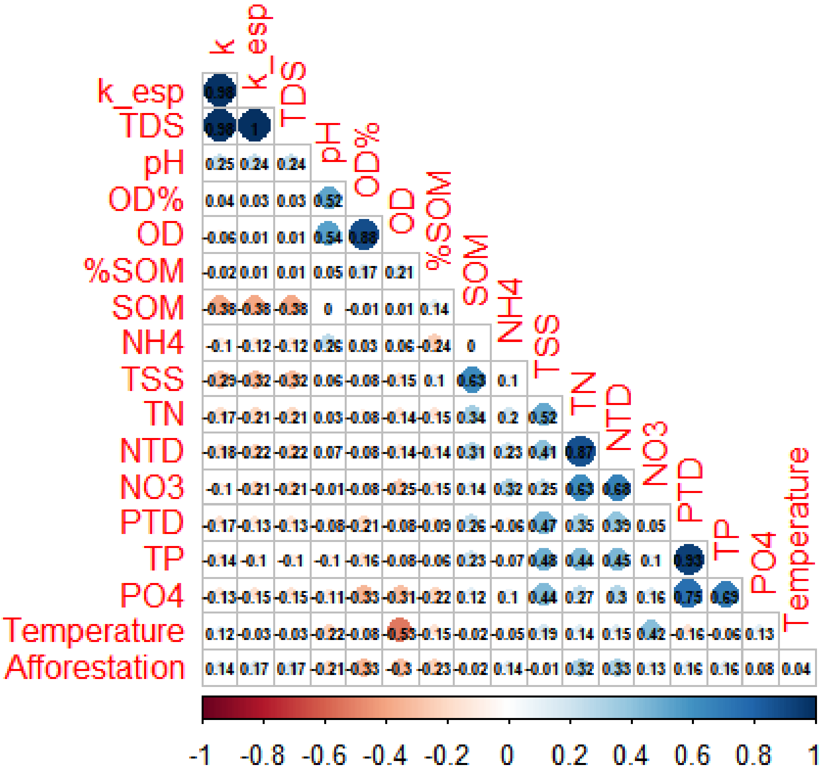
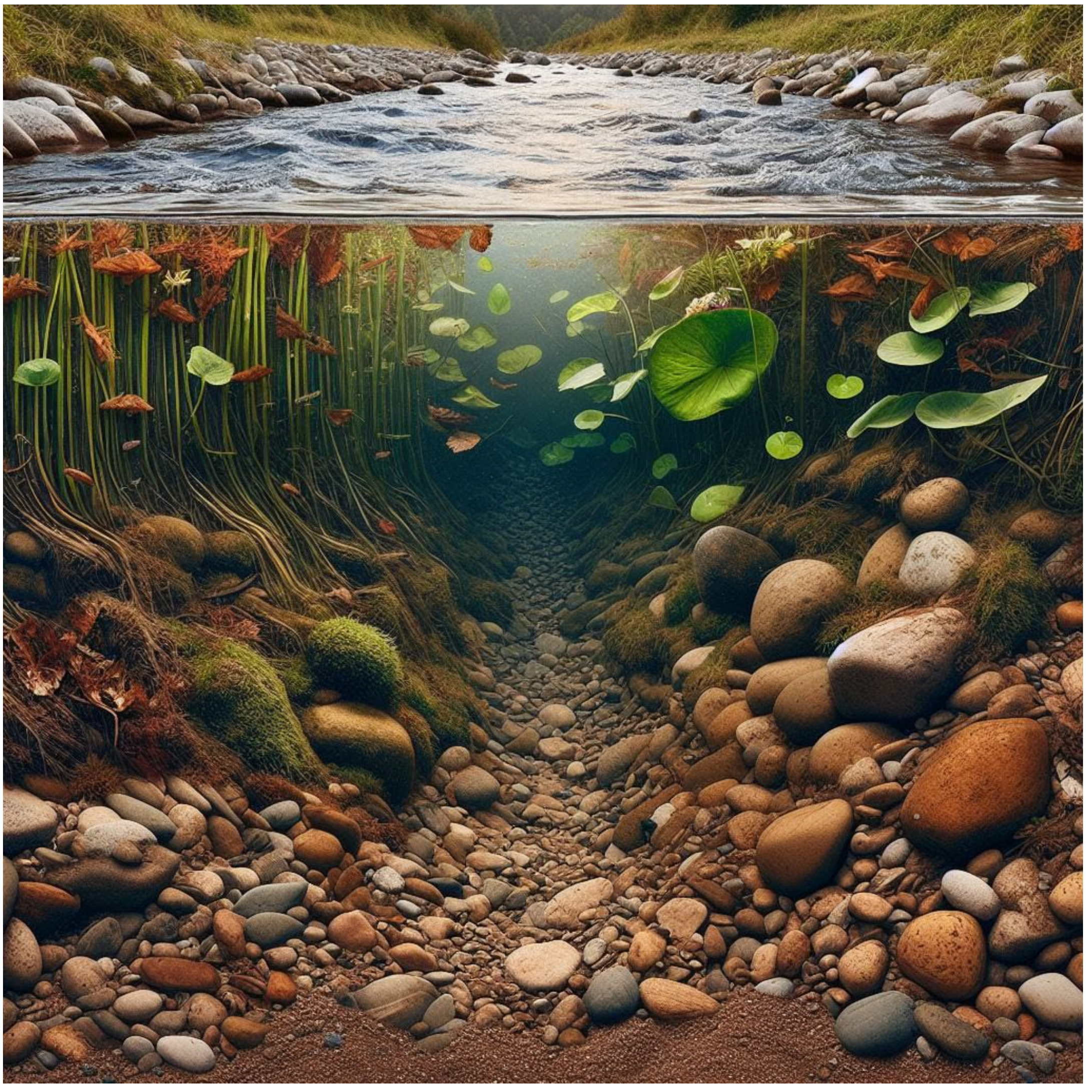


**Fig. S2**. Multiple correlation of environmental variables used in NMDS- envfit analyses. Variables selected for final analyses were based on high correlation (-0.7<P<0.7).

# *CHAPTER III: Changes in basal resources mediate the effects of Eucalyptus spp. afforestation on macroinvertebrate functional diversity in subtropical lowland streams.*



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Scope: Aquatic Science, Ranking Q1

# Changes in basal resources mediate the effects of *Eucalyptus* spp. afforestation on macroinvertebrate functional diversity in subtropical lowland streams.

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**Statements and Declarations.**

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**Conflicts of interest:** The authors declare no conflict of interest.

**Availability of data and material:** The datasets generated and analysed during the current study are available in the supplementary material. Additional information may be acquired from the corresponding authors upon a reasonable request.

**Code availability:** The code used to analyse the data that supported our results is available from the corresponding authors upon reasonable request.

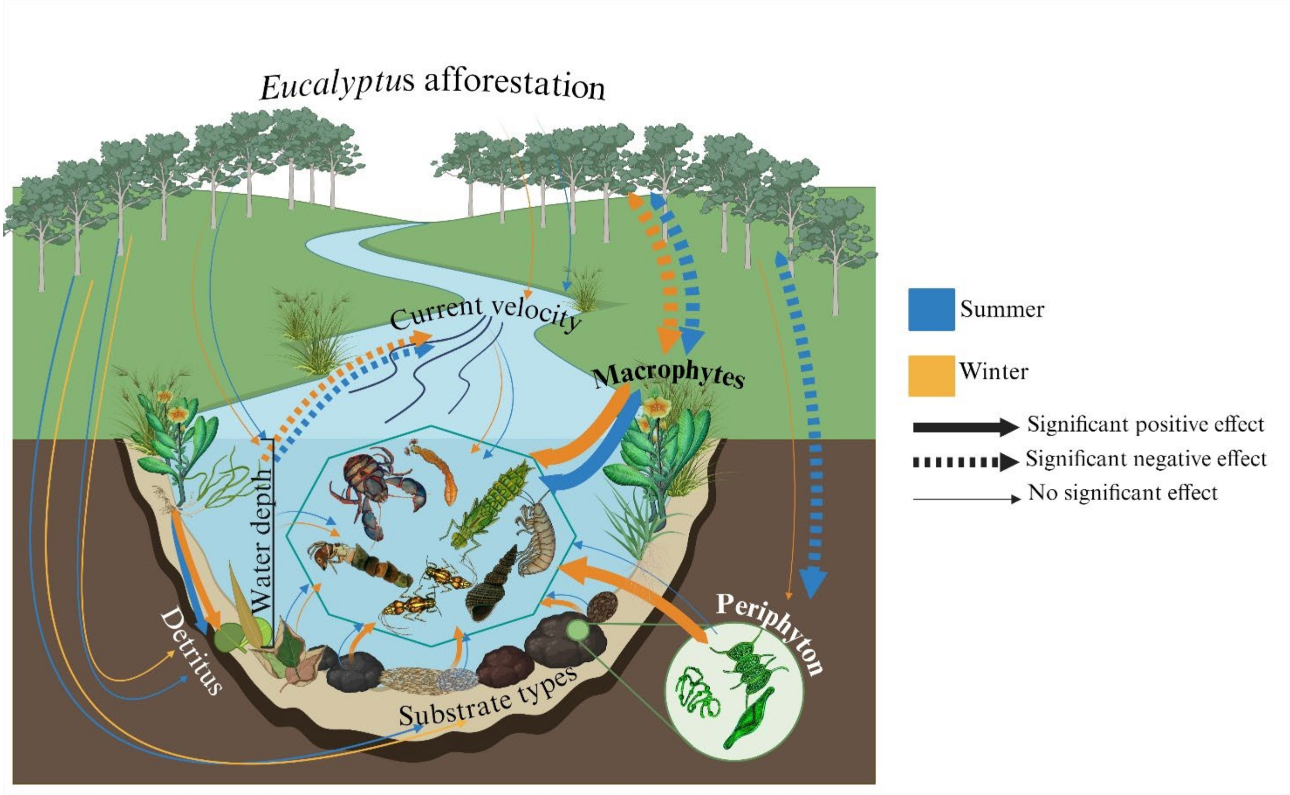
**Authors' contributions:** M.B.G.: conceptualisation, methodology, investigation, formal analyses, data curation, writing-original draft, writing-review editing, preparation of figures 2-6. B.R.T.: conceptualisation, writing-review editing, supervision. D.A.M: formal analyses, writing-review editing, preparation of figures 1 and 6. M.B.: investigation, writing-review editing. J.A.: project administration, funding acquisition, writing-review editing. F.T.M.: conceptualisation, methodology, investigation, writing-review editing, project administration, supervision.

**Ethics approval:** No approval from ethics committees was required to accomplish the goals of this study because research work was conducted with unregulated invertebrate taxa.

**Consent to participate:** All authors consent to publish this research.

**Consent for publication:** No applicable

## Graphical abstract



## Highlights

- The increase in *Eucalyptus* afforestation within stream watersheds causes indirect negative changes in macroinvertebrate functional diversity by decreasing macrophyte biomass.
- Physico-chemical water parameters, local habitat conditions, and basal resource biomass all influenced the distribution of functional traits of macroinvertebrates associated with food preference, feeding habits, adaptations to flow constraints, and maximum body size.
- Changes in macroinvertebrate functional diversity can indicate the degradation of ecological processes in subtropical lowland streams associated with *Eucalyptus* afforestation.

## Abstract

*Eucalyptus* afforestation negatively affects the ecological condition of streams by impacting macroinvertebrates, whose responses to habitat deterioration and changes in basal resources are not well understood. This study examines the effects of *Eucalyptus* afforestation on macroinvertebrate functional diversity and trait structure, driven by variations in local habitat and basal resources. The study was conducted once in summer and winter across 27 subtropical lowland streams along an *Eucalyptus* spp.-afforestation gradient. We measured physico-chemical water parameters, habitat variables (substrates, depth, and current velocity), and biomass of basal resources (macrophytes, periphyton, and detritus) simultaneously with macroinvertebrate sampling. We used co-inertia multivariate RLQ and four-corner analyses to explore relationships between physico-chemical water parameters, local habitat, and basal resources, with trait structure. Structural equation modelling- SEM was used to understand the direct and indirect effects of *Eucalyptus* afforestation. SEM revealed that *Eucalyptus* afforestation indirectly reduced macroinvertebrate functional diversity by reducing macrophyte biomass in both seasons. The RQL analysis revealed a positive correlation between the biomass of macrophytes, the density of predators, and the number of small and medium-sized animals. This suggests that the decrease in macrophyte biomass may affect the available refuges and biological interactions by affecting predators and small and medium body sizes . These findings emphasise the importance of macrophytes in sustaining a unique functional trait composition of macroinvertebrates in stream ecosystems. Despite the fact that afforestation did not influence substrate types, they positively affect functional diversity. Macroinvertebrate functional traits are effective indicators of ecological degradation in streams affected by *Eucalyptus* afforestation.

**Keywords:** *Eucalyptus* afforestation, macroinvertebrates functional traits, macrophyte biomass, predators, stream functioning.

## Introduction.

Macroinvertebrate communities have been widely employed in biomonitoring programmes to evaluate the ecological conditions of stream ecosystems (Buss et al. 2015; Feio et al. 2021). This is because they respond effectively to variations in natural stream conditions and play a crucial role in various ecological processes, including the processing of organic material, nutrient and mineral cycling, and energy processing at different trophic levels (Dolédec and Statzner 2010; Leiva et al. 2022; Bendary et al. 2022).

The relationship between macroinvertebrate communities with stream conditions, as well as their specific participation in the ecological processes, is hindered by specific traits that indicate taxa's adaptations and resilience to changes in abiotic and biotic factors (McGill et al. 2006; Forio et al. 2018). A group of traits exhibited by species is associated with the particular environmental setting in which a species can thrive, how species interact with one another, the strength of competition or the effectiveness of a predator's consumption (McGill et al. 2006; Cadotte et al. 2011). In this sense, including functional approaches in biomonitoring programmes offers better responses than taxonomic diversity (Le Provost et al. 2020; Moi et al. 2023; Paz et al. 2022) by highlighting how environmental changes affect macroinvertebrate assemblages by filtering specific traits (Menezes et al. 2010; Schmera et al. 2017; Leiva et al. 2022). In addition, evaluating trait structure enables the creation of a functional diversity index that summarises the range of traits within a community. An increase in functional diversity outcomes in a wider range of effective exploitation of existing niches and resources, resulting in improved ecosystem functioning (Schmera et al. 2017; Leiva et al. 2022).

Macroinvertebrate communities are highly influenced by factors operating at local scales, such as the substrate composition, current velocity (Poff et al. 1997), and basal resource types (autochthonous primary producers and detritus derived from allochthonous leaf litter and/or autochthonous macrophytes; Vannote et al. 1980; Leberfinger and Bohman 2010, 2011). The arrangement of these local factors strongly acts as environmental filters for macroinvertebrate traits (Li et al. 2018). For instance, food source selection is based on traits related to the ability to find them, eat them, and turn them into secondary production (Marczak et al. 2007; Thorp et al. 2015; Doretto et al. 2020; Entrekin et al. 2020; Rüegg et al. 2021). The nutritional value of the basal resources also influences this selection (Thorp and Delong 1994, 2002; Marcarelli et al. 2011; Frainer et al. 2014; Baker et al. 2023). Furthermore, particular basal resources, such as macrophytes and leaf litter, as well as larger substrate types (e.g., stones), may be used as shelters, reducing the risk of predation by minimising the interactions between predators and prey (López-Rodríguez et al. 2018; Wolters et al. 2018; Poi et al. 2021; Yofukuji et al. 2021). These kinds of refuges may also help mitigate the impact of strong water currents (Wolters et al. 2018). The ability to search for and hide also depends on several traits, particularly those related to mobility (Christie et al. 2009; Verdonschot et al. 2012).

Changes in land cover in the basin by human stressors may alter the configuration of local habitat and thus promote changes in macroinvertebrate functional diversity (García et al. 2017; Ceneviva-Bastos et al. 2017; Pye et al. 2023). Among human stressors, *Eucalyptus* spp. is the most planted forest, occupying over 20 million hectares worldwide (Myburg et al. 2014). In subtropical lowland regions, the expansion of this land use has predominantly replaced grassland ecosystems, resulting in multifaceted impacts on the natural environment (Ferreira et al. 2019; Barrios et al. 2023). These impacts encompass soil acidification, modifications in the hydrological cycle, decreased surface runoff, and local loss of grassland-characteristic terrestrial species (Engel et al. 2005; Brazeiro, 2015; Silveira et al. 2016; González-Sosa et al. 2024).

*Eucalyptus* afforestation has been associated with the simplification of macroinvertebrate community composition (Abelho and Graça 1996; Larrañaga et al. 2009; Ferreira et al. 2016, 2019). In the case of subtropical lowland streams, the structure of macroinvertebrate composition, the abundance of sensitive taxa, such as Ephemeroptera, Trichoptera, Plecoptera (EPT), sensitive families, and functional feeding groups (FFG) exhibits a decline as the afforestation watershed area expands. In contrast, the abundance of more tolerant groups like Chironominae and Oligochaeta tends to increase (Barrios et al. 2023). Alterations in macroinvertebrate community with the reduction of less tolerant taxa and changes in traits related to FFG, may promote a homogenisation of aquatic communities which is reflected in changes in macroinvertebrate functional diversity in streams (Piano et al., 2020)The responses of macroinvertebrate communities to the increase in *Eucalyptus* afforestation may also be a consequence of the alterations in habitat conditions and the availability of basal resources (Li et al. 2019). For instance, the allochthonous input of *Eucalyptus* leaf litter into streams may negatively affect the macroinvertebrate community and trait composition (Ferreira et al. 2016, 2019). This may occur through the consumption of this toxic leaf litter by detritivore shredders (Canhoto and Graça 1995), as well as the exposure to secondary compounds present in the leaf leachate (Canhoto et al. 2007; 2013). As a result, detritivores shredders may decline in their abundance leading to a lack of leaf litter transformation into biomass within the trophic network (Jonsson and Sponseller, 2021). Consequently, microorganisms may only process leaf litter, resulting in elevated respiration rates and increased $CO_2$ emissions (Tiegs et al. 2019; Woodward et al. 2012; Shah, 2021; Costello et al. 2022). The presence of *Eucalyptus* leaf litter in the streams may also have an impact on autochthonous production due to eucalypt leachates (Martínez et al. 2017), which may alter water quality and solar irradiation into the water column (González-Paz et al. 2023). Thus, the use of trait-based approaches reflects important ecological processes such as nutrient and carbon cycling and biomass production, which perform as a proxy for ecosystem functioning (Doledec & Statzner, 2010).

Therefore, changes in local conditions promoted by *Eucalyptus* afforestation may lead to a loss of trait redundancy and, consequently, a reduction in the functional diversity among macroinvertebrates (Leiva et al. 2022; Moi et al. 2023). As a result, changes in carbon and nutrients flows and bottom-up effects on aquatic consumers may arise (Kominoski and

Rosemond 2012; García et al. 2017; Ceneviva-Bastos et al. 2017; Price et al. 2019; Brauns et al. 2022; Moi et al. 2023). However, these aspects have not yet been fully understood (Ferreira et al. 2019; Barrios et al. 2022).

Here, we evaluated the effects of *Eucalyptus* afforestation on macroinvertebrate functional diversity and trait structure, driven by variations in local habitat and basal resources in subtropical lowland streams. We aim to understand how these changes in macroinvertebrate functional diversity may also differ among the most contrasting climatic seasons (summer and winter) due to possible changes in the availability of basal resources (macrophytes, periphyton, and detritus) and local habitat configuration. We hypothesise that the increase in *Eucalyptus* afforestation cover contributes to diminishing macroinvertebrate functional diversity, mediated by the relationship between functional traits with basal resources and local habitat characteristics. As physico-chemical water parameters strongly relate to the *Eucalyptus* afforestation gradient (Barrios et al. 2023), we also analysed the relationship between these parameters and the trait structure of macroinvertebrates.

## Materials and methods

### Study area.

The study was conducted in subtropical lowland streams distributed across the main basins of Uruguay. First, we determined the area occupied by *Eucalyptus* afforestation within the watershed of streams selected. For this, the QGIS "Semi-Automatic Classification Plugin" was employed using Google Earth images from within QGIS. The procedure consisted of selecting averaged spectral signatures (or ROI's), which are polygons drawn over homogeneous areas of the image that overlay pixels belonging to the same land cover class that represent the cover pixels of *Eucalyptus* plantations, water, and extensive livestock. Once we obtained the spectral signatures, we applied the plugin to the satellite images, which resulted in the raster format classification of the image. Subsequently, we selected only the pixels with afforestation from the classified image and applied the "Delete Holes" process with a minimum hole limit within the shapefile, ensuring a single homogeneous shape remained for each afforestation area (Congedo 2021). A total of 27 streams (< 30 km$^2$ of catchment area; Fig. 1; Supplementary Table 1) were selected, representing a wide range of relative area in each basin covered by *Eucalyptus* spp. ranging from 0.05 to 85.8 %. The rest of the watersheds were occupied by grassland with low-density cattle grazing (i.e. <1 ind/ha). Extensive cattle are usually used as the reference condition in Uruguay as natural or pristine areas are practically non/existing. For more information on the study area, see Barrios et al. (2023).

We use the term afforestation which is the process of creating a forest on land that has not been forested for a long period, typically at least 50 years, or in some cases, ever. This involves planting trees, and in our case, replacing natural grasslands with *Eucalyptus* plantations. Due to the fact that the selection of sites was based on the participation of forestry enterprises involved in the project that support this study, some bias in the geographic distribution of sites may arise. To account for this potential effect, we ran a regression model to test the relationship between the spatial distance the sites and the dissimilarity of macroinvertebrate community composition. In this case, no significant effects were found indicating there is not bias in the geographic distribution of sites. (For more details of the analysis and R packages used, see Supplementary Analysis 1).

In all streams associated with afforested areas in our study, the riparian zone consisted of a regulatory buffer zone (Decree N° 405–021, Art. 7, IMPO, 2021), not covered by *Eucalyptus*. This buffer area is usually equal to or higher than 20m around the stream and is dominated by grass and other herbs, and a few shrub species (Bernardi et al. 2016; Mary-Lauyé et al. 2023).

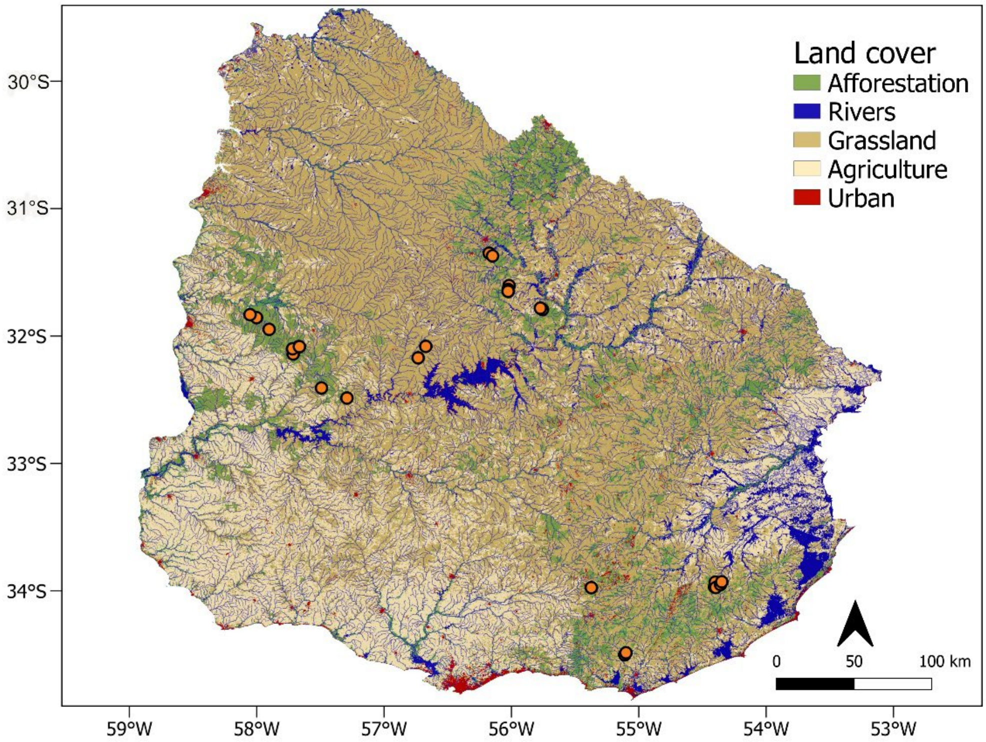


**Fig.1.** Map highlighting land cover in 27 studies streams (orange points) across Uruguay. Land cover included afforestation by *Eucalyptus* spp., natural grassland cover, human agricultural system, and urban cover. Land cover in Uruguay was estimated for 2019 using the land use cover map provided by MapBiomas (https://plataforma.brasil.mapbiomas.org/; Souza et al. 2020).

### Physicochemical water parameters and local habitat sampling

We measured the water's physico-chemical parameters (pH, conductivity, total dissolved solids, temperature, and dissolved oxygen) in situ once in each stream in both seasons using the YSI V6600 multiparameter probe. Water samples were collected to analyse nutrient concentrations such as total nitrogen (TN), total phosphorous (TP), nitrate ($NO_3$-N), ammonium ($NH_4$-N), and phosphate ($PO_4$-P). (For values of water parameters and their response to afforestation gradient, see Supplementary Table 2).

**Local habitat description and sampling of basal resources and macroinvertebrate sampling**

Field sampling campaigns were conducted in the summer (February-March) and winter (July-August) of 2019 across 27 streams, specifically during consistent baseflow conditions, excluding storm-induced high-water levels. We placed a transect of 50 meters along the stream at each site. All transects were flanked on both margins by buffer areas devoid of *Eucalyptus* trees. These buffer zones extended approximately 20 meters from the stream to the plantations. Additionally, we distributed three Surbers nets (width: 25 cm, length: 50 cm, height: 50 cm, 500 µm mesh size with the laterals closed by a 500 µm mesh size net), ensuring a minimum distance of 15 metres between each point. This modified Surber net was adapted to our streams characterised by low slopes, minimal water movement, and water depth may reach 50 cm in some points. Moreover, the large area of the Surber net allowed large stone and dense macrophytes to be enclosed inside the Surber area. Within the area of each Surbers net, local habitat variables were recorded for each Surber sample, including water depth (cm), current velocity ($m.s^{-1}$, measured with a digital flow probe), and substrate components (percentages of mud, clay, sand, gravel, and stones) (mean values are reported in Supplementary Table 3).

Subsequently, the stock of basal resources was collected alongside the macroinvertebrate community in the total area occupied by the Surber net. Basal resources consisted in detritus, macrophytes and periphyton (mean values are reported in Supplementary Table 4). In the case of detritus, the sample consisted of a mix of allochthonous leaf litter (leaves, wood, seeds, and flowers) and senescent macrophyte material. In the of the periphyton sampling, we used a field surface fluorometry (BenthoTorch$^{TM}$ optical sensor) which was placed on the streambed substrate within each Surber (we employed the BenthoTorch before the measures of local habitat and the streambed removal). This device analyses the composition of periphyton ($\mu g.cm^{-2}$ of diatoms, cyanobacteria, and green algae; Kahlert and McKie 2014). For the collecting, sediment within the Surber was disturbed and basal resources (detritus and macrophytes) were removed along with associated macroinvertebrates. Larger stones were cleaned to ensure any macroinvertebrates present were accounted for. All collected material (detritus, macrophytes, and macroinvertebrates) was stored in plastic bags and placed in coolers for transportation. The number of replicates was three per stream *27 stream sites * 2 seasonal visits = 162 samples. The total area per stream was 2.25 $m^2$, which allowed for a large variation in stream habitat conditions. Surber replicates were positioned in order to cover all dominant microhabitats (a combination of patch with macrophyte cover, or without macrophytes dominated by rocks and sand).

In the laboratory, macrophytes and leaf litter collected were rinsed under tap water using a 500 µm sieve to separate the macroinvertebrates. These organisms were then placed in plastic pots containing 70% ethanol. Subsequently, different basal resource types were separated, and in the case of macrophytes, taxonomic identification was pursued to the finest possible resolution (genus or species), except for grass species, which were identified at the family level (i.e. Poaceae). Each species was further categorised based on its growth form (submerged, rooted floating, and emergent; Feijoó and Lombardo, 2007; Trindade et al. 2018). We classified grass species independently from the emergent growth form, as they are terrestrial yet inhabit aquatic environments. Allochthonous leaf litter and macrophyte litter were combined in a single category labelled as "detritus" due to the degradation of some plant material, making it impossible to distinguish between macrophytes and terrestrial material in most cases. Macrophytes and detritus were subject to drying in an oven (at 80°C until constant weight) followed by calcination in a muffle furnace (at 500°C for 4 hours) to obtain the ash-free biomass (g). Posteriorly, the biomass of different basal resources was relativised to each Surber sampled and calculated as the mean proportion of biomass of basal resources as $g.m^{-2}$. In the case of periphyton, the biomass obtained using the BenthoTorch ($\mu g.cm^{-2}$) was also relativised in $g.m^{-2}$.

Macroinvertebrates were identified to genus level except for some groups that were classified to family (Hirudinea and some Diptera, and Coleoptera), and subfamily level (Chironomidae). Acari was identified as Hydracarina, the level used for water mites. We exclude Oligochaeta from the analysis due to this group was only classified into class level impeding to allocate it into trait categories as different species have different functional traits (Capurro et al. 2021; Guimarães et al. 2024).

To assess functional diversity, we identified 51 macroinvertebrate trait categories spanning nine trait groups associated with food item preferences, morphology, physiology, and behaviour characteristics (see Table 1). These functional traits were drawn from various sources in the existing literature (Tomanova et al. 2006, 2008; Poff et al. 2006; Tomanova and Usseglio-Polatera, 2007; Chevenet et al. 1994; Reynaga and Dos Santos, 2012; Jiang et al. 2021; Leiva et al. 2022; Ao et al. 2022), supplemented by analyses of morphological aspects such as maximum mean size, which was measured for all individuals collected. For this, body size was measured with precision to the nearest 1 mm by utilising callipers or stereo lenses. We used the following criteria: (i) the height of gastropods along the central axis of their shells, (ii) the length of bivalves from their anterior to posterior side, (iii) the total length of *Hyallela* from the tip of their rostrum to the last urosome, and (iv) the total length of insect larvae, *Aegla*, *Asellus*, Dugesiidae and mites from the end of their head to the last segment (Eklöf et al. 2017).

We selected the traits deemed relevant to changes in their habitat and carbon resource characteristics. A fuzzy coding procedure was then employed to assign affinity scores to each taxon for each trait category. Scores ranged from 0 to 3, where 0 indicated no affinity, and a score of 3 denoted maximal affinity (Chevenet et al. 1994; Tomanova et al. 2006, 2008; Tomanova and Usseglio-Polatera 2007).

**Table 1.** Functional traits groups and their trait categories codes with their corresponding explanation of the macroinvertebrate community.

| Trait group | Trait category | Explanation |
|---|---|---|
| **1) Food** | SP | Sediment particles |
| | FPOM | Fine detritus < 1mm |
| | CPOM | Coarse detritus > 1mm |
| | MiPh | Microphytes |
| | MaPh | Macrophytes |
| | DA | Dead animals |
| | MIIn | Microinvertebrates |
| | MAIn | Macroinvertebrates |
| | FsTp | Fish/tadpoles |
| **2) Feeding habits** | CG | Collector-Gatherer |
| | SH | Shredder |
| | SC | Scraper |
| | CF | Collector-Filterer |
| | PI | Piercer |
| | PR | Predator |
| | PA | Parasite |
| **3) Respiration** | Teg | Tegument |
| | Gill | Gills |
| | Plas | Plastron |
| | Stig | Stigmata |
| **4) Maximun body size (mm)** | <2.5 | |
| | 2.5-5 | |
| | 5.0-10.0 | |
| | 10.0-20.0 | |
| | 20-40 | |
| | 40-80 | |
| | >80 | |
| **5) Body flexibility** | <10 | None |
| | 10-45 | Low |
| | >45 | High |
| **6) Body form** | Stre | Streamlined |
| | Flat | Flattened |
| | Cyl | Cylindrical |
| | Sphe | Spherical |
| **7) Specific adaptation to flow constrains** | Suc | Sucker |
| | SG | Silt gland |
| | MM | Mineral material-case |
| | AH | Anal hooks |
| | TH | Tarsal hooks |
| | NA | No adaptation |
| **8) Mobility and attachment to substratum** | Flier | Flier |
| | SwS | Surface swimmer |
| | SwW | Full water swimmer |
| | CL | Crawler |
| | EpB | Epibenthic burrower |
| | EnB | Endobenthic burrower |
| | TA | Temporarily attached |
| **9) Voltinism** | Sem | Semivoltine (<1 generation/y) |
| | Uni | Univoltine (1 generation/y) |
| | Mult | Bi or multivoltine (>1 generation/y) |

We computed Functional Dispersion (FDis) to assess functional diversity based on the relative density of taxa in each trait category. FDis quantifies the taxa spread in the trait category space from the centroid of each taxon, weighted by their relative density (Laliberté and Legendre, 2010). Higher functional dispersion indicates that the most abundant species possess different functional traits, potentially enabling them to exploit various habitat and carbon resource types (Espinoza-Toledo et al. 2021). FDis provides insights into the differentiation of macroinvertebrate taxa in the community concerning their ecological roles and functional traits (Villéger et al. 2008). Compared to other components of functional diversity, such as Functional Richness (FRic), which represents the extent of functional traits within a community without being weighted by taxa abundance, the use of FDis is highly robust (Laliberté and Legendre 2010). Functional diversity indices of the invertebrate communities per season (summer and winter) were calculated using the “FD” packages in R (Laliberté et al. 2014).

### Data analyses.

To identify the bivariate association between the macroinvertebrate functional traits and (1) physico-chemical water parameters, (2) local habitat variables, and (3) basal resources, we conducted three RLQ and Fourth-corner analyses (Dray and Legendre, 2008; Dray et al. 2014). RLQ multivariate analyses aimed to identify linear combinations of traits and environmental variables onto which sites and taxa were projected to yield the most covariant new site and taxa scores. For this, RLQ analyses involved relating an environmental (R) matrix, a macroinvertebrate taxon density matrix (L), and a macroinvertebrate trait matrix (Q) to perform separate analyses with each matrix. Before RLQ analyses, three matrices were analysed separately. First, a correspondence analysis (CA) was performed on the taxa density matrix, and then a principal component analysis (PCA) on the trait’s matrix using row weights corresponding to the column weights of the previous CA. PCA was also used for the environmental matrix. Environmental variables were scaled and centred due to differences in units, and macroinvertebrate density values were transformed using the ‘constant’ function with the Hellinger method. From the RLQ multivariate biplot, we interpreted the inertia which provides information on how much variation is explained by the principal axes of the analysis, which helps us evaluate the relevance and interpretability of these axes. We conducted a Monte Carlo test using 999 random permutations to assess: (a) the significance of the relationship between environmental variables and macroinvertebrate taxa composition (association between R and L matrices; Model 2) and (b) to test whether the distribution of macroinvertebrate taxa according to their preferred sites depended on their functional traits (association between Q and L matrices; Model 4) (Dolédec et al. 1996; Dray and Legendre 2008). Additionally, we performed a fourth-corner analysis to test the bivariate associations of environmental variables and macroinvertebrate trait categories (Dray et al. 2014). The R packages used were “ad4” for the RLQ analyses (Dray et al. 2015).

Structural equation models (SEM) were employed to disentangle the direct and indirect pathways by which *Eucalyptus* spp. afforestation may affects the functional diversity of macroinvertebrate communities. The SEM was fitted based on a meta-model (Supplementary Fig. 1), in which we accounted for *Eucalyptus* spp. afforestation, macroinvertebrate functional diversity (FDis), carbon basal resource (biomass of macrophyte, periphyton, and detritus), and habitat quality variables (water velocity, stream depth, sand, clay, mud, and stone). We implicitly test whether *Eucalyptus* spp. afforestation influences macroinvertebrate functional diversity directly or indirectly by influencing carbon basal resource and local habitat variables. To test the influence of the season, we ran individual models for summer and winter. We tested multicollinearity between drivers by calculating the variance inflation factor (VIF). A value of VIF > 3 indicates possible collinearity, which was not observed in our model. Moran's test was used to check for residual spatial autocorrelation in SEMs. We found no evidence of spatial autocorrelation in our SEMs, either for the SEM in summer (Moran's, I test observed = –0.005, expected = 0.009, *p* = 0.457) or for the SEM in winter (Moran's, I test observed = –0.008, expected = –0.012, *p* = 0.566). The SEMs were fitted using a linear model in the "piecewiseSEM" package (Lefcheck, 2016). We present the standardised coefficient for each path and estimate. The indirect effects of *Eucalyptus* spp. afforestation on macroinvertebrate functional diversity were estimated by multiplying the coefficient of *Eucalyptus* spp. afforestation on carbon basal resource and local habitat variables by the coefficient of carbon basal resource and local habitat variables on macroinvertebrate functional diversity. The significance of all paths was obtained using maximum likelihood and SEM fit was examined using Shipley's test of d-separation through Fisher's C statistic (*P-value* > 0.05 indicates an adequate model). All analyses were done with 'R' version 4.3.1 (R Development Core Team 2023).

For the construction of SEMs, we used the aggregate biomass values of macrophyte growth forms and periphyton components as total macrophyte and periphyton biomass respectively. This approach aimed to mitigate issues stemming from the high number of explanatory variables, which could exceed the number of replicates available. However, we employed the biomass of individual macrophyte growth-forms and periphyton components for RLQ and fourth-corner analyses. We did not incorporate physico-chemical water parametersin the SEM construction, as water variables were measured once per stream. In contrast, local habitat and basal resources were sampled three times (using three Surber samples) per stream. Therefore, we used each replicate in the data frame to ensure an ample number of samples for the analysis of local habitat and basal resources. It is well known that *Eucalyptus* afforestation alongside livestock presence affects physico-chemical water parameters by increasing nutrient concentrations and decreasing dissolved oxygen in waters (Barrios et al. 2023). Thus, possible direct effects of the increase in *Eucalyptus* spp. afforestation on functional diversity also includes the effects of physico-chemical water parameters.

## Results

The RLQ analysis, using physico-chemical water parameters (Table 2), demonstrated a cumulative inertia of 82.59% across the first two axes (70.39 % and 13.49 %, respectively), with a total inertia of 0.387 explained by RLQ ordination. We observed a distinct separation among sites along the axis according to season gradient following axis 2 and an afforestation gradient following axis 1 (Fig. 2a). The most significant variables associated with the RLQ ordination axis, such as TN and TP, appeared to be more linked to sites with high and moderate afforestation cover, while dissolved oxygen and pH (axis 1) were associated with sites during the winter season (Fig. 2a,c).

When examining bivariate associations, the four-corner analysis indicated that taxa distribution was influenced by water variables (Model 2, *P-value* < 0.0001), while taxa composition was not significantly affected by their functional traits (Model 4, *P-value* = 0.110). However, after applying *P-value* adjustment, we identified significant bivariate associations between traits and environmental variables. For instance, traits associated with macroinvertebrate (MAIn) and carnivorous diets (fish and tadpoles), predators, parasites, and larger body sizes (> 10 mm) exhibited negative correlations with dissolved oxygen (DO) (Fig. 2d, 5). Meanwhile, food preference for fine particulate organic matter (FPOM), collector-gatherers, and scrapers was positively correlated with DO (Fig. 2d, 5). Total nutrients displayed positive correlations with larger body sizes (40–80 mm), predators (only correlations with TN), and negative correlations with scrapers and case-building macroinvertebrates, such as *Neotrichia, Oxyethira, Hydroptila*, and other Hydroptilidae taxa, *Mortoniella*, as well as larvae and adults of some taxa related to Elmidae (Fig. 2b,d, 5). Positive correlations were detected between univoltinism and anal hook traits with ammonium concentrations and food preference for animal detritus (DA) with conductivity (k) (Fig. 2d, 5).

Using local habitat variables in the RLQ analysis showed that the total inertia was 82.48% across the first two axes, which were 68.72% and 13.76%, respectively (Table 2). We observed no clear seasonal pattern among stream sites between summer and winter, nor along the afforestation gradient (Fig. 3a). However, significant associations of local habitat variables with the first two RLQ ordinations were noted, particularly with current velocity and the percentages of clay, mud, gravel, and stone (Fig. 3a,c). Bivariate associations revealed that taxa distribution was influenced by local habitat variables (Model 2, *P-value* < 0.0001) and functional traits (Model 4, *P-value* = 0.018). Associations of individual traits with local habitat (Fig. 3d, 5) revealed that current velocity, gravel, and stone percentages were negatively correlated with predators, such as taxa with macroinvertebrate diets such as Belostomatidae, Noteridae taxa, *Acanthagrion*, and *Perithemis* (Fig. 3b,d).

On the other hand, mud and clay showed a positive correlation with traits related to fish/tadpole diet, parasites, sucker adaptations to flow constraints, and larger body sizes (>10 mm). Traits related to fine particulate organic matter (FPOM) food preferences and collector-gatherer (CG) and scraper (SC) feeding habits were positively correlated with gravel and stone percentages (only SC), including taxa such as *Psephenus, Mortoniella, Helycopsyche*, and taxa from the Hydroptilidae family (Fig. 3b,d, 5). Gravel percentages were also positively correlated with traits related to coarse particulate organic matter (CPOM) food preferences, shredder (SH), and plastron respiration (Fig. 3d, 5). Additionally, stone was positively associated with case-building and tarsal hook traits, such as taxa from the Hydroptilidae family (Fig. 3b,d, 5).

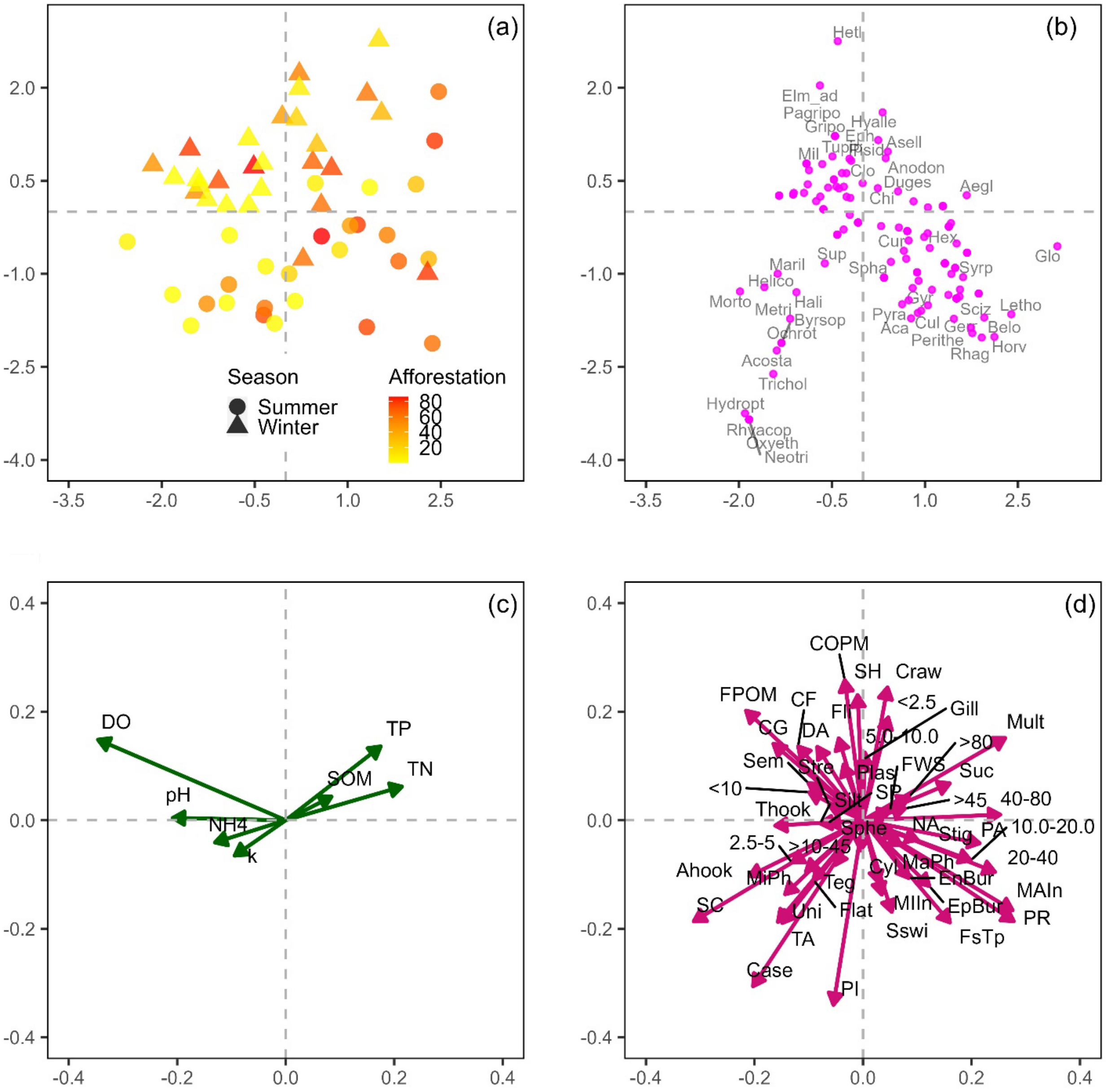


**Fig.2**. Results of the first two axes of RQL analysis: to test seasonal and *Eucalyptus* afforestation patterns in stream sites according to variations in the water parameters (a), scores of the distribution of macroinvertebrate taxa (b), coefficients for water parameters (c), and traits (d). Taxa codes are shown in Supplementary table 7, and taxa traits codes are in Table 1.

In the case of RLQ using basal resources, the total accumulated inertia was 77.81 % across the first two axes (64.56 and 13.25 %, respectively; total inertia = 0.262; Table 2). There was not a clear seasonal pattern observed among stream sites between summer and winter, nor along the afforestation gradient (Fig. 4a). Emergent, grass and rooted floating macrophytes, as well as detritus, green algae, and cyanobacteria, showed significant correlations with the axis of the RLQ ordination (Fig. 4c). Bivariate associations indicated that the taxa distribution was influenced by basal resources (Model 2, *P-value* < 0.0001) and by their functional traits (Model 4, *P-value* = 0.002).

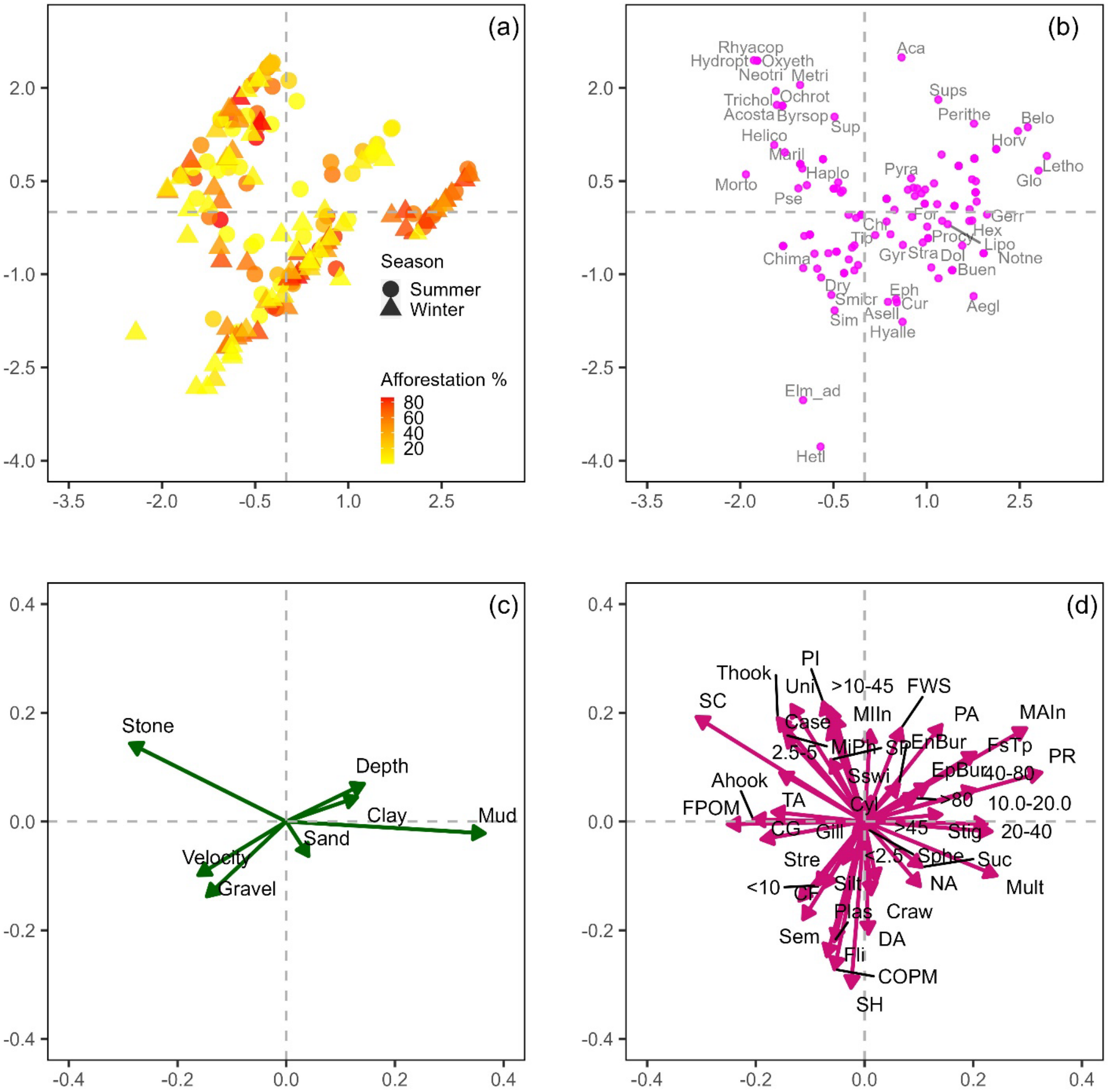


**Fig.3**. Results of the first two axes of RQL analysis: to test seasonal and *Eucalyptus* afforestation patterns in stream sites according to variations in local habitat variables (a), scores of the distribution of macroinvertebrate taxa (b), coefficients for local habitat variables and (c) and traits (d). Taxa codes are shown in Supplementary table 7, and taxa traits codes are in Table 1.

Traits related to food preferences for macroinvertebrates (MAIn) and fish/tadpoles (FsTp), as well as feeding habits such as predator (PR), exhibited positive correlations with submerged, emergent, and grass macrophytes (Fig. 4d, 5). These macrophyte growth-forms were mostly associated with body sizes ranging between 20 and 40 mm, typically observed in taxa such as Belostomatidae and Anisoptera (Aeshnidae and Libellulidae) (Fig. 4b,d, 5). Negative correlations were observed between these macrophytes and sediment particles (SP), fine particulate organic matter (FPOM) food preferences such as *Simulium*, and collector-gatherers (e.g., Orthocladiinae) and scrapers (e.g., Hydroptilidae taxa) feeding groups (Fig. 4b,d, 5). Grass was also negatively correlated with microphytes (MiPh) food items and tegument respiration, while rooted floating macrophytes showed negative correlations with tarsal hook adaptations to flow constraints and crawler mobility. Among periphyton components, negative correlations were evident between diatoms and parasites, larger body sizes (40 – 80 mm), and cyanobacteria with a cylindrical body form. Green algae exhibited a positive correlation with collector-filters (CF). Regarding detritus, there was a positive correlation with dead animals (DA, e.g., Asellidae, *Aegla, Hyallella*) and negative correlations with FPOM, MiPh food items, collector-gatherers (CG), and scrapers (SC) (Fig. 4b,d, 5).

**Table 2.** Summary of the RLQ analysis: eigenvalues and percentage of projected inertia accounted for by the first two RLQ axes. Covariation (square root of eigenvalue) is the covariance between the two new sets of factorial scores projected onto the first two RLQ axes. Correlation is the correlation between the two new sets of factorial scores projected onto the first two RLQ axis.

| Environmental variables | Axes | Eigenvalues | Projected inertia (%) | Covariance | Correlation |
|---|---|---|---|---|---|
| Water variables | Ax1 | 0.273 | 70.39 | 0.522 | 0.184 |
| | Ax2 | 0.033 | 13.49 | 0.229 | 0.112 |
| Local habitat variables | Ax1 | 0.303 | 68.72 | 0.550 | 0.203 |
| | Ax2 | 0.061 | 13.76 | 0.246 | 0.123 |
| Basal resource biomass | Ax1 | 0.169 | 64.56 | 0.411 | 0.163 |
| | Ax2 | 0.035 | 13.25 | 0,186 | 0.097 |

The increase of *Eucalyptus* spp. afforestation negatively impacts functional diversity (FDis) through indirect pathways mediated by local habitat and basal resources (Fig. 6; Supplementary Table 5). These indirect effects also exhibited variation across contrasting seasons. Specifically, afforestation indirectly reduced FDis in summer by decreasing macrophyte biomass (Fig. 6 a,b). At the same time, macrophyte biomass increased positively with the percentage of mud in both seasons (standardised coefficient: summer = 0.387; winter = 0.384). During summer, it was evident that other local habitat variables and basal resources were unaffected by afforestation, but they positively influenced functional diversity, including the percentage of stone (0.499), clay (0.408), periphyton biomass (0.347), mud (0.285), and sand (0.305) (Fig. 6 a,b). During winter (Fig. 6 c,d), afforestation negatively affected current velocity (-0.331), periphyton biomass (-0.253), and macrophyte biomass (-0.251). However, only macrophyte biomass significantly affected FDis (Fig. 6 c,d).

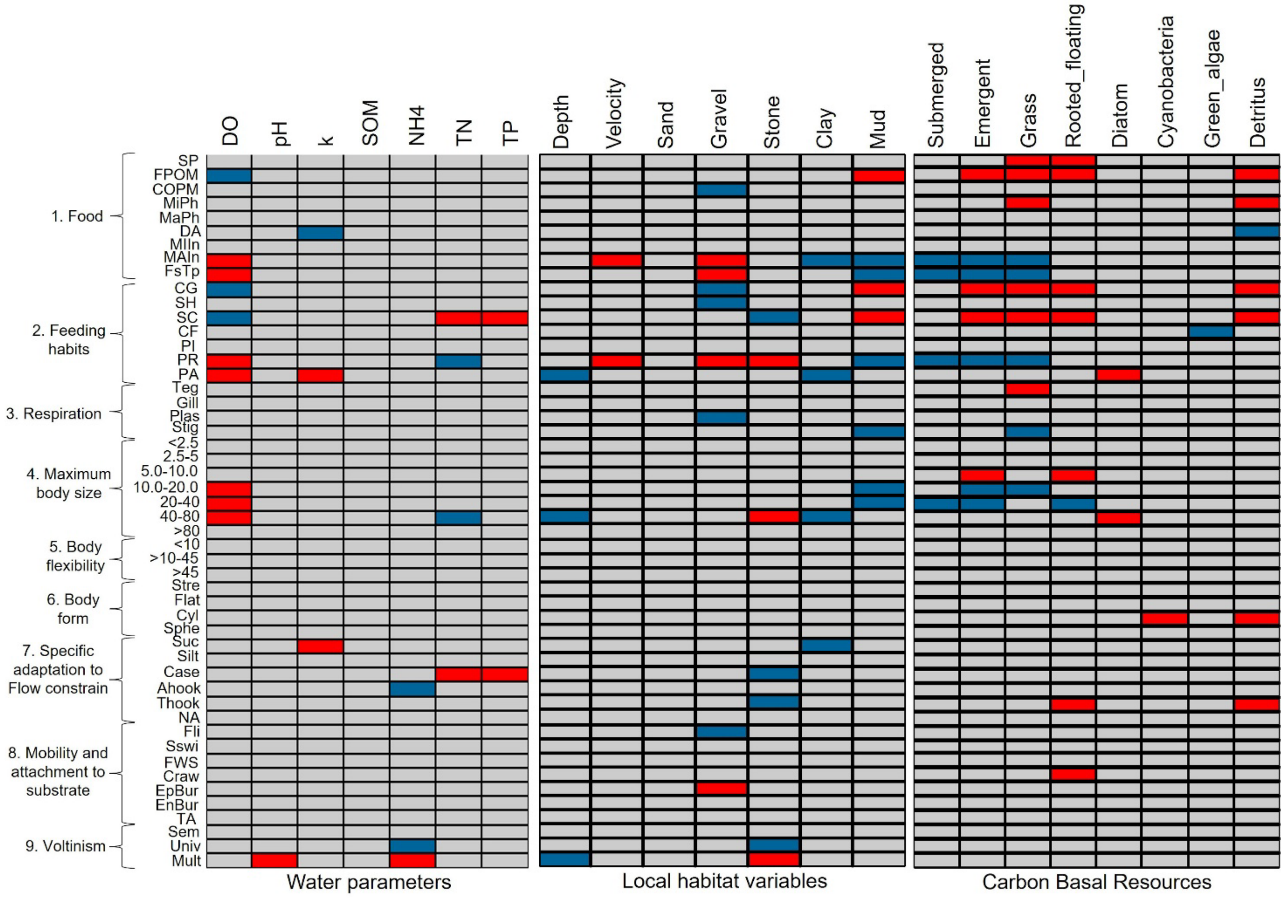


**Fig.4**. Results of the first two axes of RQL analysis: to test seasonal and *Eucalyptus* afforestation patterns in stream sites according to variations in basal resources (a), scores of the distribution of macroinvertebrate taxa (b), coefficients for basal resources and (c) and traits (d). Taxa codes are shown in Supplementary table 7, and taxa traits codes are in Table 1.

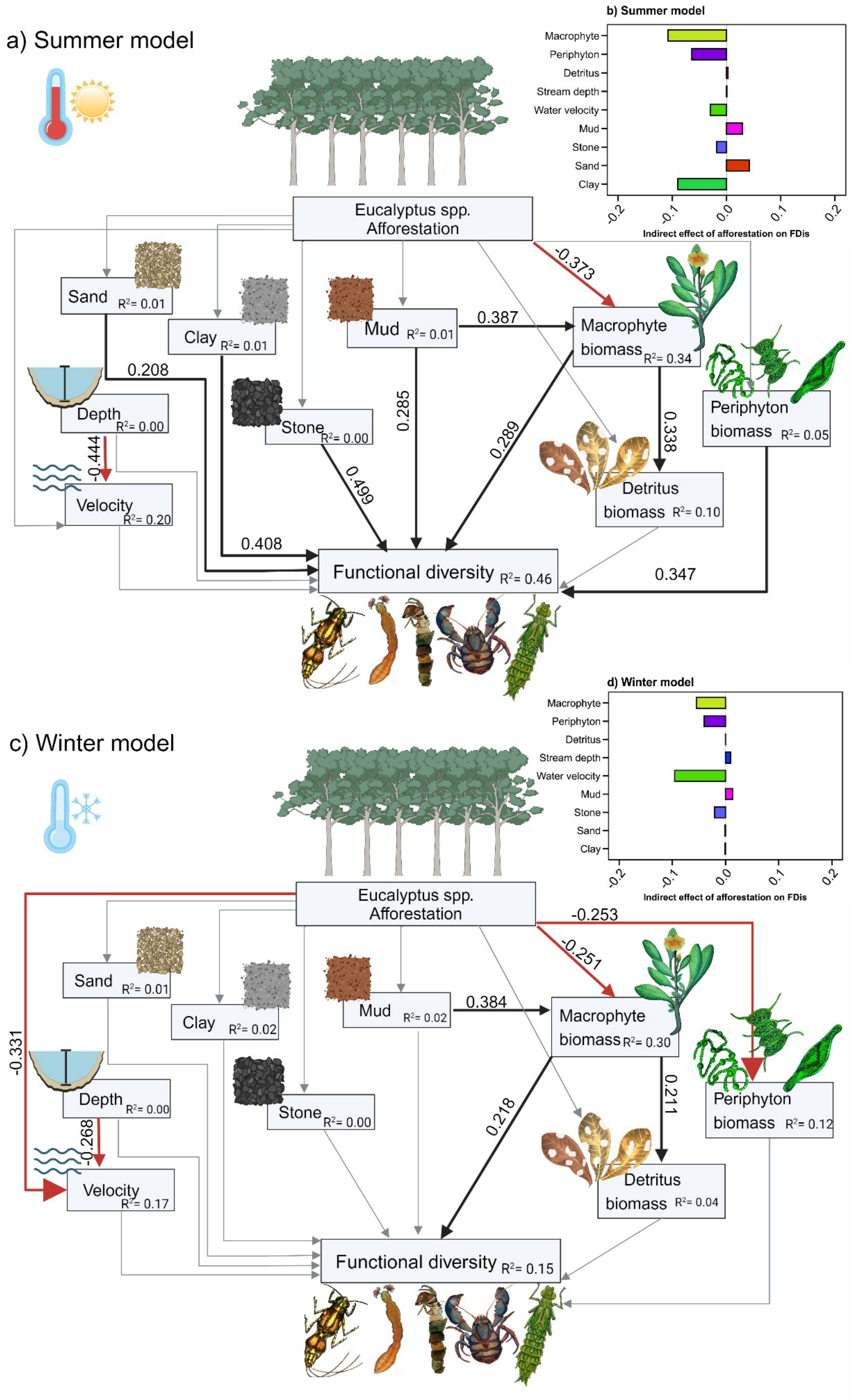


**Fig.6.** The effect of afforestation intensification on local habitat variables and basal resources and their cascading effects on macroinvertebrate functional diversity. a,c, Structural equation modelling (SEM) allowed us to disentangle the effects of *Eucalyptus* afforestation on macroinvertebrate functional diversity in (a) summer and (c) winter seasons. Standardised indirect effects of *Eucalyptus* afforestation on macroinvertebrate functional diversity mediated by local habitat variables (stream depth, current velocity, percentages of mud, stone, sand and clay) and basal resources (periphyton biomass,

macrophyte biomass and detritus biomass) in (b) summer and (d) winter season. The SEM was run separately during the summer and winter seasons. Solid black and red arrows represent significant ($P \leq 0.05$) positive and negative pathways, respectively. Solid grey arrows represent non-significant pathways ($P \geq 0.05$). The thickness of the significant pathways (arrows) represents the magnitude of the standardised regression coefficient. Numbers adjacent to arrows are the standardised effect size. $R^2$ inside the boxes represent the percentage of explanation of each endogenous variable. We found good model fits for summer (Fisher´C = 51.01, $P$ = 0.283) and winter season (Fisher´C = 44.56, $P$ = 0.532). Full model outputs are provided in Supplementary Table 5.

## Discussion

The conversion of natural landscapes to *Eucalyptus* plantations is one of the major threats to macroinvertebrate diversity in stream ecosystems worldwide (Ferreira et al. 2016, 2019). Our findings suggest that an increase in *Eucalyptus* afforestation replacing grasslands within stream watersheds, leads to indirect negative changes in macroinvertebrate functional diversity by decreasing basal resource availability, particularly macrophyte biomass. This suggests that *Eucalyptus* afforestation promotes trait homogenisation, limiting macroinvertebrate communities to use fewer niches by reducing macrophyte biomass, which poses a threat to ecological processes (Moi et al. 2023).

The negative response of macrophyte biomass to *Eucalyptus* afforestation could be mediated in multiple ways, such as the increase in nutrient concentration, which increases along the afforestation gradient (Barrios et al. 2023). In other studies, macrophytes may decrease even with a low increase in the trophic condition due to the increase in intensive agriculture practices (Urbanič et al. 2021). The increase of dissolved organic carbon (DOC) from terrestrial sources has also been negatively related to macrophyte biomass due to the release of humic substances, decreasing light irradiance (Reitsema et al. 2018). *Eucalyptus* afforestation promotes an increase in soil organic carbon, which is high in resins, waxes, and aromatic oils (González-Sosa et al. 2024). These compounds can be easily washed and drained into the waterbodies (Tesón et al. 2014) due to the increase in soil repellency (González-Sosa et al. 2024) and may affect macrophytes, especially those with submerged and emergent growth forms.

When we analysed in detail the associations of trait structure with different growth forms of macrophytes, we can better understand how the loss of the carbon basal resource may affect macroinvertebrate functional diversity. In this study case, we detected positive correlations between submerged, emergent, and grass macrophytes with traits related to predators with invertivores and fish/tadpoles' diets, as well as with small (10–20 mm) and medium sizes (20–40 mm). These macrophyte growth forms are more complex in their architecture compared to submerged-floating types (Tóth 2024). This structural complexity provides refuge for invertebrates and small fish, consequently attracting more predators (Wolters et al. 2018; Yofukuji et al. 2021). Thus, these results suggest that macrophytes can enhance predation by maintaining a high density of prey (Klecka and Boukal 2014; Rezende et al. 2019). Additionally, the ability of predators to search for food among macrophytes apparently depends on the size of both the predators and their prey (Klecka and

Boukal 2013), as small and medium sizes allow them to move through macrophyte structural elements (Klecka and Boukal 2013, 2014).

In this sense, the presence of macrophytes in our systems, especially those with more structured growth forms, increases macroinvertebrate functional diversity as they play a crucial role in shaping trait variation among taxa (Thomaz and Cunh 2010; Wolters et al. 2018; Yofukuji et al. 2021; Rideout et al. 2022). Consequently, the decline in predatory macroinvertebrates resulting from the reduction in macrophyte abundance caused by afforestation, may trigger cascading effects that alter ecosystem processes. Furthermore, the negative effects of *Eucalyptus* afforestation on macroinvertebrate functional diversity persisted in both the summer and winter seasons. Therefore, this confirms the persistent effect of this stressor and the significance of macrophytes in shaping macroinvertebrate functional diversity. We suggest that other experimental approximations, such as mesocosms or microcosms that may show the importance of different macrophyte growth forms in the interaction of prey and predator (Meerhoff et al. 2003; Wolters et al. 2018; Teixeira-de-Mello et al. 2016), and also including experimentation with possible factors related with *Eucalyptus* afforestation, such as the presence of DOC, nutrient increase, among others.

The afforestation *Eucalyptus* has a negative effect on periphyton biomass in winter, but this did not affect the macroinvertebrate functional diversity. Contrarily, the presence of this basal resource was not affected by the afforestation during the summer season, but it had a positive effect on the functional diversity. We observed that periphyton biomass did not varied among seasons except for diatoms that tended to increase in winter (Supplementary analysis 2). This demonstrated the complexity of the system and the relationships between basal resources and macroinvertebrate functional diversity. The fact that *Eucalyptus* afforestation was negatively related with periphyton in winter, but their biomass tended to be similar between both season (except for diatoms) suggest that changes in periphyton species composition or different periphyton growth-forms may occur responding to the intensification of the afforested area. Other findings suggest that macroinvertebrates better respond to specific growth forms of periphyton instead of its biomass (Tonkin et al., 2014), and this aspect should be considered in future studies.

Positive relationship between periphyton biomass and functional diversity in summer may suggest that macroinvertebrates are using this resource which is highly nutritive (Thorp and Delong 1994; 2002; Marcarelli et al. 2011). Additionally, periphyton may retain fine particulate organic matter, which might support a larger density of macroinvertebrates that feed on these resources (Tonkin et al. 2014; Tonin et al. 2017). As a result, the functional diversity within the community was enhanced (Rideout et al. 2022). This also suggests that some feeding traits profit from the seasonally available resources, supporting the notion that they might have omnivorous or generalist behaviours. Other studies have shown that omnivory may be a strategy to overcome the high variability of basal resources and reduce intraguild competition (Cortés-Guzmán et al. 2022). It is necessary to implement stable isotope techniques and experimental methods that may help to understand the importance of periphyton as a food resource for macroinvertebrate consumers (Watson and Barmuta 2011; Paice et al. 2016).

We expected that the *Eucalyptus* afforestation could affect the detritus benthic stock, especially during the summer when there is a higher *Eucalyptus* leaf fall that may enter the streams (Abelho and Graça 1996; Abehlo 2001; Ferreira et al. 2016). Additional research indicates that the entry of *Eucalyptus* leaves into aquatic ecosystems may alter the macroinvertebrate community, particularly affecting detritivorous shredders that consume these leaves due to that *Eucalyptus* leaves possess numerous secondary compounds detrimental to aquatic organisms (Canhoto and Graça 1995; Ferreira et al. 2016; 2019). However, and contrary to our expectations, detritus biomass was higher in winter than summer (Supplementary analysis 2) and we did not find significant content of *Eucalyptus* litter in the benthic stock during the processing of the collected material in Surbers. The buffer zone surrounding the stream sites may explain this by restricting the entry of *Eucalyptus* leaf material into the streams. Compliance with these management regulations to preserve the buffer zone demonstrates its effectiveness in protecting stream dynamics, at least for the direct impact of *Eucalyptus* afforestation (Sargac et al. 2021) but does not prevent the basic indirect ones on macroinvertebrate functional diversity.

Our results revealed that substrate types did not vary among seasons (Supplementary analysis 2) and they were not affected by *Eucalyptus* afforestation. Nevertheless, these variables consistently exerted a significant influence on the functional diversity of macroinvertebrates during the summer season. Meanwhile, other local habitat variables, such as current velocity was affected by *Eucalyptus* afforestation in winter, but this apparently did not affect macroinvertebrate functional diversity. This suggests that the trait structure of macroinvertebrate communities undergoes a functional reconfiguration in response to changes in the components of habitat heterogeneity over different seasons independently of changes related to *Eucalyptus* afforestation (Wang et al. 2023). Macroinvertebrate functional diversity was higher in summer than winter (Supplementary analysis 2) and could be related to its positive correlations with periphyton and macrophyte biomass and with stream bed substrates that promote a higher trait diversification for using different basal resources and local habitat conditions. Therefore, functional diversity's response to these variables provides us with insights into the functioning of our study systems. Although, it is necessary to conduct long-term research in order to gain a deeper understanding of how local habitat variables contribute to the preservation of macroinvertebrate functional diversity.

We detected that case-building (usually Hydroptilidae species) with attachment traits exhibited a positive correlation with the percentage of stones. Larger substrate sizes may facilitate the colonization of macroinvertebrates with attachment traits (Firmiano et al. 2021). Finer substrate sizes (e.g., clay) were related to parasites (e.g., *Temnocephala*), invertivores diet, sucker adaptation to flow constraints, and maximum sizes ranged from 40 to 80 mm. This suggests that substrate types are related certain traits, particularly those related to feeding habits, adaptations to flow constraints, and body size (Gao et al. 2024). Larger differences in substrate sizes play a big role in shaping traits and keeping a high functional diversity among macroinvertebrates (Li et al. 2019). In this scenario, the presence of larger substrates (e.g., stones) may foster higher taxa richness and density (Downes et al. 2000; Duan et al. 2008; Gao et al. 2024). This may be attributed to the presence of shelters for macroinvertebrates that provide bigger substrates, particularly during periods of increased water flow (Pond et al. 2022) or in the presence of predators (Milesi et al. 2016; Gao et al. 2024). In addition, larger substrates promote periphyton attachment, which serves as food for scrapers (Downes et al. 2000; Duan et al. 2009; Gao et al. 2024).

When we analysed the associations of trait structure with physico-chemical water parameters in detail, we observed that specific traits exhibited distinct responses to water quality deterioration. For instance, total nutrients (TP, TN) concentration in water had a negative effect on scrapers and case-building taxa. This may be related to their low tolerance capacity to high nutrient concentrations in water (Paz et al. 2022; Barrios et al. 2023) or due to higher nutrient concentrations affect primary producers which serves as food for scrapers (Tonin et al. 2017). Scrapers, on the other hand, responded positively to dissolved oxygen, likely because of the higher primary productivity of algae used by this feeding trait (Guo et al. 2022). physico-chemical water parameters are negatively affected by *Eucalyptus* spp. afforestation in the studied streams (Barrios et al. 2023), and due to the relationships between water variables and some traits, we expected that deterioration of water quality has negative effects on functional diversity. These possible negative effects may also be indirect, considering that water deterioration may also impair macrophyte availability (Feijoó and Lombardo 2007), which apparently is an important factor structuring macroinvertebrate functional diversity in our study.

## Conclusions

Our finding partially supports the hypothesis that the increased *Eucalyptus* afforestation cover led to a decrease in macroinvertebrate functional diversity. However, in the case of subtropical lowland streams, this effect was mediated by changes in the availability of basal resources. In this case, *Eucalyptus* afforestation can also reduce functional diversity by removing traits related to basal resource availability, especially those related to macrophytes. Thus, our study highlights the broader impacts of *Eucalyptus* afforestation on aquatic diversity, including functional diversity and macroinvertebrate community trait structure. Previous studies have shown that this type of land use can affect physico-chemical water parameters and the taxonomic diversity of macroinvertebrates (Barrios et al. 2023).

We show that an increase in *Eucalyptus* afforestation significantly impacted macrophytes, emerging as the primary driver of functional diversity. Macrophytes play a crucial role in lowland streams, sustaining macroinvertebrate communities and providing facilities for predators. Therefore, an increase in *Eucalyptus* plantation cover may decrease macrophyte availability and reduce the niche for macroinvertebrates. Future experimental studies are needed to assess the importance of different macrophyte growth forms on prey-predator interactions.

The most sensitive traits that responded to environmental variables were those related to food item preferences, feeding groups, and body size. Thus, their selection for understanding the long-term effects of *Eucalyptus* afforestation is recommended. The use of functional traits and functional diversity can act as a warning for ecological process deterioration in stream ecosystems. Reduced specialised traits may indicate the absence of specific processes in a system influenced by anthropogenic stressors.

The buffer zone apparently prevents Eucalyptus afforestation, affecting the local variables of the physical habitat, such as substrate types, current velocity, and stream width, which also determine the trait structure and functional diversity.

Our results have important implications for stream conservation since the decrease in functional diversity has profound negative effects on ecosystem productivity, such as animal biomass production (Moi et al. 2023) and the processing of organic material (Ferreira et al. 2019). We advocate for the implementation of integrated monitoring programmes considering taxonomical composition (Barrios et al., 2023) and functional diversity, as well as ecological processes, into the monitoring of streams under exotic plant plantations (Firmiano et al. 2021; Paz et al. 2022; Hepp et al. 2024).

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**Supplementary information**

# Journal: Aquatic Science

# Changes in basal resources mediate the effects of *Eucalyptus* spp. afforestation on macroinvertebrate functional diversity in subtropical lowland streams.

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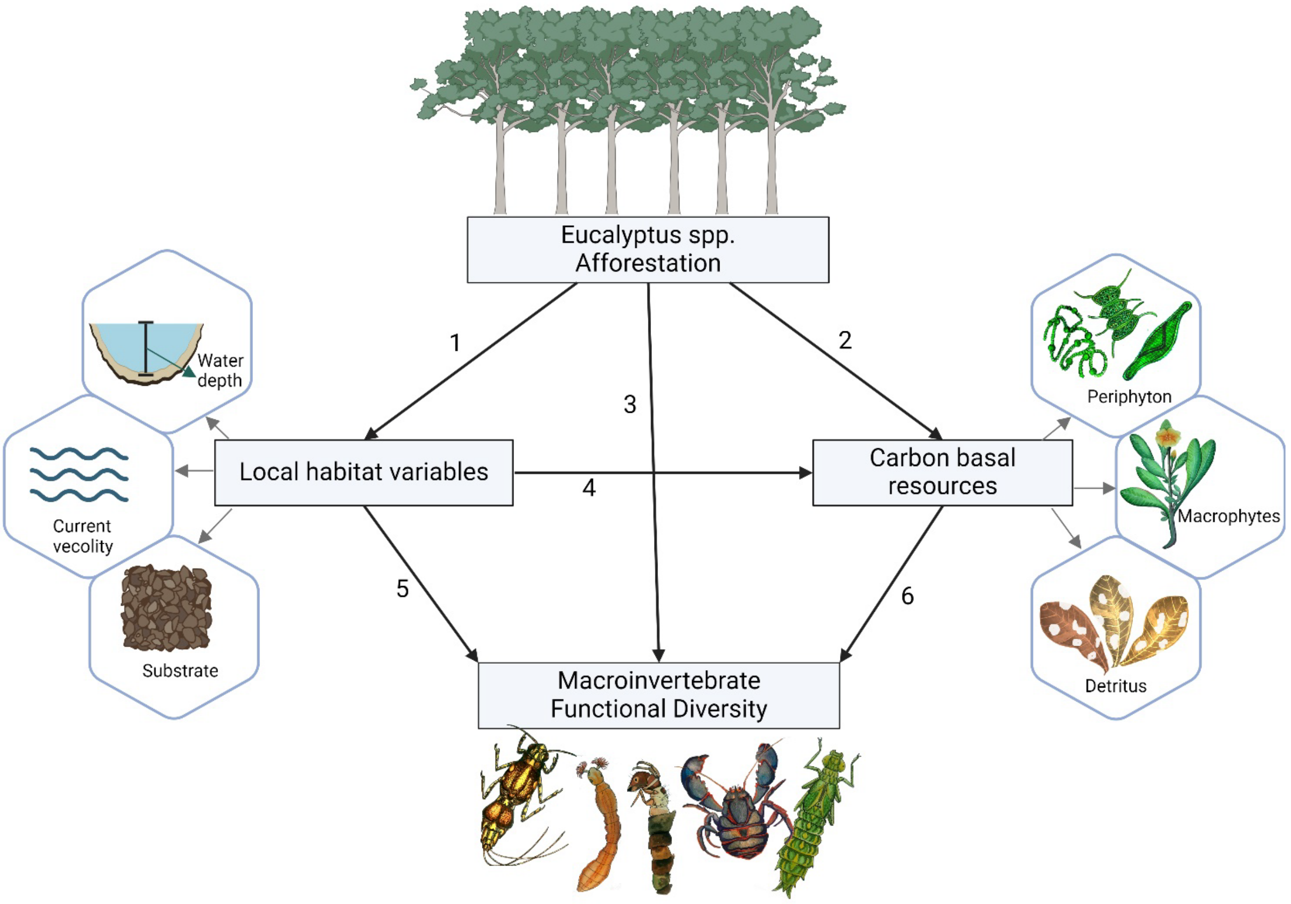

| # | Links | *Rationale* | Ref. |
|---|---|---|---|
| 1 | ***Eucalyptus* spp. afforestation → Local habitat variables** | *Eucalyptus* afforestation and its management practices may promote an increase in bank erosion levels and bank dynamics, leading to siltation, water quantity reduction, and stream flow. With this, an increase in homogenisation of the streambed occurs with the prevailing finer particulate substrates (1,2). | 1,2 |
| 2 | ***Eucalyptus* spp. afforestation → Carbon basal resources** | *Eucalyptus* spp. leaf litter may also impact autochthonous production such as periphyton and macrophytes, due to *Eucalyptus* leachates that may alter water quality (3). Siltation caused by *Eucalyptus* plantations may also promote a decrease in macrophyte production (4). The input of Eucalyptus spp. leaf litter may replace the composition of the leaf species in the natural benthic leaf litter stock (4). | 3,4 |
| 3 | ***Eucalyptus* spp. afforestation → Macroinvertebrate Functional Diversity** | Land use changes promoted by *Eucalyptus* afforestation may reduce or eliminate certain trait groups and decrease trait redundancy, leading to a simplification of functional diversity (5,6). | 5,6 |
| 4 | **Local habitat variables → Carbon basal resources** | Homogenisation of the distribution of substrate types in the streambed may affect periphyton colonisation (7), macrophyte growth (8,9), and the retention of leaf litter in the benthos. Water depth and current velocity are related to carbon basal resource retentions (10,11) | 7-11 |

| | | | |
|---|---|---|---|
| **5** | **Local habitat variables → Macroinvertebrate Functional Diversity** | Homogenisation of stream habitat structure may lead to a loss of traits-related to specific habitat configurations. For instance, the increase in fine substrate, promotes a decrease in attached traits, and those that feed on periphyton on larger substrate. Thus, the deterioration of local habitat or habitat homogenisation promotes a loss in functional diversity (12) | 12 |
| **6** | **Carbon basal resources → Macroinvertebrate Functional Diversity** | A decrease in carbon basal resources may promote changes in functional traits, especially those related to feeding groups, leading to an increase in generalist consumers. For instance, the replacement of native leaf litter species by leaf Eucalyptus may alter detritivorous traits and cause a cause a loss in species richness (5). This promotes a decrease in species/trait richness and trait redundance, affecting functional diversity (5,6,13). | 5,6,13 |

**Supplementary Figure 1.** A priori structural equation modelling (SEM) aimed to evaluate the direct and indirect effects of *Eucalyptus* spp. afforestation, as well as local habitat factors (such as water depth, current velocity, and substrate size types), along with basal carbon resources (detritus, macrophytes, and periphyton), on macroinvertebrate functional diversity within subtropical lowland streams. To streamline the model visually, predictors were grouped into composite categories (e.g., local habitat variables) while being individually considered in the analysis. The provided table offers conceptual backing for all relationships depicted in the model, drawing upon findings from prior studies. Thus, each relationship portrayed in our model reflects natural occurrences and is not merely coincidental.

**Supplementary Table 1**. Coordinates and basin area (km$^2$) of the stream sites selected.

| Basin | Stream site | Latitiud Y | Longitud X | Basin area (Km$^2$) | Afforestation (%) |
|---|---|---|---|---|---|
| Laguna Merín | DF-NF4 | -34.172 | -54.364 | 6.39 | 0.05 |
| A° Maldonado | DF-Monte 3 | -34.651 | -54.999 | 3.9 | 0.18 |
| A° Maldonado | DF- Monte 2 | -34.637 | -54.990 | 10.18 | 0.31 |
| Río Queguay | DF-CV | -32.270 | -57.601 | 1.96 | 0.36 |
| Laguna Merín | DF-NF16 | -34.138 | -54.359 | 8.79 | 0.46 |
| Río Negro | DF-C9 | -32.213 | -55.580 | 7.14 | 1.18 |
| Río Negro | DF-S19 | -32.473 | -56.405 | 0.33 | 3.34 |
| Laguna Merín | DF-NF6 | -34.176 | -54.355 | 5.39 | 3.8 |
| Río Negro | DF-S16 | -32.554 | -56.459 | 22.51 | 6.19 |
| Río Negro | DF-2018-4 | -32.044 | -55.816 | 8.21 | 7.52 |
| Río Negro | DF-2018-6 | -31.815 | -55.957 | 4.1 | 12.76 |
| Río Negro | DF-MFC | -32.203 | -55.595 | 19.34 | 19.57 |
| Río Negro | DF-2018-2 | -32.085 | -55.822 | 6.61 | 28.04 |
| Río Negro | DF-2018-3 | -32.071 | -55.822 | 7.82 | 32.88 |
| Río Negro | DF-2018-8 | -31.834 | -55.933 | 4.53 | 44.36 |
| Río Negro | DF- F8F | -32.838 | -56.962 | 14.39 | 49.21 |
| Río Negro | DF- F6F | -32.767 | -57.142 | 29.76 | 50.32 |
| Río Queguay | DF-DT | -32.249 | -57.647 | 2.17 | 56.41 |
| Laguna Merín | DF-F5 | -34.155 | -54.322 | 2.47 | 61.39 |
| Río Queguay | DF- 2018-19 | -32.353 | -57.512 | 5.79 | 62.23 |
| Río Negro | DF- 2018-20 | -32.527 | -57.342 | 3.49 | 62.89 |
| Río Negro | DF-2018-1 | -32.084 | -55.823 | 5.27 | 67.06 |
| Río Santa Lucía | DF-Forest 3 | -34.177 | -55.235 | 5.45 | 74.36 |
| Laguna Merín | DF- F7 | -34.136 | -54.313 | 2.01 | 75.75 |
| Río Negro | DF-2018-18 | -32.475 | -57.303 | 6.99 | 79.11 |
| Río Negro | DF- 2018-13 | -32.490 | -57.346 | 1.19 | 79.37 |
| Río Negro | DF- 2018-17 | -32.475 | -57.298 | 4.35 | 85.8 |

**Supplementary Table 2.** Percentage of *Eucalyptus* spp. afforestation cover and physical and chemical water quality parameters in the streams selected. TP: Total phosphorous (µg. $L^{-1}$), $PO_4$-P: Phosphate (µg. $L^{-1}$), TN: total nitrogen (µg. $L^{-1}$), $NO_3$-N: nitrate (µg. $L^{-1}$), $NH_4$-N: ammonium (mg. $L^{-1}$), SOM: suspended organic material (mg. $L^{-1}$), k: conductivity (µS. $cm^{-1}$), TDS: Total dissolved solids (mg. $L^{-1}$), DO: dissolved oxygen (mg. $L^{-1}$), Temp: temperature (°C). Table extracted and modified from Barrios et al., (2023).

| Stream | Season | Afforestation (%) | TP | $PO_4$-P | TN | $NO_3$-N | $NH_4$-N | SOM | k | TDS | pH | DO | Temp. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DF-NF4 | Summer | 0.05 | 22.4 | 11.1 | 219.5 | 151.1 | 7.3 | 3.3 | 225 | 0.172 | 7.5 | 9.5 | 17.3 |
| | Winter | 0.05 | 20.9 | 4.8 | 241.5 | 222.6 | 6.5 | 2.5 | 102 | 0.093 | 7.2 | 10.8 | 10.1 |
| DF-Monte 3 | Summer | 0.18 | 11.1 | 3.2 | 122.5 | 121.7 | 0 | 0.9 | 523 | 0.382 | 7.6 | 8.6 | 19.2 |
| | Winter | 0.18 | 29.7 | 1.7 | 173.9 | 38.2 | 7.3 | 3 | 100 | 0.086 | 7.6 | 11.4 | 12.2 |
| DF-Monte 2 | Summer | 0.31 | 9.5 | 3.2 | 118.1 | 104.9 | 1 | 1.4 | 391 | 0.275 | 7.7 | 9.5 | 21.1 |
| | Winter | 0.31 | 22.6 | 6.4 | 194.2 | 10.6 | 6.5 | 3.7 | 92 | 0.081 | 7.9 | 11.8 | 11.2 |
| DF-CV | Summer | 0.36 | 16 | 1.7 | 307.7 | 276.5 | 10.8 | 2.2 | 490 | 0.343 | 7.4 | 8.4 | 21.2 |
| | Winter | 0.36 | 43.8 | 3.3 | 471.3 | 75.1 | 4.2 | 1.6 | 422 | 0.356 | 7.4 | 10.1 | 13 |
| DF-NF16 | Summer | 0.46 | 4.7 | 3.2 | 268.1 | 100.7 | 3.4 | 2.4 | 155 | 0.114 | 6.9 | 7.4 | 19.2 |
| | Winter | 0.46 | 26.2 | 6.4 | 288.8 | 139.7 | 4.2 | 0.8 | 80 | 0.074 | 7.1 | 10.4 | 9.3 |
| DF-C9 | Summer | 1.18 | 41.7 | 9.6 | 237.2 | 130.1 | 1 | 6.2 | 86 | 0.055 | 6.8 | 7.6 | 25.9 |
| | Winter | 1.18 | 33.2 | 4.8 | 397 | 204.2 | 5 | 3 | 30 | 0.024 | 7.6 | 10.2 | 14.8 |
| DF-S19 | Summer | 3.34 | 17.6 | 8 | 493.2 | 306.6 | 10.8 | 2.2 | 344 | 0.226 | 7.4 | 8.5 | 24.4 |
| | Winter | 3.34 | 54.4 | 6.4 | 315.9 | 148.9 | 6.5 | 1.7 | 99 | 0.081 | 7.6 | 10.7 | 14.5 |
| DF-NF6 | Summer | 3.8 | 11.1 | 6.4 | 268.1 | 142.7 | 4.2 | 1.4 | 215 | 0.162 | 7.5 | 6.2 | 17.8 |
| | Winter | 3.8 | 26.2 | 6.4 | 336.1 | 241.1 | 15.1 | 2 | 113 | 0.101 | 7.5 | 11 | 10.6 |
| DF-S16 | Summer | 6.19 | 16 | 4.8 | 686.9 | 381.9 | 3.5 | 2.4 | 387 | 0.235 | 8.3 | 11.2 | 28.7 |
| | Winter | 6.19 | 27.9 | 3.3 | 302.3 | 185.8 | 6.5 | 2.3 | 143 | 0.122 | 7.8 | 11.1 | 12.6 |
| DF-2018-4 | Summer | 7.52 | 32.1 | 0.1 | 563.6 | 125.9 | 4.2 | 0.8 | 66 | 0.041 | 7 | 9.6 | 27.9 |
| | Winter | 7.52 | 61.5 | 4.8 | 484.9 | 195 | 6.5 | 3.5 | 30 | 0.028 | 7.6 | 10.6 | 9.2 |
| DF-2018-6 | Summer | 12.76 | 27.2 | 23.8 | 281.3 | 146.9 | 0 | 1.3 | 79 | 0.061 | 6.7 | 7.4 | 16.4 |
| | Winter | 12.76 | 59.8 | 25.4 | 829.6 | 204.2 | 8.1 | 6.5 | 37 | 0.032 | 7.3 | 10.6 | 12 |
| DF-MFC | Summer | 19.57 | 9.5 | 3.2 | 219.5 | 100.7 | 0 | 2.4 | 146 | 0.093 | 6.8 | 8.3 | 25.8 |
| | Winter | 19.57 | 42.1 | 0.9 | 498.4 | 379.4 | 10.4 | 5.5 | 42 | 0.034 | 7.4 | 10.6 | 14.4 |
| DF-2018-2 | Summer | 28.04 | 32.1 | 6.4 | 400.4 | 251.9 | 0 | 7.7 | 101 | 0.066 | 6.5 | 7.4 | 24.5 |
| | Winter | 28.04 | 45.6 | 6.4 | 363.2 | 167.3 | 3.4 | 3.3 | 40 | 0.036 | 7 | 9.9 | 10.8 |
| DF-2018-3 | Summer | 32.88 | 36.9 | 4.8 | 462.2 | 193.1 | 0 | 2.5 | 79 | 0.05 | 6.9 | 4.4 | 26.2 |
| | Winter | 32.88 | 42.1 | 11.2 | 741.7 | 287.2 | 4.2 | 4.5 | 35 | 0.032 | 7 | 9.6 | 9.8 |
| DF-2018-8 | Summer | 44.36 | 27.2 | 6.4 | 268.1 | 180.5 | 1.8 | 5.3 | 61 | 0.043 | 7 | 7.1 | 20.8 |
| | Winter | 44.36 | 43.8 | 8 | 606.5 | 195 | 11.2 | 5.5 | 36 | 0.032 | 8.2 | 9.8 | 11.6 |
| DF- F8F | Summer | 49.21 | 22.4 | 6.4 | 436.7 | 306.6 | 10.8 | 0.7 | 426 | 0.311 | 7.8 | 8.3 | 19.2 |
| | Winter | 49.21 | 35 | 11.2 | 248.3 | 102.8 | 0 | 1 | 373 | 0.314 | 7.7 | 13.2 | 13.1 |
| DF- F6F | Summer | 50.32 | 16 | 3.3 | 428.7 | 276.5 | 23.4 | 2.4 | 476 | 0.317 | 7.7 | 8.5 | 23.6 |
| | Winter | 50.32 | 31.5 | 1.7 | 349.7 | 185.8 | 0 | 1.1 | 409 | 0.344 | 7.6 | 11.7 | 13.1 |
| DF-DT | Summer | 56.41 | 35.3 | 4.8 | 751.4 | 306.6 | 0 | 3.6 | 533 | 0.383 | 7.1 | 6.7 | 20 |
| | Winter | 56.41 | 104 | 4.8 | 261.8 | 75.1 | 0 | 1.2 | 464 | 0.396 | 7.2 | 11.1 | 12.6 |
| DF-F5 | Summer | 61.39 | 17.6 | 9.6 | 188.7 | 134.3 | 9.7 | 2.8 | 206 | 0.147 | 7.5 | 6.5 | 20.1 |
| | Winter | 61.39 | 70.4 | 9.6 | 701.2 | 241.1 | 10.4 | 2.3 | 99 | 0.085 | 7.2 | 9.5 | 12.6 |
| DF- 2018-19 | Summer | 62.23 | 69 | 17.5 | 945.1 | 404.5 | 5.3 | 5.6 | 128 | 0.078 | 7.8 | 6.8 | 28.7 |
| | Winter | 62.23 | 31.5 | 1.7 | 491.6 | 148.9 | 0.2 | 3 | 184 | 0.163 | 7.4 | 8.2 | 11.3 |
| DF- 2018-20 | Summer | 62.89 | 30.4 | 11.2 | 444.8 | 329.2 | 0 | 1.3 | 277 | 0.179 | 6.9 | 2.3 | 23.3 |
| | Winter | 62.89 | 35 | 3.3 | 376.7 | 47.5 | 1.8 | 2.5 | 473 | 0.429 | 7.3 | 7.5 | 10.2 |
| DF-2018-1 | Summer | 67.06 | 28.8 | 3.2 | 343 | 306.5 | 0 | 0.8 | 60 | 0.041 | 6.1 | 6.5 | 22.8 |
| | Winter | 67.06 | 31.5 | 6.4 | 424 | 130.4 | 0 | 2.8 | 40 | 0.036 | 6.9 | 10.2 | 10.5 |
| DF-Forest 3 | Summer | 74.36 | 3.1 | 1.7 | 533.6 | 299.1 | 26.5 | 1.6 | 159 | 0.125 | 7.2 | 7.4 | 16.1 |
| | Winter | 74.36 | 17.3 | 8 | 397 | 93.6 | 8.1 | 4 | 82 | 0.08 | 7.7 | 11 | 7.6 |
| DF- F7 | Summer | 75.75 | 20.8 | 8 | 356.3 | 172.1 | 2.6 | 3.3 | 376 | 0.277 | 7 | 4.6 | 18.7 |
| | Winter | 75.75 | 36.8 | 4.8 | 613.3 | 213.4 | 6.5 | 1.5 | 115 | 0.099 | 6.8 | 9.8 | 12.5 |
| DF-2018-18 | Summer | 79.11 | 31.4 | 7.7 | 410.1 | 204.8 | 4.2 | 2.6 | 53 | 0.035 | 6.9 | 7.3 | 23.3 |
| | Winter | 79.11 | 36.8 | 8 | 363.2 | 130.4 | 1 | 2.5 | 418 | 0.32 | 7.5 | 13 | 17 |
| DF- 2018-13 | Summer | 79.37 | 70.6 | 30.1 | 694.9 | 306.6 | 6.1 | 3 | 100 | 0.061 | 7.2 | 6.3 | 28 |
| | Winter | 79.37 | 40.3 | 1.7 | 471.3 | 75.1 | 0 | 4 | 361 | 0.326 | 6.9 | 4.3 | 10.5 |
| DF- 2018-17 | Summer | 85.8 | 30.4 | 1.7 | 533.6 | 261.5 | 25.7 | 3 | 64 | 0.043 | 7 | 7.6 | 23.3 |
| | Winter | 85.8 | 35 | 0.1 | 424 | 112 | 0.2 | 3.7 | 318 | 0.231 | 7.6 | 10.8 | 19.4 |

**Supplementary Table 3.** Mean values of the local habitat components in the three Surber samples. Mean width was obtained by measuring 6 transects distributed each 10 meters along the 50 transect used in the study.

| Stream site | Season | Width m | Depth cm | Current velocity $m.s^{-1}$ | Sand % | Gravel % | Stone % | Clay % | Mud % |
|---|---|---|---|---|---|---|---|---|---|
| DF-NF4 | Summer | 3.11 | 20.17 | 0.10 | 20.00 | 20.00 | 53.33 | 0.00 | 6.67 |
| DF-NF4 | Winter | 1.91 | 20.33 | 0.23 | 21.67 | 56.67 | 3.33 | 0.00 | 18.33 |
| DF-Monte 3 | Summer | 4.99 | 13.67 | 0.07 | 11.67 | 31.67 | 56.67 | 0.00 | 0.00 |
| DF-Monte 3 | Winter | 4.17 | 23.00 | 0.17 | 35.00 | 31.67 | 33.33 | 0.00 | 0.00 |
| DF- Monte 2 | Summer | 2.55 | 9.00 | 0.13 | 0.00 | 8.33 | 91.67 | 0.00 | 0.00 |
| DF- Monte 2 | Winter | 2.26 | 12.67 | 0.20 | 25.00 | 13.33 | 61.67 | 0.00 | 0.00 |
| DF-CV | Summer | 4.87 | 24.33 | 0.05 | 36.67 | 5.00 | 25.00 | 0.00 | 33.33 |
| DF-CV | Winter | 6.75 | 19.00 | 0.08 | 63.33 | 3.33 | 0.00 | 0.00 | 33.33 |
| DF-NF16 | Summer | 2.53 | 17.00 | 0.15 | 43.33 | 41.67 | 15.00 | 0.00 | 0.00 |
| DF-NF16 | Winter | 1.96 | 22.33 | 0.40 | 36.67 | 30.00 | 33.33 | 0.00 | 0.00 |
| DF-C9 | Summer | 7.55 | 30.33 | 0.00 | 40.00 | 30.00 | 0.00 | 30.00 | 0.00 |
| DF-C9 | Winter | 6.20 | 32.33 | 0.40 | 13.33 | 53.33 | 0.00 | 33.33 | 0.00 |
| DF-S19 | Summer | 2.72 | 21.33 | 0.13 | 0.00 | 20.00 | 80.00 | 0.00 | 0.00 |
| DF-S19 | Winter | 2.88 | 20.33 | 0.57 | 0.00 | 46.67 | 53.33 | 0.00 | 0.00 |
| DF-NF6 | Summer | 2.27 | 37.00 | 0.00 | 40.00 | 51.67 | 3.33 | 0.00 | 5.00 |
| DF-NF6 | Winter | 3.36 | 18.67 | 0.07 | 0.00 | 26.67 | 38.33 | 0.00 | 35.00 |
| DF-S16 | Summer | 4.71 | 14.67 | 0.10 | 3.33 | 30.00 | 66.67 | 0.00 | 0.00 |
| DF-S16 | Winter | 5.98 | 24.00 | 0.40 | 0.00 | 23.33 | 76.67 | 0.00 | 0.00 |
| DF-2018-4 | Summer | 6.45 | 26.00 | 0.00 | 3.33 | 0.00 | 0.00 | 96.67 | 0.00 |
| DF-2018-4 | Winter | 4.54 | 16.00 | 0.15 | 33.33 | 0.00 | 0.00 | 66.67 | 0.00 |
| DF-2018-6 | Summer | 5.41 | 11.17 | 0.03 | 0.00 | 8.33 | 25.00 | 66.67 | 0.00 |
| DF-2018-6 | Winter | 3.75 | 12.67 | 0.53 | 43.33 | 23.33 | 33.33 | 0.00 | 0.00 |
| DF-MFC | Summer | 1.90 | 26.00 | 0.10 | 41.67 | 23.33 | 35.00 | 0.00 | 0.00 |
| DF-MFC | Winter | 1.40 | 23.67 | 0.23 | 66.67 | 0.00 | 33.33 | 0.00 | 0.00 |
| DF-2018-2 | Summer | 4.05 | 51.67 | 0.00 | 0.00 | 0.00 | 100.00 | 0.00 | 0.00 |
| DF-2018-2 | Winter | 2.43 | 39.00 | 0.10 | 6.67 | 26.67 | 66.67 | 0.00 | 0.00 |
| DF-2018-3 | Summer | 2.77 | 31.33 | 0.02 | 0.00 | 53.33 | 3.33 | 43.33 | 0.00 |
| DF-2018-3 | Winter | 3.94 | 32.00 | 0.13 | 0.00 | 66.67 | 26.67 | 6.67 | 0.00 |
| DF-2018-8 | Summer | 2.17 | 25.00 | 0.03 | 53.33 | 43.33 | 0.00 | 0.00 | 3.33 |
| DF-2018-8 | Winter | 2.56 | 37.67 | 0.30 | 33.33 | 16.67 | 16.67 | 0.00 | 33.33 |
| DF- F8F | Summer | 3.78 | 23.00 | 0.17 | 0.00 | 0.00 | 83.33 | 0.00 | 16.67 |
| DF- F8F | Winter | 3.34 | 14.00 | 0.20 | 0.00 | 6.67 | 93.33 | 0.00 | 0.00 |
| DF- F6F | Summer | 5.07 | 16.67 | 0.12 | 16.67 | 16.67 | 66.67 | 0.00 | 0.00 |
| DF- F6F | Winter | 8.13 | 14.00 | 0.17 | 8.33 | 33.33 | 58.33 | 0.00 | 0.00 |
| DF-DT | Summer | 6.83 | 27.67 | 0.03 | 13.33 | 20.00 | 6.67 | 0.00 | 60.00 |
| DF-DT | Winter | 2.55 | 7.33 | 0.17 | 6.67 | 83.33 | 0.00 | 0.00 | 10.00 |
| DF-F5 | Summer | 2.10 | 27.00 | 0.07 | 21.67 | 28.33 | 50.00 | 0.00 | 0.00 |
| DF-F5 | Winter | 8.13 | 19.33 | 0.08 | 25.00 | 45.00 | 30.00 | 0.00 | 0.00 |
| DF- 2018-19 | Summer | 9.19 | 25.33 | 0.02 | 3.33 | 0.00 | 30.00 | 0.00 | 66.67 |
| DF- 2018-19 | Winter | 4.17 | 24.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 100.00 |
| DF- 2018-20 | Summer | 2.77 | 38.00 | 0.07 | 3.33 | 0.00 | 0.00 | 0.00 | 96.67 |
| DF- 2018-20 | Winter | 3.75 | 35.67 | 0.00 | 16.67 | 0.00 | 0.00 | 0.00 | 83.33 |
| DF-2018-1 | Summer | 3.17 | 40.33 | 0.00 | 0.00 | 5.00 | 95.00 | 0.00 | 0.00 |
| DF-2018-1 | Winter | 6.56 | 21.33 | 0.13 | 40.00 | 26.67 | 33.33 | 0.00 | 0.00 |
| DF-Forest 3 | Summer | 7.17 | 23.00 | 0.13 | 0.00 | 11.67 | 88.33 | 0.00 | 0.00 |
| DF-Forest 3 | Winter | 7.13 | 14.67 | 0.20 | 0.00 | 25.00 | 46.67 | 0.00 | 28.33 |
| DF- F7 | Summer | 4.62 | 17.00 | 0.05 | 80.00 | 16.67 | 3.33 | 0.00 | 0.00 |
| DF- F7 | Winter | 7.91 | 20.67 | 0.13 | 5.00 | 86.67 | 1.67 | 0.00 | 6.67 |
| DF-2018-18 | Summer | 5.77 | 26.67 | 0.30 | 81.67 | 18.33 | 0.00 | 0.00 | 0.00 |
| DF-2018-18 | Winter | 1.99 | 20.33 | 0.03 | 73.33 | 26.67 | 0.00 | 0.00 | 0.00 |
| DF- 2018-13 | Summer | 2.52 | 9.00 | 0.07 | 16.67 | 3.33 | 0.00 | 0.00 | 80.00 |
| DF- 2018-13 | Winter | 9.56 | 21.00 | 0.00 | 0.00 | 0.00 | 3.33 | 0.00 | 96.67 |
| DF- 2018-17 | Summer | 2.60 | 23.00 | 0.10 | 35.00 | 20.00 | 45.00 | 0.00 | 0.00 |
| DF- 2018-17 | Winter | 4.77 | 20.00 | 0.03 | 36.67 | 3.33 | 60.00 | 0.00 | 0.00 |

**Supplementary Table 4.** Mean values of the biomass (g.m$^{-2}$) of different basal resources collected in the three Surber samples. Basal resources were classified into detritus, periphyton (Diatom, Cyanobacteria, and green algae), and macrophytes (submerged, rooted floating, emergent, and grass). We separated grass from the emergent as they are part of the terrestrial grassland area surrounding the streams, but they are also tolerate growing in aquatic systems.

| Stream site | Season | Detritus | Diatom | Cyanobacteria | Green algae | Sumerged | Rooted floating | Emergent | Grass |
|---|---|---|---|---|---|---|---|---|---|
| DF-NF4 | Summer | 0.566 | 0.010 | 0.003 | 0.003 | 66.234 | 0.000 | 33.297 | 0.000 |
| DF-NF4 | Winter | 32.690 | 0.020 | 0.010 | 0.003 | 4.763 | 0.000 | 28.566 | 25.624 |
| DF-Monte 3 | Summer | 1.241 | 0.003 | 0.003 | 0.000 | 11.517 | 0.000 | 20.823 | 65.965 |
| DF-Monte 3 | Winter | 0.000 | 0.013 | 0.003 | 0.003 | 23.578 | 7.664 | 9.672 | 33.391 |
| DF- Monte 2 | Summer | 4.253 | 0.010 | 0.003 | 0.003 | 16.995 | 9.487 | 11.981 | 21.528 |
| DF- Monte 2 | Winter | 1.268 | 0.010 | 0.007 | 0.010 | 3.070 | 16.378 | 16.824 | 21.100 |
| DF-CV | Summer | 2.516 | 0.010 | 0.003 | 0.010 | 16.529 | 18.563 | 33.303 | 29.639 |
| DF-CV | Winter | 32.021 | 0.023 | 0.007 | 0.007 | 25.743 | 25.444 | 33.126 | 5.082 |
| DF-NF16 | Summer | 4.188 | 0.013 | 0.007 | 0.010 | 33.319 | 22.062 | 33.327 | 8.426 |
| DF-NF16 | Winter | 9.238 | 0.017 | 0.010 | 0.013 | 0.000 | 22.126 | 24.547 | 35.718 |
| DF-C9 | Summer | 0.000 | 0.000 | 0.007 | 0.007 | 0.000 | 20.186 | 20.585 | 45.909 |
| DF-C9 | Winter | 0.000 | 0.003 | 0.010 | 0.003 | 0.000 | 0.000 | 0.000 | 47.062 |
| DF-S19 | Summer | 1.243 | 0.010 | 0.007 | 0.003 | 52.568 | 9.125 | 0.000 | 1.444 |
| DF-S19 | Winter | 12.876 | 0.023 | 0.007 | 0.017 | 11.842 | 44.018 | 0.000 | 12.504 |
| DF-NF6 | Summer | 0.000 | 0.000 | 0.000 | 0.000 | 33.328 | 0.000 | 0.000 | 33.331 |
| DF-NF6 | Winter | 48.189 | 0.003 | 0.000 | 0.000 | 0.000 | 0.032 | 0.000 | 30.560 |
| DF-S16 | Summer | 12.987 | 0.027 | 0.013 | 0.010 | 48.403 | 0.000 | 0.000 | 38.598 |
| DF-S16 | Winter | 10.686 | 0.017 | 0.003 | 0.003 | 28.277 | 0.000 | 0.000 | 0.000 |
| DF-2018-4 | Summer | 0.321 | 0.003 | 0.003 | 0.010 | 33.320 | 0.000 | 0.000 | 33.170 |
| DF-2018-4 | Winter | 0.000 | 0.020 | 0.013 | 0.013 | 23.223 | 0.000 | 33.330 | 10.087 |
| DF-2018-6 | Summer | 0.209 | 0.010 | 0.010 | 0.020 | 24.592 | 37.673 | 4.258 | 0.000 |
| DF-2018-6 | Winter | 20.184 | 0.017 | 0.010 | 0.027 | 4.568 | 43.210 | 23.633 | 24.380 |
| DF-MFC | Summer | 1.991 | 0.000 | 0.000 | 0.000 | 0.000 | 11.572 | 51.274 | 35.492 |
| DF-MFC | Winter | 4.184 | 0.017 | 0.007 | 0.007 | 17.316 | 24.392 | 25.946 | 29.044 |
| DF-2018-2 | Summer | 0.000 | 0.013 | 0.010 | 0.007 | 0.000 | 0.000 | 31.132 | 35.344 |
| DF-2018-2 | Winter | 2.679 | 0.013 | 0.010 | 0.007 | 0.000 | 0.000 | 25.783 | 12.688 |
| DF-2018-3 | Summer | 0.000 | 0.007 | 0.003 | 0.003 | 0.000 | 4.173 | 49.530 | 12.957 |
| DF-2018-3 | Winter | 0.898 | 0.000 | 0.000 | 0.000 | 0.050 | 28.923 | 53.383 | 13.233 |
| DF-2018-8 | Summer | 0.000 | 0.007 | 0.003 | 0.003 | 5.582 | 34.535 | 1.042 | 47.345 |
| DF-2018-8 | Winter | 286.709 | 0.010 | 0.003 | 0.007 | 0.887 | 38.262 | 0.298 | 25.043 |
| DF- F8F | Summer | 0.000 | 0.000 | 0.007 | 0.000 | 31.322 | 0.000 | 0.000 | 26.466 |
| DF- F8F | Winter | 0.000 | 0.040 | 0.007 | 0.013 | 20.659 | 0.000 | 0.000 | 67.216 |
| DF- F6F | Summer | 2.009 | 0.023 | 0.003 | 0.000 | 0.267 | 7.417 | 22.718 | 49.474 |
| DF- F6F | Winter | 0.344 | 0.027 | 0.003 | 0.000 | 21.137 | 0.000 | 16.953 | 26.484 |
| DF-DT | Summer | 61.051 | 0.010 | 0.010 | 0.017 | 6.102 | 0.432 | 0.000 | 37.633 |
| DF-DT | Winter | 6.594 | 0.037 | 0.010 | 0.003 | 0.000 | 4.511 | 0.479 | 57.104 |
| DF-F5 | Summer | 0.455 | 0.000 | 0.003 | 0.000 | 0.000 | 32.138 | 0.000 | 64.608 |
| DF-F5 | Winter | 13.836 | 0.007 | 0.003 | 0.003 | 0.000 | 33.870 | 0.000 | 29.414 |
| DF- 2018-19 | Summer | 22.550 | 0.000 | 0.000 | 0.000 | 4.239 | 32.186 | 0.597 | 16.958 |
| DF- 2018-19 | Winter | 31.349 | 0.013 | 0.007 | 0.007 | 45.281 | 8.998 | 2.173 | 24.199 |
| DF- 2018-20 | Summer | 150.461 | 0.003 | 0.000 | 0.000 | 14.207 | 5.313 | 39.146 | 22.694 |
| DF- 2018-20 | Winter | 208.512 | 0.020 | 0.003 | 0.003 | 17.534 | 16.021 | 10.460 | 25.553 |
| DF-2018-1 | Summer | 0.000 | 0.033 | 0.010 | 0.010 | 0.000 | 0.000 | 33.325 | 33.302 |
| DF-2018-1 | Winter | 0.308 | 0.007 | 0.003 | 0.017 | 29.886 | 0.000 | 33.330 | 7.440 |
| DF-Forest 3 | Summer | 7.198 | 0.010 | 0.003 | 0.007 | 28.613 | 0.522 | 5.344 | 27.654 |
| DF-Forest 3 | Winter | 9.376 | 0.007 | 0.003 | 0.003 | 1.144 | 0.000 | 28.107 | 29.812 |
| DF- F7 | Summer | 1.854 | 0.003 | 0.000 | 0.003 | 14.582 | 0.000 | 0.000 | 83.223 |
| DF- F7 | Winter | 8.603 | 0.013 | 0.003 | 0.000 | 33.327 | 0.000 | 0.000 | 52.303 |
| DF-2018-18 | Summer | 0.000 | 0.010 | 0.007 | 0.007 | 0.000 | 0.000 | 0.000 | 99.482 |
| DF-2018-18 | Winter | 1.005 | 0.027 | 0.003 | 0.000 | 31.259 | 39.705 | 15.557 | 15.557 |
| DF- 2018-13 | Summer | 4.691 | 0.013 | 0.003 | 0.010 | 8.387 | 7.875 | 23.205 | 59.245 |
| DF- 2018-13 | Winter | 32.293 | 0.017 | 0.007 | 0.017 | 34.464 | 16.426 | 0.109 | 9.952 |
| DF- 2018-17 | Summer | 18.664 | 0.007 | 0.010 | 0.007 | 3.007 | 0.000 | 0.000 | 33.302 |
| DF- 2018-17 | Winter | 2.337 | 0.007 | 0.003 | 0.000 | 41.741 | 8.311 | 0.000 | 49.069 |

**Supplementary Table 5.** Standardised direct effects of *Eucalyptus* spp. afforestation intensification on water quality variables and carbon basal resource availability and the consequences to functional diversity of stream macroinvertebrates. This table includes all significant and nonsignificant path considered by our model and includes those variables which were allow to covary. We ran separate SEMs summer and winter seasons. The different models are represented by different colours, summer model = green colour; winter model = pink colour. Double-headed arrows indicate the variables that covary (error correlations).

| **Predictors** | | **Response** | **Standardis cocoefficien** | **Std.Error** | ***P*-value** |
|---|---|---|---|---|---|
| **Summer model; Fisher`s C= 51.01, *P* = 0.283** | | | | | |
| richness path: **$r^2$ = 0.37** | | | | | |
| Afforestation | → | Periphyton biomass | –0.191 | 0.000 | 0.041 |
| Depth | → | Periphyton biomass | –0.071 | 0.000 | 0.567 |
| Velocity | → | Periphyton biomass | –0.069 | 0.025 | 0.580 |
| Afforestation | → | Detritus | 0.068 | 0.002 | 0.548 |
| Macrophyte biomass | → | Detritus | 0.338 | 0.041 | 0.003 |
| Afforestation | → | Macrophyte biomas | –0.373 | 0.005 | **<0.001** |
| Depth | → | Macrophyte biomas | 0.189 | 0.936 | 0.084 |
| Velocity | → | Macrophyte biomas | 0.099 | 127.97 | 0.376 |
| Sand | → | Macrophyte biomas | –0.027 | 0.617 | 0.854 |
| Stone | → | Macrophyte biomas | –0.163 | 0.489 | 0.298 |
| Clay | → | Macrophyte biomas | 0.047 | 0.570 | 0.692 |
| Mud | → | Macrophyte biomas | 0.387 | 0.544 | **0.004** |
| Afforestation | → | Depth | –0.003 | 0.000 | 0.976 |
| Afforestation | → | Velocity | 0.068 | 0.000 | 0.500 |
| Depth | → | Velocity | –0.444 | 0.000 | **<0.001** |
| Afforestation | → | Sand | 0.080 | 0.001 | 0.474 |
| Afforestation | → | Stone | –0.037 | 0.001 | 0.741 |
| Afforestation | → | Clay | –0.106 | 0.001 | 0.346 |
| Afforestation | → | Mud | 0.103 | 0.001 | 0.358 |
| Detritus | → | functional diversity | 0.042 | 0.000 | 0.669 |
| Periphyton | → | functional diversity | 0.347 | 0.459 | **<0.001** |
| Macrophyte | → | functional diversity | 0.289 | 0.000 | **0.004** |

| | | | | | |
|---|---|---|---|---|---|
| Depth | → | functional diversity | –0.169 | 0.000 | 0.101 |
| Velocity | → | functional diversity | –0.044 | 0.101 | 0.667 |
| Sand | → | functional diversity | 0.305 | 0.000 | **0.030** |
| Stone | → | functional diversity | 0.499 | 0.000 | **0.001** |
| Clay | → | functional diversity | 0.408 | 0.000 | **<0.001** |
| Mud | → | functional diversity | 0.285 | 0.000 | **0.033** |
| Stone | ↔ | Sand | –0.495 | – | <0.001 |
| Mud | ↔ | Detritus | 0.284 | – | 0.005 |
| Clay | ↔ | Stone | –0.193 | – | 0.042 |
| Mud | ↔ | Stone | –0.359 | – | <0.001 |
| **Winter model; Fisher`s C= 44.56, *P* = 0.532** | | | | | |
| Afforestation | → | Periphyton biomass | -0.253 | 0.000 | **0.028** |
| Depth | → | Periphyton biomass | -0.261 | 0.000 | 0.021 |
| Velocity | → | Periphyton biomass | -0.019 | 0.010 | 0.870 |
| Afforestation | → | Detritus | 0.049 | 0.005 | 0.660 |
| Macrophyte biomass | → | Detritus | 0.211 | 0.120 | 0.062 |
| Afforestation | → | Macrophyte biomass | -0.251 | 0.004 | **0.020** |
| Depth | → | Macrophyte biomass | 0.145 | 0.789 | 0.168 |
| Velocity | → | Macrophyte biomass | -0.144 | 47.15 | 0.210 |
| Sand | → | Macrophyte biomass | 0.178 | 0.364 | 0.175 |
| Stone | → | Macrophyte biomass | -0.077 | 0.318 | 0.562 |
| Clay | → | Macrophyte biomass | -0.027 | 0.545 | 0.802 |
| Mud | → | Macrophyte biomass | 0.384 | 0.362 | **0.004** |
| Afforestation | → | Depth | -0.053 | 0.000 | 0.633 |
| Afforestation | → | Velocity | -0.331 | 0.000 | **0.001** |
| Depth | → | Velocity | -0.268 | 0.001 | 0.011 |
| Afforestation | → | Sand | -0.039 | 0.001 | 0.728 |
| Afforestation | → | Stone | -0.043 | 0.002 | 0.697 |
| Afforestation | → | Clay | -0.126 | 0.000 | 0.259 |
| Afforestation | → | Mud | 0.150 | 0.001 | 0.179 |
| Detritus | → | functional diversity | -0.001 | 0.000 | 0.989 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| Periphyton | → | functional diversity | 0.159 | 0.517 | 0.170 |
| Macrophyte | → | functional diversity | 0.218 | 0.000 | 0.048 |
| Depth | → | functional diversity | -0.181 | 0.000 | 0.138 |
| Velocity | → | functional diversity | 0.159 | 0.05 | 0.194 |
| Sand | → | functional diversity | 0.030 | 0.000 | 0.838 |
| Stone | → | functional diversity | 0.231 | 0.000 | 0.126 |
| Clay | → | functional diversity | 0.007 | 0.000 | 0.949 |
| Mud | → | functional diversity | 0.088 | 0.000 | 0.591 |
| Stone | → | Sand | -0.420 |  | <0.001 |
| Mud | → | Detritus | -0.081 |  | 0.237 |
| Clay | → | Stone | -0.136 |  | 0.114 |
| Mud | → | Stone | -0.146 |  | 0.097 |

**Supplementary Table 6**. Fourth-corner tests between the first two axes for the RLQ ordination (AxcQ1 and AxcQ2) and the three environmental variables (Afforestation and water variables, local habitat variables, and basal resource biomass). Positive significant associations are represented by blue cells and negative significant associations by violet cells.

| Environmental variable categories | Variables | Axis | Obs | Std.Obs | *P*-value | *P*-value.adj | |
|---|---|---|---|---|---|---|---|
| Afforestation and water variables | DO | AxcQ1 | -0.159 | -4.384 | 0.001 | **0.001** | ** |
| | pH | | -0.096 | -2.599 | 0.008 | **0.029** | * |
| | k | | -0.043 | -1.173 | 0.246 | 0.351 | |
| | SOM | | 0.039 | 1.046 | 0.302 | 0.385 | |
| | NH4 | | -0.060 | -1.666 | 0.099 | 0.198 | |
| | TN | | 0.099 | 2.704 | 0.005 | **0.025** | * |
| | TP | | 0.081 | 2.209 | 0.026 | 0.059 | |
| | DO | AxcQ2 | -0.085 | 2.171 | 0.026 | 0.051 | . |
| | pH | | 0.002 | 0.078 | 0.938 | 0.938 | |
| | k | | -0.037 | -1.209 | 0.226 | 0.351 | |
| | SOM | | 0.025 | 0.795 | 0.427 | 0.471 | |
| | NH4 | | -0.023 | -0.783 | 0.437 | 0.471 | |
| | TN | | 0.036 | 1.160 | 0.251 | 0.351 | |
| | TP | | 0.078 | 2.526 | 0.012 | **0.033** | * |
| Local habitat variables | Depth | AxcQ1 | 0.065 | 1.971 | 0.049 | 0.076 | . |
| | Velocity | | -0.074 | -2.606 | 0.007 | **0.019** | * |
| | Sand | | 0.019 | 0.697 | 0.481 | 0.518 | |
| | Gravel | | -0.067 | -2.721 | 0.006 | **0.019** | * |
| | Stone | | -0.131 | -3.813 | 0.001 | **0.005** | ** |
| | Clay | | 0.060 | 2.311 | 0.02 | **0.031** | * |
| | Mud | | 0.167 | 4.718 | 0.001 | **0.005** | ** |
| | Depth | AxcQ2 | 0.043 | 1.349 | 0.186 | 0.237 | |
| | Velocity | | -0.060 | -2.176 | 0.024 | **0.042** | * |
| | Sand | | -0.039 | -1.619 | 0.107 | 0.150 | |
| | Gravel | | -0.084 | -3.493 | 0.001 | **0.005** | ** |
| | Stone | | 0.088 | 2.493 | 0.012 | **0.024** | * |
| | Clay | | 0.027 | 1.138 | 0.251 | 0.293 | |
| | Mud | | -0.013 | -0.372 | 0.722 | 0.722 | |
| Basal resource biomass | Submerged | AxcQ1 | -0.041 | -1.904 | 0.061 | 0.139 | |
| | Emergent | | -0.080 | -3.701 | 0.001 | **0.005** | ** |
| | Grass | | -0.086 | -3.923 | 0.001 | **0.005** | ** |
| | Rooted_floating | | -0.105 | -4.848 | 0.001 | **0.005** | ** |
| | Diatom | | 0.014 | 0.781 | 0.431 | 0.530 | |
| | Cyanobacteria | | -0.020 | -0.832 | 0.413 | 0.512 | |
| | Green_algae | | 0.006 | 0.271 | 0.8 | 0.853 | |
| | Detritus | | -0.072 | -3.213 | 0.003 | **0.010** | ** |
| | Submerged | AxcQ2 | -0.035 | -1.735 | 0.091 | 0.146 | |
| | Emergent | | -0.036 | -1.767 | 0.084 | 0.146 | |
| | Grass | | -0.004 | -0.170 | 0.882 | 0.882 | |
| | Rooted_floating | | 0.017 | 0.682 | 0.508 | 0.581 | |
| | Diatom | | 0.031 | 1.458 | 0.148 | 0.215 | |
| | Cyanobacteria | | 0.054 | 2.537 | 0.012 | **0.032** | * |
| | Green_algae | | 0.070 | 3.378 | 0.003 | **0.010** | ** |
| | Detritus | | 0.037 | 1.774 | 0.086 | 0.146 | |

**Supplementary Table 7.** Taxa codes used for macroinvertebrate communities.

| Phyllum | Class | Order | Family | Taxa | Code |
|---|---|---|---|---|---|
| Annelida | Hirudinea | | Glossiphoniidae | Glossiphoniidae | Hir |
| Arthropoda | Arachnida | Trombidiformes | Hydrachnidia | Hydrachnidia | Aca |
| Arthropoda | Insecta | Coleoptera | Curculionidae | Curculionidae | Cur |
| Arthropoda | Insecta | Coleoptera | Dryopidae | Dryops | Dry |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Celina | Celi |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Colymbetinae | Coly |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Copelatus | Cope |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Hydroporus | Hyr |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Hypodessus | Hypo |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Laccophilus | Lacc |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Laccornellus | Lacr |
| Arthropoda | Insecta | Coleoptera | Dytiscidae | Vatellus | Vat |
| Arthropoda | Insecta | Coleoptera | Elmidae | Elmidae_adult | Elm_ad |
| Arthropoda | Insecta | Coleoptera | Elmidae | Heterelmis_larvae | Hetl |
| Arthropoda | Insecta | Coleoptera | Elmidae | Hexacylloepus_larvae | Hexl |
| Arthropoda | Insecta | Coleoptera | Elmidae | Hydora_larvae | Hydor |
| Arthropoda | Insecta | Coleoptera | Elmidae | Luchoelmis_larvae | Lucho |
| Arthropoda | Insecta | Coleoptera | Elmidae | Macroelmis_larvae | Mcl |
| Arthropoda | Insecta | Coleoptera | Elmidae | Microcylloepus_larvae | Mil |
| Arthropoda | Insecta | Coleoptera | Elmidae | Neolmis_larvae | Neol |
| Arthropoda | Insecta | Coleoptera | Elmidae | Xenelmis_larva | Xene |
| Arthropoda | Insecta | Coleoptera | Gyrinidae | Gyretes | Gyre |
| Arthropoda | Insecta | Coleoptera | Haliplidae | Haliplus | Hali |
| Arthropoda | Insecta | Coleoptera | Heteroceridae | Heteroceridae | Hete |
| Arthropoda | Insecta | Coleoptera | Hydrochidae | Hydrochidae | Hychy |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Berosus | Bero |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Crenilis | Creni |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Derallus | Dera |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Enocrus | Eno |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Hydrophilus | Hylu |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Hydrovatus | Hyva |
| Arthropoda | Insecta | Coleoptera | Hydrophilidae | Paracymus | Para |
| Arthropoda | Insecta | Coleoptera | Noteridae | Suphis | Suph |
| Arthropoda | Insecta | Coleoptera | Noteridae | Suphisellus | Suse |
| Arthropoda | Insecta | Coleoptera | Psephenidae | Psephenus | Pse |
| Arthropoda | Insecta | Coleoptera | Scirtidae | Scirtidae | Scirt |
| Arthropoda | Insecta | Coleoptera | Sphaeriusidae | Sphaeriusidae | Spha |
| Arthropoda | Insecta | Diptera | Ceratopogonidae | Ceratopogoninae | Cerat |
| Arthropoda | Insecta | Diptera | Ceratopogonidae | Forcipomyiinae | For |
| Arthropoda | Insecta | Diptera | Chaoboridae | Chaoboridae | Chao |
| Arthropoda | Insecta | Diptera | Chironomidae | Chironominae | Chi |
| Arthropoda | Insecta | Diptera | Chironomidae | Orthocladiinae | Ort |
| Arthropoda | Insecta | Diptera | Chironomidae | Tanypodinae | Tan |
| Arthropoda | Insecta | Diptera | Culicidae | Culicidae | Cul |
| Arthropoda | Insecta | Diptera | Dolichopodidae | Dolichopodidae | Dol |
| Arthropoda | Insecta | Diptera | Empididae | Empididae | Emp |
| Arthropoda | Insecta | Diptera | Ephydridae | Ephydridae | Eph |
| Arthropoda | Insecta | Diptera | Limoniidae | Hexatoma | Hex |
| Arthropoda | Insecta | Diptera | Psychodidae | Clogmia | Clo |
| Arthropoda | Insecta | Diptera | Psychodidae | Maurina | Mrn |
| Arthropoda | Insecta | Diptera | Sciomyzidae | Sciomyzidae | Sci |

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|---|---|---|---|---|---|
| Arthropoda | Insecta | Diptera | Simuliidae | Simulium | Sim |
| Arthropoda | Insecta | Diptera | Stratiomyidae | Stratiomyidae | Str |
| Arthropoda | Insecta | Diptera | Syrphidae | Syrphidae | Syr |
| Arthropoda | Insecta | Diptera | Tabanidae | Tabanus | Tab |
| Arthropoda | Insecta | Diptera | Tipulidae | Tipula | Tip |
| Arthropoda | Insecta | Ephemeroptera | Baetidae | Americabaetis | Ame |
| Arthropoda | Insecta | Ephemeroptera | Baetidae | Baetodes | Bae |
| Arthropoda | Insecta | Ephemeroptera | Baetidae | Callibaetis | Cal |
| Arthropoda | Insecta | Ephemeroptera | Baetidae | Camelobaetidius | Cam |
| Arthropoda | Insecta | Ephemeroptera | Caenidae | Caenis | Cae |
| Arthropoda | Insecta | Ephemeroptera | Leptohyphidae | Haplohyphes | Hap |
| Arthropoda | Insecta | Ephemeroptera | Leptohyphidae | Tricorythodes | Tri |
| Arthropoda | Insecta | Ephemeroptera | Leptohyphidae | Tricorythopsys | Try |
| Arthropoda | Insecta | Ephemeroptera | Leptophlebiidae | Farrodes | Far |
| Arthropoda | Insecta | Ephemeroptera | Leptophlebiidae | Needhamellus | Nee |
| Arthropoda | Insecta | Ephemeroptera | Leptophlebiidae | Ulmeritoides | Ulm |
| Arthropoda | Insecta | Ephemeroptera | Leptophlebiidae | Ulmeritus | Ulm |
| Arthropoda | Insecta | Ephemeroptera | Polymitarcyidae | Campsurus | Camp |
| Arthropoda | Insecta | Hemiptera | Belostomatidae | Belostoma | Bel |
| Arthropoda | Insecta | Hemiptera | Belostomatidae | Horvathinia | Hor |
| Arthropoda | Insecta | Hemiptera | Belostomatidae | Lethocerus | Let |
| Arthropoda | Insecta | Hemiptera | Corixidae | Corixidae | Crx |
| Arthropoda | Insecta | Hemiptera | Gerridae | Gerridae | Ger |
| Arthropoda | Insecta | Hemiptera | Hebridae | Lipogomphus | Lip |
| Arthropoda | Insecta | Hemiptera | Helotrephidae | Neotrephes | Neo |
| Arthropoda | Insecta | Hemiptera | Hydrometridae | Hydrometra | Hyd |
| Arthropoda | Insecta | Hemiptera | Hydrometridae | Limnobatodes | Lim |
| Arthropoda | Insecta | Hemiptera | Naucoridae | Ambrysus | Amb |
| Arthropoda | Insecta | Hemiptera | Naucoridae | Procryphocricos | Pro |
| Arthropoda | Insecta | Hemiptera | Notonectidae | Buenoa | Bue |
| Arthropoda | Insecta | Hemiptera | Notonectidae | Notonecta | Not |
| Arthropoda | Insecta | Hemiptera | Pleidae | Neoplea | Neo |
| Arthropoda | Insecta | Hemiptera | Pleidae | Paraplea | Par |
| Arthropoda | Insecta | Hemiptera | Veliidae | Rhagovelia | Rha |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Argyractis | Arg |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Crambinae | Cra |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Elophila | Elo |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Neargyractis | Nea |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Oxyelophila | Oxy |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Paraponyx | Par |
| Arthropoda | Insecta | Lepidoptera | Crambidae | Petrophila | Pet |
| Arthropoda | Insecta | Lepidoptera | Erebidae | Paracles | Par |
| Arthropoda | Insecta | Lepidoptera | Pyralidae | Pyralidae | Pyr |
| Arthropoda | Insecta | Megaloptera | Corydalidae | Corydalus | Cor |
| Arthropoda | Insecta | Odonata | Aeshnidae | Aeshnidae | Aes |
| Arthropoda | Insecta | Odonata | Aeshnidae | Anax | Ana |
| Arthropoda | Insecta | Odonata | Aeshnidae | Castoraeschna | Cas |
| Arthropoda | Insecta | Odonata | Aeshnidae | Coryphaeschna | Cor |
| Arthropoda | Insecta | Odonata | Aeshnidae | Limnetron | Lim |
| Arthropoda | Insecta | Odonata | Aeshnidae | Neuraeshna | Neu |
| Arthropoda | Insecta | Odonata | Calopterygidae | Mneserate | Mne |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Acanthagrion | Aca |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Argia | Arg |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Coenagrionidae | Coe |

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|---|---|---|---|---|---|
| Arthropoda | Insecta | Odonata | Coenagrionidae | Cyanallagma | Cya |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Enallagma | Ena |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Idioneura | Idi |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Neoneura | Neo |
| Arthropoda | Insecta | Odonata | Coenagrionidae | Oxagrion | Oxa |
| Arthropoda | Insecta | Odonata | Gomphidae | Aphylla | Aph |
| Arthropoda | Insecta | Odonata | Gomphidae | Archaeogomphus | Arc |
| Arthropoda | Insecta | Odonata | Gomphidae | Cacoides | Cac |
| Arthropoda | Insecta | Odonata | Gomphidae | Gomphidae | Gom |
| Arthropoda | Insecta | Odonata | Gomphidae | Gomphoides | Gom |
| Arthropoda | Insecta | Odonata | Gomphidae | Phyllocycla | Phy |
| Arthropoda | Insecta | Odonata | Gomphidae | Phyllogomphoides | Phy |
| Arthropoda | Insecta | Odonata | Gomphidae | Tibiagomphus | Tib |
| Arthropoda | Insecta | Odonata | Libellulidae | Argyrothemis | Arg |
| Arthropoda | Insecta | Odonata | Libellulidae | Dythemis | Dyt |
| Arthropoda | Insecta | Odonata | Libellulidae | Erythemis | Ery |
| Arthropoda | Insecta | Odonata | Libellulidae | Erythrodiplax | Ery |
| Arthropoda | Insecta | Odonata | Libellulidae | Gynothemis | Gyn |
| Arthropoda | Insecta | Odonata | Libellulidae | Libellula | Lib |
| Arthropoda | Insecta | Odonata | Libellulidae | Libellulidae | Lib |
| Arthropoda | Insecta | Odonata | Libellulidae | Macrothemis | Mac |
| Arthropoda | Insecta | Odonata | Libellulidae | Micrathyria | Mic |
| Arthropoda | Insecta | Odonata | Libellulidae | Oligoclada | Oli |
| Arthropoda | Insecta | Odonata | Libellulidae | Orthemis | Ort |
| Arthropoda | Insecta | Odonata | Libellulidae | Pachydiplax | Pac |
| Arthropoda | Insecta | Odonata | Libellulidae | Perithemis | Per |
| Arthropoda | Insecta | Odonata | Libellulidae | Planiplax | Pla |
| Arthropoda | Insecta | Odonata | Libellulidae | Sympetrum | Sym |
| Arthropoda | Insecta | Odonata | Macromiidae | Macromia | Mac |
| Arthropoda | Insecta | Plecoptera | Gripopterygidae | Gripopteryx | Gri |
| Arthropoda | Insecta | Plecoptera | Gripopterygidae | Paragripopteryx | Par |
| Arthropoda | Insecta | Plecoptera | Gripopterygidae | Tupiperla | Tup |
| Arthropoda | Insecta | Plecoptera | Perlidae | Anacroneuria | Ana |
| Arthropoda | Insecta | Trichoptera | Calamoceratidae | Phylloicus | Phy |
| Arthropoda | Insecta | Trichoptera | Glossosomatidae | Mortoniella | Mor |
| Arthropoda | Insecta | Trichoptera | Helicopsychidae | Helicopsyche | Hel |
| Arthropoda | Insecta | Trichoptera | Hydropsychidae | Leptonema | Lep |
| Arthropoda | Insecta | Trichoptera | Hydropsychidae | Macronema | Mac |
| Arthropoda | Insecta | Trichoptera | Hydropsychidae | Smicridea | Smi |
| Arthropoda | Insecta | Trichoptera | Hydropsychidae | Synoestropsis | Syn |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Acostatrichia | Aco |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Byrsopteryx | Byr |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Hydroptila | Hyd |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Metrichia | Met |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Neotrichia | Neo |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Ochrotrichia | Och |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Oxyethira | Oxy |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Rhyacopsyche | Rhy |
| Arthropoda | Insecta | Trichoptera | Hydroptilidae | Tricholeiochiton | Tri |
| Arthropoda | Insecta | Trichoptera | Leptoceridae | Brachysetodes | Bra |
| Arthropoda | Insecta | Trichoptera | Leptoceridae | Grumichella | Gru |
| Arthropoda | Insecta | Trichoptera | Leptoceridae | Netopsyche | Net |
| Arthropoda | Insecta | Trichoptera | Leptoceridae | Oecetis | Oec |
| Arthropoda | Insecta | Trichoptera | Odontoceridae | Marilia | Mar |

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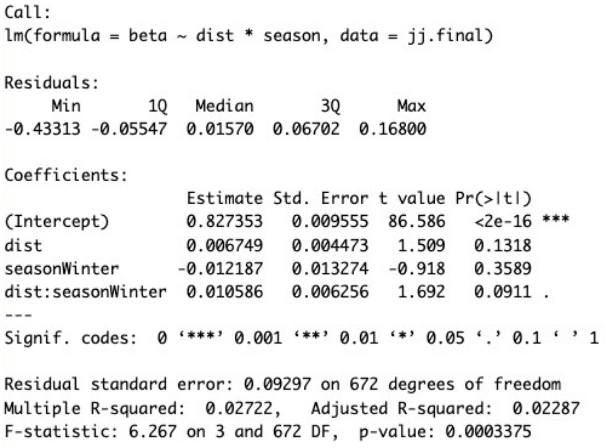
|---|---|---|---|---|---|
| Arthropoda | Insecta | Trichoptera | Philopotamidae | Chimarra | Chi |
| Arthropoda | Insecta | Trichoptera | Polycentropodidae | Cyrnellus | Cyr |
| Arthropoda | Insecta | Trichoptera | Polycentropodidae | Polycentropus | Pol |
| Arthropoda | Malacostraca | Amphipoda | Hyalellidae | Hyalella | Hya |
| Arthropoda | Malacostraca | Decapoda | Aeglidae | Aegla | Aeg |
| Arthropoda | Malacostraca | Decapoda | Palaemonidae | Palaemonetes | Pal |
| Arthropoda | Malacostraca | Decapoda | Trichodactylidae | Trichodactylus | Tri |
| Arthropoda | Malacostraca | Isopoda | Asellidae | Asellus | Ase |
| Mollusca | Bivalvia | Sphaeriida | Sphaeriidae | Pisidium | Pis |
| Mollusca | Bivalvia | Unionoidea | Mycetopodidae | Anodontites | Ano |
| Mollusca | Bivalvia | Veneroida | Corbiculidae | Corbicula | Cor |
| Mollusca | Gastropoda | Architaenioglossa | Ampullariidae | Pomacea | Pom |
| Mollusca | Gastropoda | Basommatophora | Planorbidae | Ancylinae | Anc |
| Mollusca | Gastropoda | Basommatophora | Planorbidae | Planorbidae | Pla |
| Mollusca | Gastropoda | Coenogastropoda | Cochliopidae | Heleobia | Hel |
| Mollusca | Gastropoda | Littorinimorpha | Tateidae | Potamolithus | Pot |
| Mollusca | Gastropoda | | Chilinidae | Chilina | Chi |
| Mollusca | Gastropoda | | Lymnaeidae | Lymnaea | Lym |
| Mollusca | Gastropoda | | Physidae | Physa | Phy |
| Nematoda | | | Nematoda | Nematoda | Nem |
| Platyhelminthes | Rhabditophora | Tricladida | Dugesiidae | Dugesiidae | Pla |

**Supplementary Analysis 1**. Using a multiple regression model, the response variable was the Jaccard dissimilarity of the macroinvertebrate community composition, and the explanatory variables were the Euclidian distance between the pair of sites and the season (summer and winter) as a covariable. For this, we used the R package "vegan." Results demonstrated that neither the Euclidean distance between pairs of sites (dist), nor the season and their interaction (dist:season) affect the macroinvertebrate composition. Thus, there is no spatial autocorrelation between sites. The regression plot (right panel) showed two slopes corresponding to the season: summer: orange, winter: blue.

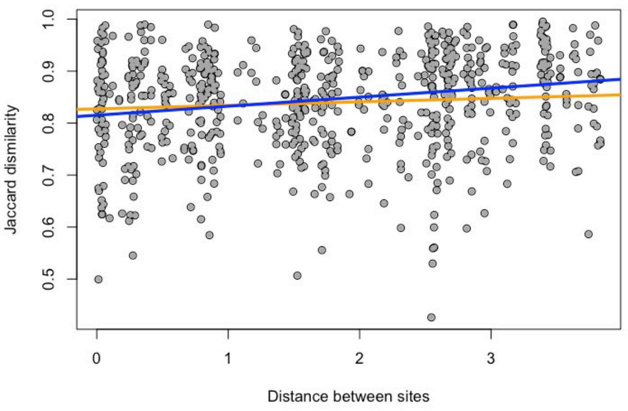


```
Call:
lm(formula = beta ~ dist * season, data = jj.final)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.43313 -0.05547  0.01570  0.06702  0.16800 

Coefficients:
                   Estimate Std. Error t value Pr(>|t|)    
(Intercept)        0.827353   0.009555  86.586   <2e-16 ***
dist               0.006749   0.004473   1.509   0.1318    
seasonWinter      -0.012187   0.013274  -0.918   0.3589    
dist:seasonWinter  0.010586   0.006256   1.692   0.0911 .  
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.09297 on 672 degrees of freedom
Multiple R-squared:  0.02722,	Adjusted R-squared:  0.02287 
F-statistic: 6.267 on 3 and 672 DF,  p-value: 0.0003375
```



**Supplementary Analysis 2**. U-Mann and Whitney test to compare means of basal resources, local habitat variables, and functional diversity between summer and winter seasons.

| Variable | Mean | | SD | | W | *p-value* | Differences |
|---|---|---|---|---|---|---|---|
| | Summer | Winter | Summer | Winter | | | |
| **Basal resources** | | | | | | | |
| Submergent g.m-2 | 17.57 | 17.83 | 22.48 | 22.52 | 373 | 0.8892 | |
| Emergent g.m-2 | 18.84 | 16.72 | 34.71 | 29.26 | 394.5 | 0.5931 | |
| Grass g.m-2 | 29.99 | 33.2 | 27.05 | 36.14 | 383 | 0.7555 | |
| Rooted floating g.m-2 | 9.81 | 12.07 | 14.15 | 16.55 | 329 | 0.5359 | |
| Diatom g.m-2 | 0.0093 | 0.0157 | 0.008 | 0.009 | 190 | 0.003 | winter>summer |
| Green algae g.m-2 | 0.0062 | 0.0071 | 0.005 | 0.007 | 345 | 0.7424 | |
| Cyanobacteria g.m-2 | 0.0057 | 0.0063 | 0.003 | 0.003 | 307.5 | 0.3283 | |
| Detritus g.m-2 | 5.691 | 28.74 | 12.71 | 65.34 | 246 | 0.04 | winter>summer |
| **Local habitat** | | | | | | | |
| Stones % | 37.9 | 29.75 | 41.54 | 40.13 | 3642 | 0.1953 | |
| Gravel % | 18.77 | 27.96 | 25329 | 34.4 | 3031 | 0.3839 | |
| Sand % | 20.93 | 21.85 | 31.54 | 34.53 | 339 | 0.6806 | |
| Clay % | 8.76 | 3.95 | 27.44 | 19.08 | 3441 | 0.2392 | |
| Mud% | 13.64 | 16.48 | 32.28 | 34.51 | 3255 | 0.9064 | |
| Current velocity m.s-1 | 0.075 | 0.0189 | 0.114 | 0.234 | 2298 | 0.0006 | winter>summer |
| Water depth cm | 24.27 | 21.72 | 15.23 | 12.75 | 3548 | 0.3709 | |
| **Functional diversity** | 0.16 | 0.15 | 0.04 | 0.03 | 3956 | 0.024 | summer>winter |

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# *CHAPTER IV: Riparian cover buffers the effects of abiotic and biotic predictors of leaf decomposition in subtropical streams.*

This image was created by M. Barrios using Bing Designer by Microsoft.

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Scope: Aquatic Science, Ranking Q1

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Aquatic Sciences

RESEARCH ARTICLE

# Riparian cover buffers the effects of abiotic and biotic predictors of leaf decomposition in subtropical streams

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## Abstract

Stream functioning is energetically dependent on terrestrial vegetation due to the input of leaves. The decomposition process of this allochthonous resource may be controlled by leaf identity and abiotic and biological predictors that are also influenced by the presence of riparian cover. In subtropical Uruguayan streams, most of the riparian zones have been reduced, and the response of the decomposition process to the predictors may depend on the presence of riparian cover. We analyzed the importance of leaf identity and riparian cover on the abiotic and biotic predictors of leaf decomposition in rangeland streams, comparing two stream types (open canopy stream, OCS, and riparian forest stream, RFS). Decomposition experiments of native species (*Eryngium pandanifolium* and *Schoenoplectus californicus*) and the exotic *Eucalyptus globulus* were carried out. There were no significant differences in decomposition rate between the stream types; however, some predictors had significant, albeit differential, effects on the decomposition process depending on the presence of riparian forest. In OCS, the decomposition rates were positively influenced by $NH_4$-N and streamflow but negatively by $PO_4$-P, conductivity, and proportions of scrapers. Most of these variables had nonsignificant effects on decomposition rates in RFS. Experimentation procedures are needed to establish the mechanisms by which the presence of riparian cover modulates the response of the leaf decomposition to the effects of abiotic and biotic variables in subtropical streams. Leaf decomposition is much more affected by changes in leaf identity, suggesting that riparian changes that are accompanied by changes in leaf inputs may strongly affect this ecosystem function.

**Keywords** Leaf physicochemical characteristics · Open canopy streams · Riparian forest stream · Subtropical Uruguayan stream

## Introduction

The functioning of streams is largely controlled by their connection with terrestrial ecosystems, which determines biogeochemical, physical, and biological patterns (Gessner et al. 1999; Graça and Canhoto 2006; Graça et al. 2015; Boyero et al. 2016; Tonin et al. 2021). This aquatic and terrestrial connection is energetically dependent on riparian cover controlling light and organic matter entry in the form of green and senescent leaves (Lopes et al. 2015; Rezende et al. 2016; Ottoni-Boldrini 2018). Therefore, the riparian cover controls the relative importance of autochthonous

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and allochthonous carbon subsidies in the aquatic food web (Vannote et al. 1980; Marcarelli et al. 2011).

The decomposition of allochthonous leaves is a fundamental process in headwater and small streams (Gessner et al. 1999). This process involves the leaching of dissolved compounds and the colonization of leaves by microorganisms and invertebrates (Vannote et al. 1980; Abelho 2001; Graça and Canhoto 2006; Cardinale et al. 2011; Tiegs et al., 2019). A great part of the allochthonous carbon that entry into the stream is respired by the microorganisms (Marcarelli et al. 2011), but the remaining coarse organic material sustains the food web due to its ingestion and assimilation by invertebrates contributing to the carbon and nutrient cycle (Marks et al. 2019). The rate at which organic matter decomposes depends primarily on intrinsic factors such as the physicochemical and morphological characteristics of the leaf (e.g., nutrients, secondary metabolites, and lignin concentration) (Ostrofsky 1997; Gessner et al. 2010; Ardón and Pringle 2008; Ramos et al. 2021) which vary between species and biogeographical regions (Boyero et al. 2017). Other extrinsic factors related to the decomposition process are controlled by biotic (bacteria, aquatic hyphomycetes, shredder macroinvertebrates) and abiotic (temperature, water chemistry, current velocity) variables (Graça et al. 2015; Boyero et al. 2016; Costello et al. 2022). In turn, the interaction of water chemistry and leaf characteristics also influences the activity of microorganisms and macroinvertebrates involved in the decomposition process (Royer and Minshall 2001; Gulis et al. 2004; 2006; Tant et al. 2013; Biasi et al. 2017).

The elimination or the replacement of native riparian vegetation areas by anthropogenic activities threatens the aquatic dependency on terrestrial carbon and the environmental and biotic predictors related to the decomposition process (Silva-Junior et al. 2014; Silva-Araujo et al. 2020; Ferreira et al. 2020; Sargac et al. 2021; Tonin et al. 2021; Boyero et al. 2021). The replacement of native allochthonous resources with exotic leaves may impair the activity of microorganisms and detritivore macroinvertebrates due to differences in the physicochemical characteristics of exotic leaves compared to native species (Ferreira et al. 2016, 2018; Kominoski et al. 2021). When riparian vegetation is removed, some physical changes in streams occur as a higher increase in nutrient concentrations and sediment inputs and a rise in temperature (Bleich et al. 2014) due to higher light incidence (Warren et al. 2016). These physical changes in streams may affect the microbial (Halvorson et al. 2016) and shredder composition (Benstead and Pringle 2004; Encalada et al. 2010; Giling et al. 2011; Cornejo et al. 2020). Riparian removal also promotes alterations in habitat heterogeneity and current velocity by promoting the growth of aquatic plants (Dosskey et al. 2010), with consequences for macroinvertebrates leaf consumers (Sargac et al. 2021) and the physical fragmentation of leaves (Tonin et al. 2021).

Subtropical Uruguayan streams are found within the grassland biome (Pampean ecoregion), where most of the riparian vegetation consists of herbaceous and grass species (Haretche et al. 2012; Bernardi et al. 2016). Also, in hilly areas (such as the upper watershed of Arroyo Maldonado) there are still some patches of riparian vegetation that included native woody trees, and in areas with open canopy streams, the riparian forests have been replaced by pastures for extensive livestock farming and some prairie areas were occupied with *Eucalyptus* spp. afforestation. On the other hand, Uruguay's streams are subject to the stochasticity of hydrological pulses (Goyenola et al. 2015) that contribute significantly to the input of plant material usually in the form of green leaves dominated by herbaceous and graminoid species (Burwood 2019). This green leaf input can also be important in streams with riparian cover due to hydrological drift from upstream and to the non-continuous riparian cover in some reaches allowing the growth of these herbaceous species (*personal observation*). In this sense, possible sources of subsidies in rangeland streams come from different origins, which are dominated by terrestrial-herbaceous and aquatic species, in addition to the possible entry of *Eucalyptus* spp. leaves from afforestation areas.

The functioning of these subtropical rangeland streams in terms of the decomposition of leaves from different origins and the effects of native riparian forest removal on the main abiotic and biotic factors determining this process are poorly understood. This research aimed to analyze the importance of native riparian forest cover on the abiotic (habitat variables) and biotic factors (microorganisms and macroinvertebrates) controlling the decomposition of leaves of different origins in rangeland streams subjected to extensive livestock farming (low cattle density per hectare).

We hypothesize that the responses of the decomposition process to abiotic and biotic factors depend on the presence of native riparian forest cover and leaf species. Thus, we analyzed the decomposition process of green leaves of two native species (terrestrial *Eryngium pandanifolium* and aquatic *Schoenoplectus californicus*) and the exotic *Eucalyptus globulus*. We selected these species because they are found in large quantities in the leaf stock in the streams, especially in the form of green leaves that enter the streams with the hydrological flood pulses (Burwood 2019; Barrios *unpublished data*). In addition, green leaves may be important as a potential carbon resource for aquatic organisms (Kochi and Yanai 2006; Lopes et al. 2015; Ottoni-Boldrini 2018; Feng et al. 2018). We also selected these species due to differences in physical and chemical leaf characteristics which can moderate riparian cover effects on litter decomposition.

To analyze the effects of riparian cover on the decomposition process, we experimented with two stream types: streams with riparian cover (hereafter referred to as riparian forest streams, RFS) and streams without riparian cover with grasses and herbaceous vegetation and some shrubs (hereafter referred to as open canopy streams, OCS). We also used two mesh bag sizes to assess the biotic effects on leaf decomposition, analyzing the relative contribution of the microorganisms (fine mesh bags) and microorganisms plus macroinvertebrates (coarse mesh bags).

We predict that: (1) differences in litter decomposition rates will vary among leaf species due to differences in their origin and physicochemical characteristics; (2) differences in litter decomposition will vary among mesh bag sizes due to the participation of macroinvertebrates in the decomposition process; and (3) differences in litter decomposition rates will vary among RFS and OCS, due to that in the RFS the variation of controlling abiotic and biotic predictor will affect in less proportion the leaf decomposition rates due to a greater capacity of the riparian zone to buffer environmental changes in comparison to OCS were the alteration on the controlling predictors affect negatively the decomposition rate.

## Methods

### Study area

The Arroyo Maldonado basin is located in the departments of Maldonado and Lavalleja in southeastern Uruguay. The course of the Arroyo Maldonado flows through the Caracoles highland system, and its headwaters are in the highest altitude areas of the country (Cerro Catedral, 513 m a.s.l.). (Grattarola et al. 2020). A substantial catchment area is used for extensive cattle production, and a small area is utilized for *Eucalyptus* spp. afforestation and agriculture (Haretche et al. 2012; Bernardi et al. 2016). Nevertheless, the conservation status of the catchment is good in terms of water quality and aquatic biota (Barrios *unpublished data*), despite the historical impact of cattle introduction with colonization (Bernardi et al. 2016). Riparian forest streams are restricted to some reaches with an average width of 42.7 m on each side of the stream. The composition of riparian vegetation is mainly shrub species that are predominantly deciduous and mixed in phytogeographical terms (Heretche et al. 2012; Bernardi et al. 2016) such as *Pouteria salicifolia, Scutia buxifolia, Allophylus edulis, Sebastiana* sp., *Myrsine* sp., *Blepharocalyx salicifolius, Myrrhinium atropurpureum, Lithraea brasiliensis,* and *Salix* sp. (Grattarola et al., 2020). Open canopy streams are characterized by herbaceous and graminoid species such as *Eryngium pandanifolium, Eupatorium buniifolium, Baccharis trimera, Erianthus angustifolium, Schoenoplectus californicus,* and *Schinus lentiscifolium* (Grattarola et al., 2020). This study was carried out in 3–4 order streams: three RFS and three OCS (Table S1).

### Stream characterization

We measured in situ physicochemical parameters of the water (pH, conductivity, dissolved solids, temperature, and dissolved oxygen) during each of the eight days of leaf sampling using a YSI multiparameter probe (YSI Inc.). Water samples were also taken for subsequent nutrient analysis, such as dissolved ($NO_3$-N, $NH_4$-N) and total nitrogen (TN) (Müller and Wiedemann 1955), total (TP) and dissolved (TDP) phosphorous, and orthophosphate ($PO_4$-P) (Valderrama 1981). The temperature was recorded every 15 min during the whole study using HOBO loggers. Streamflow (Q, $m^3.s^{-1}$) was estimated by measuring area (width and depth) together with velocity (measured every 0.25 m). The components of periphyton biomass were measured *in* situ using an optical sensor (BenthoTorch™) on a surface of 0.78 $cm^2$ in ten points randomly selected including patches of sediments and some leaf bags. This device measures the fluorescence of algal pigments like chlorophytes, diatoms, and cyanobacteria (Table 1; S1).

### Leaf litter decomposition experiment

#### Leaf selection and collection

For the decomposition experiment, green leaves were used due to their high abundance in the littoral zone and their importance in the leaf stock in the streams due to the leaf input during flood pulses (Burwood 2019; Barrios *unpublished data*). Three leaf species of different origins were selected: native terrestrial *E. pandanifolium*, native aquatic *S. californicus,* and exotic terrestrial *E. globulus*. These species are rare in the littoral zone of RFS, but they are in high abundance in OCS; however, there is a potential input of these species (especially of green leaves) in both stream types during rainfall events and the drift from upstream (Teixeira de Mello, *personal observation*). Eucalyptus leaves were used as a reference model due to their wide use in decomposition experiments and because of the risk posed by plantations and the introduction of their litter into aquatic ecosystems (Ferreira et al. 2016; 2018). Based on previous chemical analyses of leaf species (Burwood 2019), *S. californicus* are poor in nitrogen and phosphorous content (% TN = 0.72 ± 0.03; %TP = 0.17 ± 0.01), the percentage of lignin is low (% Lignin = 11.3 ± 0.83), and the carbon and nutrients relationships are high (C:N = 65.62 ± 2.7; C:P = 292.4 ± 19.9). Conversely, *E. globulus* and *E. pandanifolium* leaves are richer in nutrients (% TN = 2.13 ± 0.13; %TP = 0.16 ± 0.00,

**Table 1** Physicochemical and habitat variables of riparian forest streams (RFS; $n=3$) and open canopy streams (OCS; $n=3$) in streams from the Arroyo Maldonado watershed, Uruguay

| Variables | RFS | | OCS | | *T* test | |
|---|---|---|---|---|---|---|
| *Water-habitat variables* | Mean | sd | Mean | sd | *t*(df) | *p* |
| Temperature (°C) | 16.9 | 1.0 | 20.3 | 2.25 | – 5.88 (24.32) | < 0.001 *** |
| Conductivity (μS. cm$^{-3}$) | 172.3 | 73.7 | 130.7 | 25.9 | – 2.01 (26) | 0.053 |
| Dissolved oxygen (mg. L$^{-1}$) | 10.2 | 0.2 | 9.49 | 0.2 | 11.15 (33.24) | < 0.001 *** |
| TN (μg. L$^{-1}$) | 213.3 | 11.8 | 172.3 | 39.4 | – 4.11 (18.85) | < 0.001 *** |
| TDN (μg. L$^{-1}$) | 180.3 | 5.54 | 128.57 | 34.02 | 5.9 (17.45) | < 0.001 *** |
| $NO_3$-N (μg. L$^{-1}$) | 17.3 | 2.73 | 6.81 | 5.85 | – 5.55 (17.76) | < 0.001 *** |
| $NH_4$-N (μg. L$^{-1}$) | 13.6 | 6.0 | 6.4 | 3.5 | – 5.23 (32.47) | < 0.001 *** |
| TP (μg. L$^{-1}$) | 21.6 | 5.7 | 19.9 | 1.4 | – 0.91 (19.48) | 0.372 |
| TDP (μg. L$^{-1}$) | 18.1 | 4.1 | 15.6 | 0.7 | – 2.46 (18.42) | 0.024 * |
| $PO_4$-P (μg. L$^{-1}$) | 2.8 | 1.3 | 2.3 | 1.9 | – 1.46 (32.80) | 0.155 |
| pH | 7.36 | 0.25 | 7.40 | 0.24 | 0.51 (33.66) | 0.614 |
| Q (m$^3$. s$^{-1}$) | 0.20 | 0.26 | 0.08 | 0.05 | – 0.68 (20.15) | 0.506 |
| Total Periphyton (μg. cm$^{-2}$) | 1.67 | 1.23 | 1.21 | 0.65 | 0.14 (24.26) | 0.887 |
| Chlorophyte (μg. cm$^{-2}$) | 0.016 | 0.01 | 0.24 | 0.04 | 18.34 (19.42) | < 0.001 *** |
| Diatom (μg. cm$^{-2}$) | 1.13 | 0.87 | 0.55 | 0.36 | – 1.37 (25.6) | 0.182 |
| Cyanobacteria (μg. cm$^{-2}$) | 0.51 | 0.37 | 0.41 | 0.25 | – 0.09 (27.9) | 0.931 |

*TN* total nitrogen, *TDN* total dissolved nitrogen, *$NO_3$-N* nitrate, *$NH_4$-N* ammonium, *TP* total phosphorous, *TDP* total dissolve phosphorous, *$PO_4$-P* Orthophosphate, *Q* streamflow

*p values significant:* *: < 0.05; **: 0.01; ***: < 0.001

and % TN = 1.33 ± 0.10; %TP = 0.21 ± 0.00, respectively), with lower CN ratios (26.64 ± 1.53; 33.23 ± 2.18, respectively). However, *E. globulus* contains higher percentage of lignin (21.63 ± 0.63%) and C:P ratios (357.70 ± 11.34) when compared to *E. pandanifolium* leaves (lignin = 13.68 ± 0.5%, C:P = 200.94 ± 16.11).

In the field, fresh leaves of the three species were collected directly from the plants and then air-dried in a dark room at room temperature (approx. 25 °C) up to constant weight (Yavitt et al. 2019; Du et al. 2020). Both leaf collection and the experiment were carried out during austral spring (October–November 2017).

### Preparation of the litter bags

Leaf mass was estimated using the litter bag technique (Bärlocher 2020). Leaf bags of two mesh sizes were used: 10 mm coarse mesh (CM) litter bags to measure litter decomposition driven by both microbes and macroinvertebrates and 0.5 mm fine mesh (FM) leaf bags to allow for assessing microbial-driven leaf decomposition. The total number of leaf bags was 216 CM bags (2 stream types × 3 streams × 3 leaf species × 3 leaf bags × 4 samplings) and 72 FM bags (2 stream types × 3 streams × 3 leaf species × 1 leaf bag × 4 samplings). Each leaf type was placed in each bag with approximately 3.06 ± 0.01 g of dry leaf (initial weight or air-dry weight). The initial ash-free dry mass (AFDM) was determined on one control set of leaves of each species. For this, leaves were dried to a constant mass (oven-dry for 48 h in the oven at 50 °C), and then, they were calcinated to determine the ash-free dry mass (4 h in the muffle at 500 °C). An average correction factor (*D*) was determined as the proportion of ash-free dry mass and air-dried leaves.

Leaf bags were transported in cool boxes to the study area and the second set of leaf bags (three replicates per leaf species) was recovered immediately upon exposure to the stream bed in each stream. These leaves were oven-dried (50 °C for 48 h) and calcinated (500 °C for 4 h), which allowed for the calculation of the mass lost during handling (*H*) as the proportion of leaf mass before and after handling. The initial ash-free dry mass of each leaf pack brought to the streams was estimated by multiplying the measured air-dry mass by the average correction factor *D* and the correction factor for manipulation *H* (Bärlocher 2020).

Each leaf bag used for the experiment was tied to a pole and placed into the streambed using plastic clamps. Subsequent samples were taken according to a preselected schedule where three CM litter bags and one FM litter bag of each species were collected at 7, 13, 21, and 26 days of the experiment. Each leaf bag was placed in a plastic bag, ensuring that the remaining material between bags was not mixed, and transported to the laboratory in a cool box at 4 °C. Laboratory analysis consisted of rinsing the recovered leaves under tap water using a 500 μm sieve to separate the macroinvertebrates that colonized the leaves. The remaining leaf was dried in an oven (50 °C until a constant weight was reached) and then calcined in a muffle (500 °C for 4 h) to determine the remanent ash-free dry mass (AFDMr) of the leaves.

The proportion of AFDMr per day of incubation was calculated for each leaf species using the equation: % AFDMr = $(M_f/M_0)*100$, where % AFDMr = percentage of AFDM of remaining mass, $M_0$ = initial dry mass, $M_f$ = final dry mass. The litter decomposition rate (*k degree*-days) was then calculated using exponential regression models $M_t = M_0 * e^{-kt}$, where $M_t$ = the mass at time t; $M_0$ = mass at time 0 (approximately 3 g/bag), *k* = exponential loss coefficient, and *t* = thermal sums calculated by summing average daily temperatures (*degree-days*) for the period the leaf bags were submerged in water. Decomposition rates (*k days*) were also provided only to compare *k* values with the literature, but they were not used in the analyses.

Macroinvertebrates associated with the leaf bags were preserved in Kahle solution (Wegner 2004) for subsequent taxonomic identification, if possible, to the genus level using the keys of Hamada et al. (2018). Functional feeding groups of macroinvertebrates (FFG) and their density (no. ind. g AFDMr$^{-1}$), as well as the exponential of the Shannon diversity of functional feeding groups (eFFG diversity), were assessed (Callisto et al. 2001; Ramírez and Gutierrez- Fonseca 2014; Ferru and Fierro 2015).

## Statistical analyses

Differences in water parameters, water flow, and biomass of periphyton (and its composition of diatoms, cyanobacteria, and green algae) between stream types were determined using a two-tailed unequal-variance *t* test. The data were evaluated for normal distribution (Shapiro–Wilk test) and homoscedasticity of variances (Levene test), and when necessary, the variables were log-transformed (Zuur et al. 2009). The percentage of AFDMr was compared between leaf species, as well as for litter mesh bag sizes and stream type (RFS and OCS) using ANCOVA, with the thermal sum by sampling day (*degree-days*) as a covariable (Bärlocher 2020).

Nonmetric multidimensional scaling (NMDS) ordinations and a three-way factorial permutational multivariate analysis of variance (PERMANOVA, $\alpha = 0.05$) were used to visualize (dis)similarities and to test variations in the composition of macroinvertebrate taxa between systems, leaf species, and mesh bag types (Anderson 2011). Indicator species analyses were used to detect the macroinvertebrate taxa that separated each group. For this, 9999 permutations were used. The Bray–Curtis similarity distance was used for both the NMDS and PERMANOVAs using the functions 'metaMDS' and 'adonis', respectively, in the R package 'vegan' (Oksanen et al. 2007). For indicator species analyses, the "indicspecies" R package was used with the "multipatt" command and the Monte Carlo test with 9999 permutations (de Caceres et al. 2010).

In the case of the decomposition rate (*k degree-days*), the density of macroinvertebrates, the diversity, and the proportion of functional feeding groups, linear mixed models were carried out to test differences between leaf species and stream type. Models were fitted using stream identity as a simple random intercept. For this, models estimate parameters via residual maximum likelihood (REML), and the "lme" R package was used (Pinheiro et al. 2017). These analyses were carried out after checking normality distribution and homoscedasticity of variances (Zuur et al. 2009), and when necessary, the variables were log-transformed.

To determine the overall influence of leaf species, abiotic variables, and biotic variables on the decomposition rate (*k degree days*), we used regression tree analysis (CART; James et al. 2013) using the "rpart" (Therneau and Atkinson 2019) and "partykit" (Hothorn and Zeileis 2015) R packages. After checking if variables followed a normal distribution (Shapiro Test), Spearman's rank correlation was used to examine simple relationships between environmental and biotic variables. Highly correlated variables were discarded (correlation coefficient $P \geq 0.7$). Using this new subset of variables, a recursive partitioning of data was made based on the best predictor variables minimizing the variance in the response variable. Then, an exhaustive search of all the possible partitions on all the explanatory variables was performed, choosing those that increased the 'purity' of the terminal nodes (*k degree-days*). In this case, the partition that minimizes the impurity measures was analyzed by the classification error that consists of the proportion of poorly classified data.

To test whether the relationship of the different abiotic and biotic variables on decomposition rates (*k degree-days*) depends on the presence of riparian cover generalized linear models (GLM) were employed using stream type (RFS, OCS) as an explanatory variable, and the abiotic or biotic variable was used as covariates. For this, separate models were built for each FM and CM leaf bags. Leaf species (Sc: *S. californicus*, Ep: *E. pandanifolium*, Eg: *E. globulus*) were also used as explanatory variables since leaf species could be more important than modulating factors in determining the decomposition process (Sargac et al. 2021). Before running the models, the exponential distribution family of the response variable (*k*-rates) was checked using the "fitdistrplus" R package (Delignette-Muller et al. 2015). Model selections were based on the visual behavior of the residuals and the Akaike selection criterion (Zuur et al. 2009). Given the low number of replicates per system, individual models were used for each predictor variable. Then, a Benjamini–Hochter procedure was used to correct *p* values and reduce the false discovery rate. This procedure calculated the expected proportion of tests (individual GLM) that were incorrectly called significant (type I error) (Benjamini and Hochter 1995). All analyses were performed using R software version 4.0.3 (R Core Team 2020).

## Results

### Stream characterization

Both RFS and OCS drained waters registered circumneutral pH, low conductivity, and low nutrient concentrations (Table 1). In the RFS, the water temperature was significantly lower, and the nutrient concentrations (TN, TND, $NO_3$-N, $NH_4$-N, TDP), and conductivity, were significantly higher than those in the OCS (Table 1). TP, $PO_4$-P, streamflow (Q), and periphyton biomass showed no significant differences between the two-stream types (Table 1). In terms of periphyton components, the biomass of diatoms and cyanobacteria was similar between both systems, but green algae biomass was significantly higher in the OCS than in the RFS (Table 1; Table S1).

### Leaf litter decomposition

After 26 days of incubation of leaves in the stream, the proportion of AFDMr across three species varied between 31.8 and 43.2% for RFS and 31.4 and 45.6% for OCS; and in the case of mesh bag, AFDMr varied between 31.4 and 45.6% of the initial AFDM for CM bags, and 27.5 and 48.4% for FM bags (Table 2). There were no significant differences in *k degree-days* between stream types (linear mixed model: $F_{1\text{-}4} = 1.38$, $p = 0.306$), as well as between mesh bag sizes ($F_{1\text{-}20} = 0.01$, $p = 0.921$), and the interaction among all factors ($F_{2\text{-}20} = 0.75$, $p = 0.485$) (Table 2; Fig. 1). The leaf litter decomposition rates differed between leaf species ($F_{2\text{-}20} = 31.04$, $p < 0.001$) and the *k degree-days* of *S. californicus* were the lowest, meanwhile the exotic *E. globulus* and the native terrestrial *E. pandanifolium* had similar *k degree-days* (Table 2; Fig. 1).

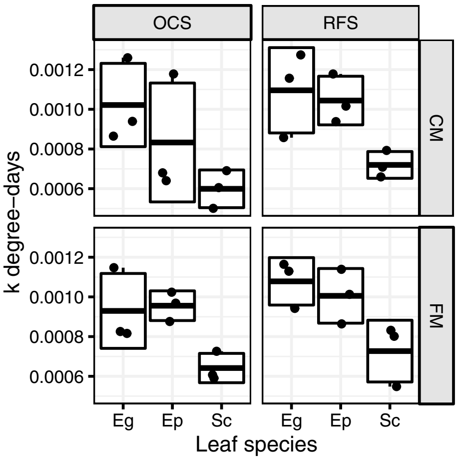

**Fig. 1** Decomposition rates (*k degree-days*) of *Eucalyptus globulus* (Eg), *Eryngium pandanifolium* (Ep), and *Schoenoplectus californicus* (Sc) in coarse (CM) and fine (FM) bag sizes incubated in riparian forest streams (RFS) and open canopy streams (OCS) (Stream types)

**Table 2** Results of linear mixed model analysis performed on the decomposition rate (*k-degree-days*) of leaf species in coarse and fine mesh bag sizes, incubated in riparian forest streams and open canopy streams (stream type)

| Variables | numDF | denDF | *F* value | *p* |
|---|---|---|---|---|
| Intercept | 1 | 20 | 328.33 | <0.001 *** |
| Leaf species | 2 | 20 | 31.04 | <0.001 *** |
| Mesh size | 1 | 20 | 0.01 | 0.921 |
| Stream type | 1 | 4 | 1.38 | 0.306 |
| Leaf species × Mesh bag | 2 | 20 | 0.57 | 0.574 |
| Leaf species × Stream type | 2 | 20 | 0.04 | 0.958 |
| Mesh bag × Stream type | 1 | 20 | 0.26 | 0.613 |
| Leaf species × Mesh bag × Stream type | 2 | 20 | 0.75 | 0.485 |

*p values significant:* *: <0.05; **: 0.01; ***: <0.001

$R^2$ marginal = 0.53; $R^2$ conditional = 0.75

### Colonization of macroinvertebrates in leaf bags

Macroinvertebrates were found in the CM bags and, unexpectedly, in the FM bags. During the experiment, the total density for *E. pandanifolium* CM litter bags was 27.3 and 54.7 ind. g $AFDMr^{-1}$ for RFS and OCS, respectively; and FM litter bags was RFS: 30.7 ind. g $AFDMr^{-1}$; OCS: 41.8 ind. g $AFDMr^{-1}$. In the case of *S. californicus*, total density for CM litter bags was RFS: 22 ind. g $AFDMr^{-1}$; OCS: 44.3 ind. g $AFDMr^{-1}$, and for FM litter bags was RFS: 30.7 ind. g $AFDMr^{-1}$; OCS: 26 ind. g $AFDMr^{-1}$. For *E. globulus* total density in CM litter bags was RFS: 32.7 ind. g $AFDMr^{-1}$; OCS: 43.4 ind. g $AFDMr^{-1}$ and for FM litter bags was RFS: 21.4 ind. g $AFDMr^{-1}$; OCS: 20.8 ind. g $AFDMr^{-1}$.

Litter bags with *E. pandanifolium* presented a higher density and taxa richness (38.7 ind. g $AFDMr^{-1}$, 47 taxa), followed by *S. californicus* (30.7 ind. g $AFDMr^{-1}$, 44 taxa) and *E. globulus* (29.6 ind. g $AFDMr^{-1}$, 43 taxa). The most abundant taxa were Chironomidae (Chironominae + Orthocladiinae = 33.6% of the total abundance), *Americabaetis* (Baetidae; 27.8%), *Simulium* (Simuliidae), and *Caenis* (Caenidae) (both with 8%).

Functional feeding groups were composed mainly of collector-gatherers (CG, 8 taxa richness; 72.9% of the total abundance), followed by predators (Pr, 19; 8.6%), scrapers (Sc, 14; 6%), filterers (Ft, 2, 5.9%), and to a lesser extent, shredders (Sh, 9; 2.5%). The last group included *Hyalella*

(Hyalellidae)*, Aegla* (Aeglidae)*, Tupiperla, Gripopteryx* (Gripopterygidae)*, Tipula* (Tipulidae)*, Phylloicus* (Calamoceratidae)*, Triplectides,* and *Nectopsyche* (Leptoceridae).

Spatial ordination of the macroinvertebrate assemblage and the linear mixed models were built solely on CM litter bags data. This is because comparisons between CM and FM litter bags may have some bias in the results, as FM bags strongly restrict macroinvertebrates > 2 mm in size versus larger sizes that can fit into the 10 mm CM litter bags. Ordination analysis reported for the macroinvertebrates CM bags displayed two main groups in the first NMDS that separated RFS and OCS (PERMANOVA, system: pseudo-$F = 2.93$, $r^2 = 0.17$, $p = 0.03$; Fig. 2) but there were no significant differences in macroinvertebrate assemblage between leaf species (PERMANOVA, pseudo-$F = 0.31$, $r^2 = 0.04$, $p = 0.975$; Fig. 2). Indicator species analyses showed that among the 53 taxa, 11 showed significant differences between the stream types (Table 3). RFS were characterized by six indicator taxa, with *Farrodes* (Leptophlebiidae), *Anacroneuria* (Perlidae), and *Aegla* (Aeglidae) having the greatest indicator value. OCS were characterized by five indicator taxa, with *Nectopsyche*, *Hydroptila* (Hydroptilidae)*,* and *Oxyethira* (Hydroptilidae) exhibiting the highest scores. The remaining 42 taxa had a broader system preference (Table 3).

Linear mixed models for macroinvertebrate attributes in CM bags (Table S3) showed that differences in the density of macroinvertebrates (no. ind. g $\text{AFDMr}^{-1}$) between leaf species, systems, and the interaction of these predictors were not significant (Fig. 3). Differences in eFFG diversity were found between *Schoenoplectus californicus* and *Eryngium pandanifolium* leaves in RFS but there were not significant difference between the system and the interactions between these variables. Differences in the proportions of shredders, filters, and predators were not significant between leaf species, systems, and the interaction of these predictors (Table S3; Fig. 3). For the proportion of scrapers, significantly higher values were found for *S. californicus* and *E. pandanifolium* leaves (Table S3; Fig. 3). In the case of the proportion of collectors-gatherers, there were significant differences between leaf and stream types, but the interaction of these variables was not significant. In this case, a higher proportion of collectors-gatherers was found for *S. californicus* and OCS (Table S2; Fig. 3).

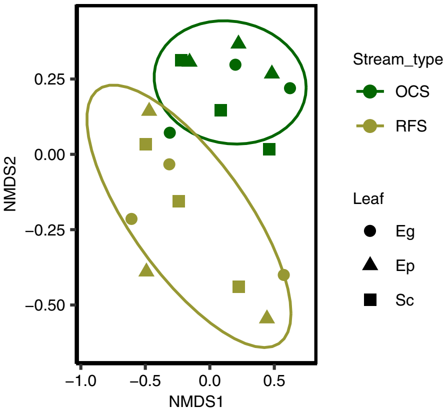

**Fig. 2** Non-metric Multidimensional Scaling (NMDS) ordination of macroinvertebrate assemblage in different stream types **a** riparian forest streams (RFS) and open canopy streams (OCS)), leaf species **b** Eucalyptus globulus (Eg), Eryngium pandanifolium (Ep), and Schoenoplectus californicus (Sc)) and mesh bag sizes **c** coarse (CM) and fine (FM) mesh bag sizes), Stress: 0.11

**Table 3** Indicator species analysis on the macroinvertebrate community between riparian forest streams (RFS) and open canopy streams (OCS) on Arroyo Maldonado, Uruguay

| Taxon | FFG | Factor association | $r_{pb}$ | *p* |
|---|---|---|---|---|
| *Farrodes* | Sc | RFS | 0.478 | 0.002 ** |
| *Anacroneuria* | Pr | RFS | 0.497 | 0.020 * |
| *Aegla* | Sh | RFS | 0.462 | 0.009 ** |
| Empididae | Pr | RFS | 0.455 | 0.011 * |
| *Gyrinus* | Pr | RFS | 0.385 | 0.017 * |
| *Baetodes* | CG | RFS | 0.374 | 0.038 * |
| *Nectopsyche* | Sh | OCS | 0.677 | 0.003 ** |
| *Hydroptilia* | Sc | OCS | 0.561 | 0.006 ** |
| *Oxyethira* | Sc | OCS | 0.466 | 0.009 ** |
| *Psephenus* | Sc | OCS | 0.444 | 0.030 * |
| *Mortoniella* | Sc | OCS | 0.388 | 0.030 * |

$r_{pb}$ point biserial correlation value of each taxon and its statistical significance of the association (*p*) with factors: stream types (RFS, OCS). The classification of the functional feeding groups (FFG) for each taxon is shown, *CG* collector- gathering, *Ft* filters, *Sc* scrapers, *Sh* shredders, *Pr* predators

*p* values significant: *: <0.05; **: 0.01; ***: <0.001

## Abiotic and biotic determinants of leaf litter decomposition

Regression tree analysis with selected variables (Fig. S2) showed that leaf species, $NH_4$-N, and functional feeding groups of macroinvertebrates were good predictors for decomposition rates (*k- degree days*) with a low mean error regression (0.0001) (Fig. 4). Initially, leaf species were separated into two groups: *S. californicus* on one side and *E. pandanifolium* and *E. globulus* together on the other side. Then, for *S. californicus*, $NH_4$-N values higher than 9.5 µg. $L^{-1}$ were associated with higher decomposition rates (mean *k degree days* = 0.00076). For the other two leaf species, when $NH_4$-N values were lower than 6.7 µg. $L^{-1}$, the proportion of CG was lower than 83%, the proportion of shredders was ≥ 3.4%, and the mean decomposition rate was 0.00085. In contrast, when $NH_4$-N values were higher than or equal

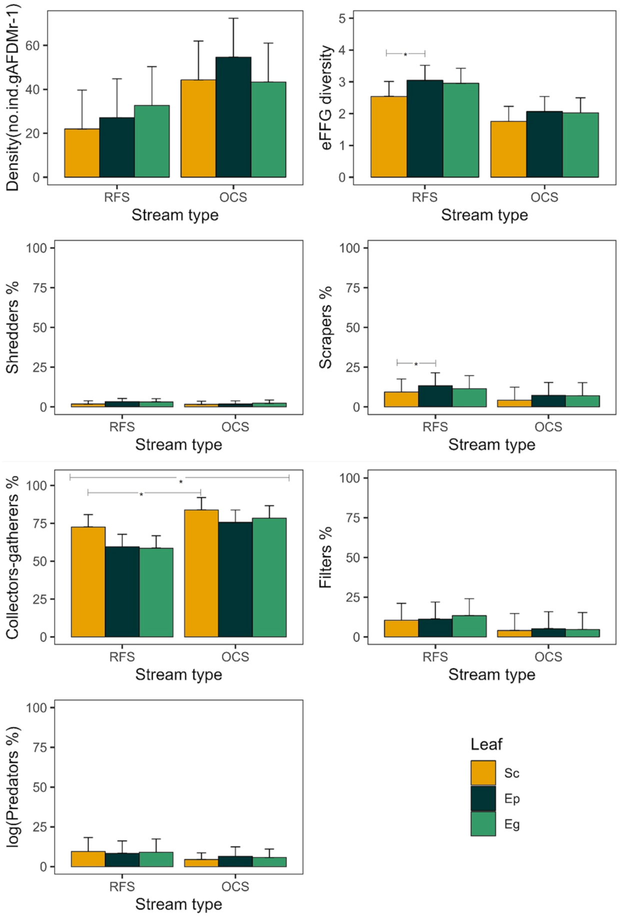

**Fig. 3** Mean (+SE) of the proportion of macroinvertebrate density (no. ind. gAFDMr$^{-1}$), Shannon diversity of functional feeding groups (eFFG diversity), and proportion (%) of shredders, scrapers, collector gathering, filters, and predators that colonized leaf litter of *Eucalyptus globulus* (Eg), *Eryngium pandanifolium* (Ep), and *Schoenoplectus californicus* (Sc) in coarse (CM) and fine (FM) mesh bag sizes incubated riparian forest streams (RFS) and open canopy streams (OCS) (Stream type). Asteriks (*) indicate significant differences between variables

to 6.7 μg. L$^{-1}$, predator proportions became relevant, and when their values were between 3.8 and 21.9%, decomposition rates were the highest (mean *k degree days* = 0.0012) (Fig. 4).

Results from the best models using CM leaf bags or FM bags data showed similar tendencies in the responses of the decomposition rate to the abiotic and biotic factors, which in most cases, varied according to the presence of riparian cover. Due to the low number of replicates in the FM leaf bag experiments, the results of the models for FM leaf bags data are shown in Table S4. The models obtained in the experiments with CM leaf bags data showed that for abiotic and biotic predictors, between 82% and 57.6% of the explained variance accounted for each GLM. All models were determined to be truly significant with the Benjamini–Hochter procedure. The interactions of the abiotic and biotic factors with leaf species were not significant in all cases. For abiotic predictors, the response of decomposition rate to the interaction of $NH_4$-N with the riparian forest was positive and significant in OCS; meanwhile the response to the interaction of $NO_3$-N and riparian forest was negative but not significant in OCS. In both nitrogen fraction, the responses of decomposition rate in RFS were not significant. The response of decomposition rate to conductivity, pH, and $PO_4$-P concentrations were negative in the OCS but only significant for conductivity and $PO_4$-P and there were no significant effects observed in the RFS (Table S4; Fig. 5). The decomposition rate was positively related to streamflow (Q) in the OCS, but no significant response was observed for these variables in RFS.

For the biotic predictors, the density of macroinvertebrates and FFG diversity did not significantly affect decomposition rates, and the interaction of these two biotic variables with the stream type was not significant. For functional feeding groups, shredder, collector-gatherers, and predators were not related to decomposition rates in any stream type or leaf species (Table S4; Fig. 6). Scraper proportion was significantly and negatively related to decomposition rates in the OCS but was not significantly related to the RFS (Table S4; Fig. 6).

## Discussion

Our experiment showed no significant differences between riparian forest streams (RFS) and open canopy streams (OCS) in leaf dry mass remaining after 26 days of exposure to leaf litter. Similar decomposition rates between both stream types were an unexpected result, considering that litter decomposition is affected negatively by riparian loss in other subtropical systems (Casotti et al. 2015; Tanaka et al. 2015; Silva-Araujo et al. 2020). However, some abiotic and biotic predictors had significant, albeit differential, effects on the decomposition process depending on the presence of riparian forest.

Leaf species identity was more important in determining differences between decomposition rates than stream types (Table 2). All leaves lost less than 50% of their initial biomass. During the early stages of the decomposition process, leaf physicochemical characteristics may be a more important predictor than the other biotic and abiotic variables involved (Boyero et al. 2017), especially when the leaves are highly recalcitrant (Burwood et al. 2021). *Eucalyptus globulus* leaves are poor in nutrients and contain high concentrations of resins and secondary metabolites (e.g., tannins, phenols), affecting decomposer organisms and ultimately slowing down the decomposition process (Canhoto and Graça 1999; Canhoto and Laranjeira 2007).

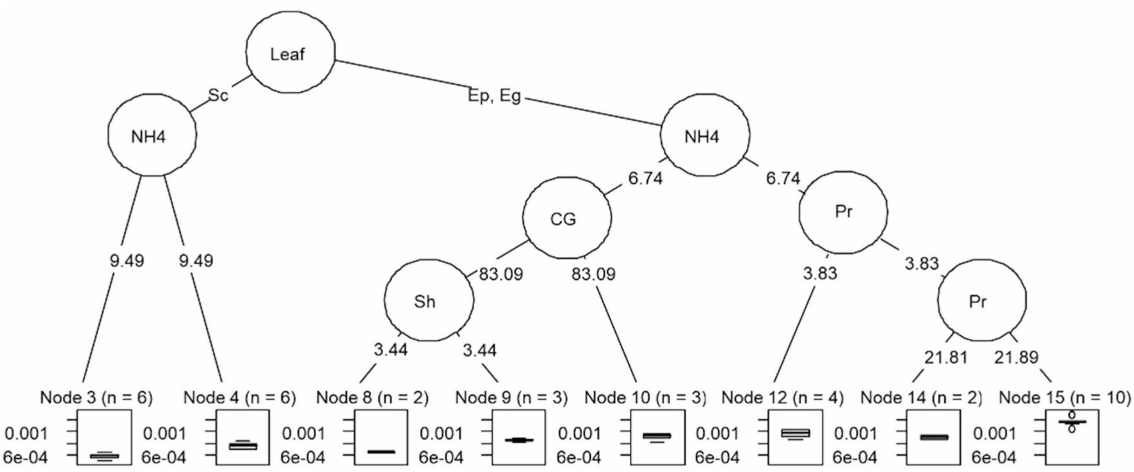

**Fig. 4** Regression tree analysis for decomposition rate of leaf litter based on most important predictors like leaf species (*Eucalyptus globulus* (Eg), *Eryngium pandanifolium* (Ep), and *Schoenoplectus californicus* (Sc)), $NH_4$-N water concentration, and functional feeding groups of macroinvertebrates (collectors-gatherers (CG), shredders (Sh), and predators (Pr))

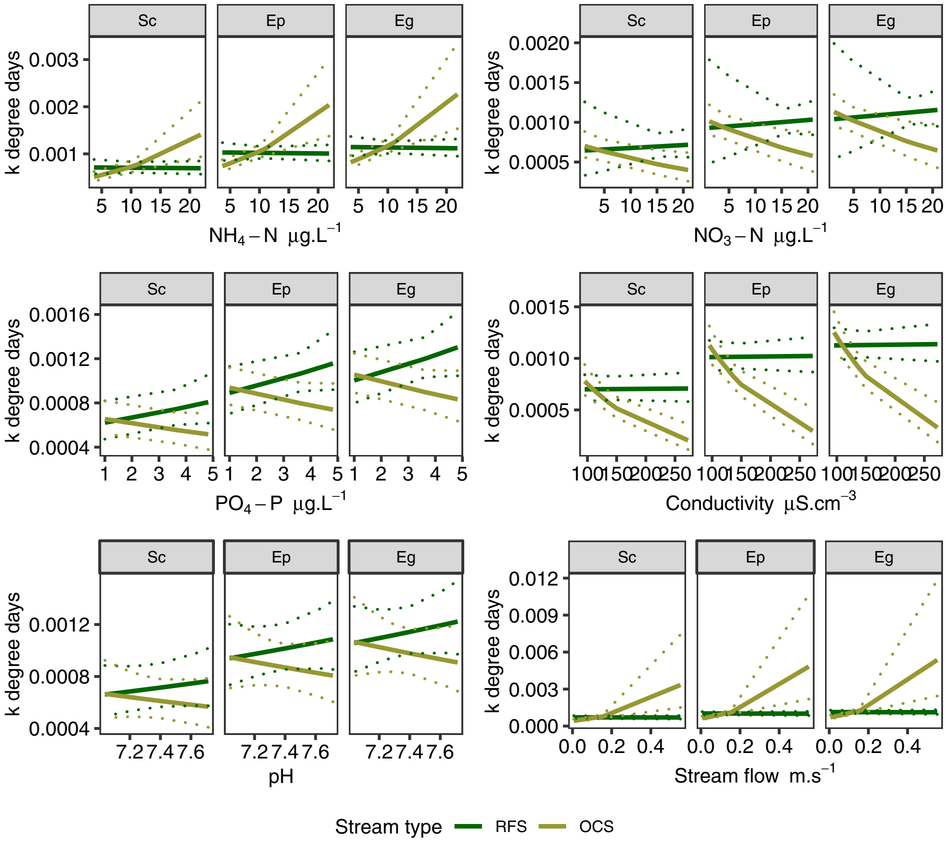

**Fig. 5** Beta-generalized linear models for predicting the joint effects of the presence of riparian cover (System) and leaf species (*Eucalyptus globulus* (Eg), *Eryngium pandanifolium* (Ep), and *Schoenoplectus californicus* (Sc)) with abiotic and biomass of periphyton (periphyton) variables on decomposition rate (*k degree-days*). Nitrate ($NO_3$-N), ammonium ($NH_4$-N), Phosphate ($PO_4$-P), conductivity (K), streamflow (Q)

*Eryngium pandanifolium* leaves are also poor in nutrients (Burwood 2019) and contain high levels of tannins, essential oils, and other secondary compounds that have antibacterial effects (Erdem et al. 2015). However, both terrestrial exotic and native leaves had similar decomposition rates but higher than aquatic *S. californicus*, which had the lowest decomposition rate due to higher C:N ratios (Arcagni et al. 2013; Burwood et al., 2021). This indicates

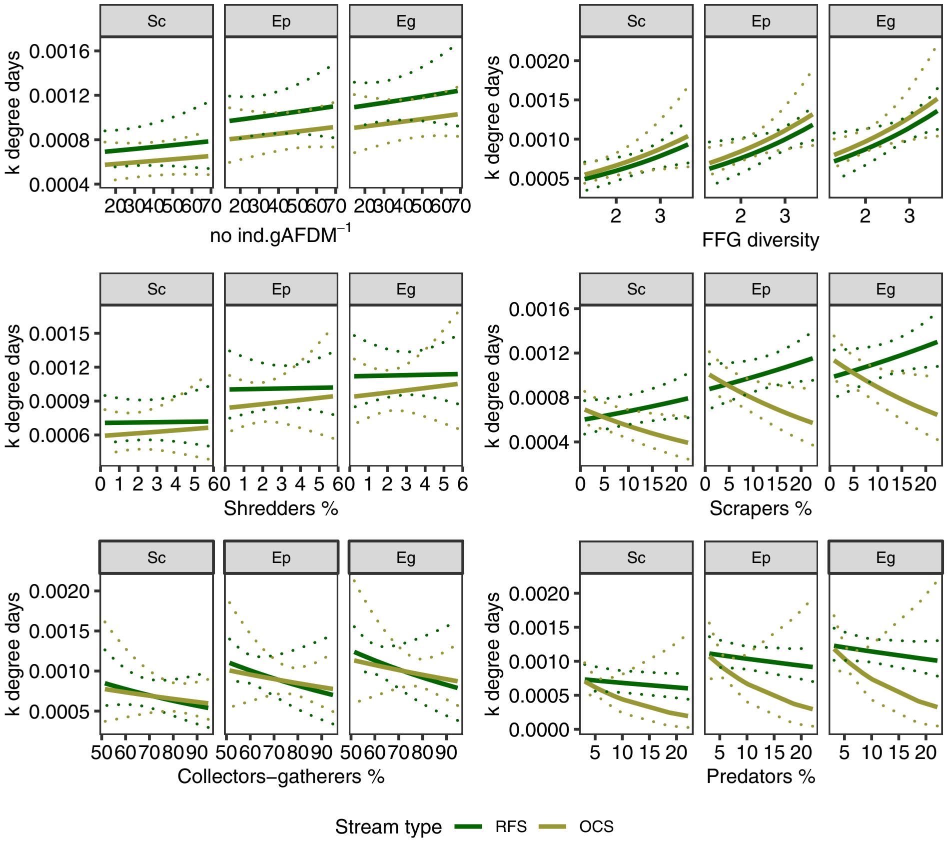

**Fig. 6** Beta-generalized linear models for predicting the joint effects of the presence of riparian cover (System) and leaf species (*Eucalyptus globulus* (Eg), *Eryngium pandanifolium* (Ep), and *Schoenoplectus californicus* (Sc)) with biotic variables on decomposition rate (*k degree-days*). The density of macroinvertebrates (no. ind. gAFDMr.$^{-1}$), Trophic diversity (FFG diversity)

that litter quality and plant identity could be more important in the decomposition process than growth form (tree vs. herbaceous) or exotic status (Kuglerová et al. 2017).

There were no differences in *k*-rates between CM and FM litter bags, and neither its interaction with system nor leaf type, which may indicate that microbial activity, could be more important than macroinvertebrate activity in the decomposition process. However, we could demonstrate the presence of invertebrates inside the FM litter bags, leading to confusing results and questioning the participation of macroinvertebrates in the process. Small invertebrates, or meiofauna, could pass through fine FM litter bags (Wang et al. 2020; Seer et al. 2021) and could feed on the associated biofilms in leaves, indirectly affecting the litter decomposition. In addition, these small invertebrates can influence microbial colonization due to alterations in the microenvironment (e.g., oxygen and organic concentrations) (Bonaglia et al. 2014; Wang et al. 2020). Thus, it is necessary to incorporate other studies and experimental manipulation to assess the role of macroinvertebrates in the decomposition process in our streams (Graça 2001). For instance, mesocosm experiments use electrical invertebrate exclusion (e.g., Moulton et al. 2019) as a substitution for FM litter bags. Besides the biological forces, other factors like physical abrasion also can play an important role in leaf decomposition, and these effects can only be detected in CM litter bags since FM litter bags offer protection of confined leaves against abrasive forces (e.g. water flow; Ferreira et al. 2006; Lecerf 2017). In our case, similar decomposition rates between both FM and CM litter bags suggest that abrasive forces may have poor effects on decomposition compared to other factors. Omoniyi et al., (2021) found that physical forces mediating the decomposition rates were the same for both litter bags, and these abiotic factors only explain less than 20% of the total effect, being the biotic factors the main drivers of litter decomposition. However, we found positive effects of streamflow (Q) on *k-rates,* possibly related to an increase in physical abrasion (Ferreira et al. 2006), and this effect was stronger in the OCS than in the RFS system. This may be explained by the homogenization of local conditions in OCS such as the dominance of fine sediment in the streambed, which led to greater leaf scouring and abrasion. In contrast, in the RFS system, no significant effects could be related to the presence of tree roots and more heterogeneous substrates creating leaf retention zones (Tonin et al. 2021), thus decreasing the leaf abrasion process.

Differences between RFS and OCS in the effects of some abiotic predictors on the decomposition process were significant. The presence of riparian vegetation in RFS contributed to improving some habitat conditions, such as lower temperature and more oxygenated waters, but conductivity and nutrient water concentrations were higher than those in OCS. This could indicate that riparian forests have a limited capacity to retain nutrients and sediment inputs from livestock practices (Suga and Tanaka 2013; Sargac et al. 2021). However, nutrient concentrations in water, such as $NO_3$-N, had no significant effects on the decomposition rates in both stream types, but for OCS, the effects of $NH_4$-N were positive but for $PO_4$-P were negative, which indicates that the decomposition rates in OCS could be readily affected by $PO_4$-P enrichment. These results could be confusing considering that water nutrient concentrations remain low for both RFS and OCS (Table S1) compared to other Uruguayan streams with different land uses (Chalar et al. 2011).

On the other hand, the ammonium concentration at which the decomposition rate is favored depends on the litter species. For *S. californicus*, $NH_4$-N concentrations greater than 9.49 µg. $L^{-1}$ are required for higher decomposition rates (Fig. 4), and for both terrestrial leaf litters, decomposition rates increase with concentrations less than or close to 6.73 µg. $L^{-1}$ (Fig. 4). Therefore, the effects of the water nutrient concentration on the decomposition process depend on the leaf nutrient composition (Costello et al. 2022). For more recalcitrant leaves with low initial nutrient concentrations, the effects of water nutrient concentration could be relatively important (Manning et al. 2021). In this respect, it is necessary to formulate hypotheses concerning the mechanisms by which the presence of riparian cover influences the response of decomposition rates considering working in the context of streams with relatively low nutrient concentrations in water. It is also necessary to assess the nutrient concentrations that may stimulate the activity of decomposer organisms in both types of systems (Woodward et al., 2012; Sena et al. 2021).

High water conductivity values have been associated with the suppression of microbial activity (Usher et al. 2020) and have also been linked to the decreased reproductive activity of hyphomycetes at the beginning of leaf decomposition (Sales et al. 2014; Breda et al. 2021). These effects may be occurring strongly in OCS, but no significant effects were evident for RFS, which could be related to the interaction of conductivity with other factors that may affect microbial activity (e.g., dissolved oxygen and nutrients; Breda et al. 2021).

The presence of riparian cover had important effects on the macroinvertebrate composition (Fig. 3), as documented in other studies (Suga and Tanaka 2013; Silva-Araujo et al. 2020; Turunen et al. 2021; Sargac et al. 2021). However, the effect of the stream type was not observed for macroinvertebrate density, FFG diversity, and functional feeding groups, except for the proportions of collector-gatherer groups, which tended to be higher in the OCS system than in the RFS system. Shredders were only associated with decomposition rates when all data were analyzed with no consideration of stream types (Fig. 4). This association was detected only for less recalcitrant terrestrial leaves (*E. globulus* and *E. pandanifolium*) and could indicate that, despite the low abundance of shredders, they may play an important role in leaf litter decomposition. However, there was no significant response of decomposition rates to the proportion of shredders and their interaction with the RFS and OCS (Fig. 6). Shredders in lower latitudes usually expand their leaf litter diet with other types of resources to supplement nutrient deficiencies due to the recalcitrancy of leaf litter in these regions (Tonin et al. 2018). In the RFS, shredders were represented mainly by *Aegla* crabs, and feeding diet experiments (e.g., Colpo et al. 2012) revealed that they fed on leaf litter but also on algae and chironomid larvae, expanding their trophic niche. *Nectopsyche* was highly represented in the OCS, but it is usually classified as a shredder-herbivore and collector-gatherer (Holzenthal 1995).

Similarly, when whole data were analyzed with no consideration of stream types, collector-gatherers and predators were associated with higher *k*-rates in native and exotic terrestrial leaves, suggesting that these invertebrates enhance leaf fragmentation (Fig. 4). The interaction of these groups with stream type was not significant in the models (Fig. 6). Collectors-gatherers were the most abundant group, and their relationship with decomposition rates in both *E. globulus* and *E. pandanifolium* leaves could be related to the apparent use of litter as a microhabitat providing shelter and food supply in the form of fine particulate organic matter (Chauvet et al. 1993; Ligeiro et al. 2010; Biasi et al. 2013). Predators may impair detritivore activity through top-down effects, which affect the decomposition process (Seer et al. 2021). However, in this case, higher relative proportions of predators were associated with higher decomposition rates (Fig. 5). This relationship is not necessarily causality, and predators (most of them Tanypodinae) may just feed on other functional groups, such as CG, due to their higher abundance concerning the shredders.

Scraper activity on biofilms on detrital surfaces enhances decomposition (Tonin et al. 2018; Seer et al. 2021), but in our case, scrapers had lower proportions and a negative effect on *k-rates* in the OCS than in the RFS. In OCS, there was a higher proportion of chlorophyte algae (Fig. S1) which may be more preferred by scrapers mainly represented by small Trichoptera (*Mortoniella, Neotrichia*) and *Psephenus* (Table 3). These scrapers are typically found feeding on biofilms, and they may prefer to feed on highly abundant and nutritious autochthonous carbon subsidies like chlorophyte algae instead of litter biofilms (Lau et al. 2009; Guo et al. 2016); therefore, their low occurrence in litter bags was possibly due to the use of litter as shelter. Scrapers in the RFS system were represented by *Farrodes*, and a lower supply of autochthonous subsidies can promote higher consumption of leaf biofilm, contributing indirectly to the decomposition of plant tissue (Tonin et al. 2018; Seer et al. 2021). In this regard, experimental procedures are needed to assess the role of the macroinvertebrates functional feeding groups in the decomposition process. For example, experiments in microcosms determine the feeding preference and behavior (growth rate) of primary consumers when allochthonous resources are supplied (Graça 2001; Sena et al. 2020, 2021). It is also necessary to use stable isotope techniques to analyze the importance of litter in the biomass of these organisms in both the RFS and the OCS systems (Neres-Lima et al. 2016). The importance of both allochthonous and autochthonous pathways in Uruguay has been explored in large rivers (González-Bergonzoni et al. 2019) where the relevance of allochthonous carbon subsidy occurs after flood pulses. In the case of small streams, a similar response to larger rivers is expected, however, autochthonous carbon may be more important as a resource, even in shading streams where algae growth is limited. In general, at lower latitudes, higher temperatures and light availability allow for sustaining considerable biomass of autochthonous production (Neres-Lima et al. 2016). However, the increase of autochthonous algae may affect the colonization of decomposer microorganisms, and consequently the decomposition rates (Tonin et al. 2018). Especially in higher light and nutrient concentrations (Halvorson et al. 2016). In our case, the higher light entry in OCS promoted higher periphyton biomass than RFS. However, with the increase of nutrient concentrations ($PO_4$-P and $NO_3$-N), decomposition rates tended to decrease, indicating possible restrictive effects of biofilm growth on colonization by decomposer microorganisms. Therefore, it is also necessary to test the interaction between the biofilm and the decomposer microorganisms in both OCS and RFS with different nutrient concentrations (Halvorson et al. 2016).

Even though most of the riparian forest in small streams of Uruguay is restricted to small patches, the decomposition process in stream ecosystems is indirectly regulated by its presence, which indicates that riparian cover can act as a buffer area for the variations in the physical, chemical, and biological variables that modulate this process (Turunen et al. 2021). This study showed relationships among variables that mostly affect the decomposition process, and the consistency of the models indicate that the direction and magnitude of the effects of these variables depend on the presence of riparian cover. However, the effects of some predictors are not well understood. This makes it necessary to establish the mechanisms by which the decomposition rate responds differentially to abiotic and biotic variables according to the presence of riparian forest cover. Thus, we can better understand how different variables modulate leaf litter decomposition in subtropical streams with different riparian zone conditions in the context of streams with low nutrient concentrations in water. On the other hand, different responses of decomposition rates to abiotic and biotic predictors between OCS and RFS, suggest a compensation mechanism of the stream system which ensures the stability of the decomposition process. This means that, while some factors negatively affected the *k-rates* in OCS (e.g., $NO_3$-N, $PO_4$-P), other variables promoted the decomposition in this system (e.g., $NH_4$-N, stream flow). Leaf decomposition is much more affected by changes in leaf identity, suggesting that riparian changes accompanied by changes in leaf inputs may strongly affect the processing capacity of allochthonous organic matter and thus the metabolism of streams. In this sense, the decomposition process seems to be more affected by leaf inputs than abiotic or biotic variables.

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**Data availability** Upon a reasonable request, the data that supported our results are available from the corresponding authors.

**Code availability** Upon a reasonable request, the code used to analyze the data that supported our results is available from the corresponding authors.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** No approval of ethics committees was required to accomplish the goals of this study because research work was conducted with unregulated invertebrate taxa.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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# Riparian cover buffers the effects of abiotic and biotic predictors of leaf decomposition in subtropical streams.

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## Supplementary Information

**Table S1**. Geographical coordinates (latitude and longitude) and mean ± standard deviation of physicochemical and habitat variables of each stream in riparian forest stream (RFS) and open canopy stream (OCS) systems. K: conductivity, DO: dissolved oxygen, TN: total nitrogen, TDN: total dissolved nitrogen, NO$_3$-N: nitrate; NH$_4$-N: ammonium; TP: total phosphorous; TDP: total dissolve phosphorous; PO$_4$-P: Orthophosphate; Q: streamflow.

| **System** | **RFS** | | | **OCS** | | |
|---|---|---|---|---|---|---|
| **Stream** | **RFS-1** | **RFS-2** | **RFS-3** | **OCS-1** | **OCS-2** | **OCS-3** |
| Latitude | 34°41'12.15"S | 34°31'28.49"S | 34°34'24.40"S | 34°37'46.89"S | 34°38'11.36"S | 34°39'4.02"S |
| Longitude | 55°0'1.21"O | 55°6'6.92"O | 55°4'9.89"O | 55°3'8.72"O | 54°59'22.95"O | 54°59'56.48"O |
| Temperature (°C) | 16.2±1.3 | 16.36±1.2 | 18.2±1.4 | 17.2±1.2 | 21.9±2.3 | 21.8±1.9 |
| K (µs/cm) | 272.4±45.8 | 136±34.4 | 108.6±22.4 | 95.2±25.5 | 146.6±41.2 | 150.2±33.8 |
| pH | 7.6±0.6 | 7.4±0.3 | 7.0±0.4 | 7.3±0.3 | 7.7±0.3 | 7.2±0.4 |
| DO (mg. L$^{-1}$) | 10.4±0.4 | 10.2±0.4 | 9.9±1.4 | 9.7±0.5 | 9.3±0.6 | 9.4±0.4 |
| TN (µg. L$^{-1}$) | 226.7±62.67 | 214.6±74.5 | 198.6±71.7 | 123.7±17.3 | 175.8±39.8 | 217.3±52.1 |
| TDN (µg. L$^{-1}$) | 187.9±55.0 | 177.2±43.2 | 175.8±42.8 | 91.6±37.7 | 122.3±36.6 | 171.8±68.0 |
| NO$_3$-N (µg. L$^{-1}$) | 20.9±44.5 | 16.3±25.4 | 14.6±18.7 | 1.3±0.6 | 4.5±7.9 | 14.6±29.7 |
| NH$_4$-N (µg. L$^{-1}$) | 21.9±44.5 | 9.8±11.1 | 9.2±12.1 | 11.2±18.7 | 4.3±7.8 | 3.7±5.1 |
| TP (µg. L$^{-1}$) | 18.4±3.0 | 17.07±7.3 | 29.4±9.9 | 21.7±6.0 | 18.7±3.8 | 19.2±2.9 |
| TDP (µg. L$^{-1}$) | 16.2±3.3 | 14.4±9.7 | 23.5±8.9 | 15.5±6.8 | 16.4±2.4 | 14.8±6.1 |
| PO$_4$-P (µg. L$^{-1}$) | 3.7±5.7 | 3.6±5.8 | 1±0 | 1±0 | 1±0 | 4.8±8.6 |
| Q (m$^3$. s$^{-1}$) | 0.55±0.5 | 0.04±0.02 | 0.01±0.01 | 0.16±0.1 | 0.05±0.05 | 0.05±0.07 |
| Total Periphyton (µg cm$^{-2}$) | 3.2±2.8 | 1.5±1.4 | 0.3±0.5 | 2.01±4.2 | 0.8±0.5 | 0.8±0.7 |
| Chlorophyte (µg cm$^{-2}$) | 0.006±0.02 | 0.02±0.03 | 0.03±0.03 | 0.3±0.6 | 0.2±0.15 | 0.22±0.3 |
| Diatom (µg cm$^{-2}$) | 2.2±1.9 | 1.0±1.0 | 0.15±0.16 | 1.0±2.3 | 0.34±0.17 | 0.27±0.11 |
| Cyanobacteria (µg cm$^{-2}$) | 1.0±0.9 | 0.45±0.4 | 0.11±0.3 | 0.76±1.22 | 0.21±0.15 | 0.27±0.3 |

**Table S2**. Decomposition rates and % mass loss after 26 days of exposure of *S. californicus* (Sc), *E. pandanifolium* (Ep), and *E. globulus* (Eg) in riparian forest stream (RFS) and open canopy stream (OCS).

| **System** | **Leaf species** | **Stream** | ***k-days*** | | ***k-degree-days*** | | **% Mass loss** | |
|---|---|---|---|---|---|---|---|---|
| | | | **CM** | **FM** | **CM** | **FM** | **CM** | **FM** |
| RFS | Sc | RFS-1 | 0.0125 | 0.0139 | 0.0007 | 0.0008 | 31.75 | 33.31 |
| | | RFS-2 | 0.0131 | 0.0137 | 0.0008 | 0.0008 | 34.53 | 32.55 |
| | | RFS-3 | 0.0120 | 0.0100 | 0.0007 | 0.0005 | 31.84 | 27.51 |
| OCS | | OCS-1 | 0.0133 | 0.0140 | 0.0007 | 0.0007 | 34.21 | 35.48 |
| | | OCS-2 | 0.0103 | 0.0119 | 0.0005 | 0.0006 | 31.54 | 30.87 |
| | | OCS-3 | 0.0123 | 0.0123 | 0.0006 | 0.0006 | 31.43 | 31.43 |
| RFS | Ep | RFS-1 | 0.0163 | 0.0176 | 0.0009 | 0.0010 | 37.66 | 40.69 |
| | | RFS-2 | 0.0192 | 0.0144 | 0.0012 | 0.0009 | 40.35 | 36.75 |
| | | RFS-3 | 0.0179 | 0.0203 | 0.0010 | 0.0011 | 40.74 | 42.78 |
| OCS | | OCS-1 | 0.0223 | 0.0196 | 0.0012 | 0.0010 | 45.22 | 43.35 |
| | | OCS-2 | 0.0135 | 0.0193 | 0.0007 | 0.0010 | 32.02 | 43.76 |
| | | OCS-3 | 0.0130 | 0.0176 | 0.0006 | 0.0009 | 33.82 | 41.32 |
| RFS | Eg | RFS-1 | 0.0200 | 0.0205 | 0.0012 | 0.0012 | 43.19 | 45.14 |
| | | RFS-2 | 0.0210 | 0.0186 | 0.0013 | 0.0011 | 41.47 | 43.67 |
| | | RFS-3 | 0.0155 | 0.0172 | 0.0009 | 0.0009 | 37.78 | 40.57 |
| OCS | | OCS-1 | 0.0239 | 0.0220 | 0.0013 | 0.0011 | 45.62 | 48.44 |
| | | OCS-2 | 0.0186 | 0.0166 | 0.0009 | 0.0008 | 40.23 | 39.98 |
| | | OCS-3 | 0.0173 | 0.0167 | 0.0009 | 0.0008 | 38.90 | 39.37 |

**Table S3.** Linear mixed models for testing differences between leaf species and stream type in macroinvertebrate density (no. ind. g $\text{AFDMr}^{-1}$), Shannon diversity of functional feeding groups (eFFG diversity), and the proportion of macroinvertebrate functional feeding groups (%): shredders, scrapers, collectors-gatherers, filters, predators.

| Macroinvertebrates | Factors | numDF | denDF | F-value | p-value | |
|---|---|---|---|---|---|---|
| **No. ind. g $\text{AFDMr}^{-1}$** | (Intercept) | 1 | 8 | 40.168 | <0.001 | *** |
| R2 marginal= 0.35 | Leaf | 2 | 8 | 1.731 | 0.237 | |
| R2 conditional= 0.86 | System | 1 | 4 | 2.935 | 0.162 | |
| | Leaf: System | 2 | 8 | 2.089 | 0.186 | |
| **eFFG diversity** | (Intercept) | 1 | 8 | 237.06 | <.0001 | *** |
| R2 marginal= 0.56 | Leaf | 2 | 8 | 6.70 | 0.019 | * |
| R2 conditional= 0.93 | System | 1 | 4 | 8.32 | 0.045 | . |
| | Leaf: System | 2 | 8 | 0.37 | 0.703 | |
| **Shredders (%)** | (Intercept) | 1 | 8 | 14.32 | 0.005 | ** |
| R2 marginal= 0.13 | Leaf | 2 | 8 | 1.482 | 0.284 | |
| R2 conditional= 0.66 | System | 1 | 4 | 0.494 | 0.521 | |
| | Leaf: System | 2 | 8 | 0.490 | 0.630 | |
| **Scrapers (%)** | (Intercept) | 1 | 8 | 9.302 | 0.016 | * |
| R2 marginal= 0.15 | Leaf | 2 | 8 | 5.369 | 0.033 | * |
| R2 conditional= 0.94 | System | 1 | 4 | 0.819 | 0.417 | |
| | Leaf: System | 2 | 8 | 0.297 | 0.751 | |
| **Collectors-Gatherers (%)** | (Intercept) | 1 | 8 | 943.37 | <0.001 | *** |
| R2 marginal= 0.64 | Leaf | 2 | 8 | 7.007 | 0.017 | * |
| R2 conditional= 0.80 | System | 1 | 4 | 11.421 | 0.028 | * |
| | Leaf: System | 2 | 8 | 0.921 | 0.437 | |
| **Filters (%)** | (Intercept) | 1 | 8 | 4.857 | 0.059 | . |
| R2 marginal= 0.14 | Leaf | 2 | 8 | 0.548 | 0.598 | |
| R2 conditional= 0.92 | System | 1 | 4 | 0.904 | 0.396 | |
| | Leaf: System | 2 | 8 | 0.360 | 0.709 | |
| **Log(Predators (%))** | (Intercept) | 1 | 8 | 79.54 | <0.001 | *** |
| R2 marginal= 0.19 | Leaf | 2 | 8 | 0.345 | 0.718 | |
| R2 conditional= 0.83 | System | 1 | 4 | 1.258 | 0.325 | |
| | Leaf: System | 2 | 8 | 1.288 | 0.327 | |

**Table S4**. Best models resulted from the model selection procedure in Log-Normal- generalized linear models for predicting the joint effects of the presence of riparian cover (Stream type: riparian forest stream (RFS) and open canopy stream (OCS)) and leaf species (*S. californicus* (Sc), *E. pandanifolium* (Ep), and *E. globulus* (Eg)) with abiotic and biotic variables on decomposition rate (k-dd: *k-degree-days*). Nitrate ($NO_3$-N), ammonium ($NH_4$-N), phosphate ($PO_4$-P), conductivity, pH, stream flow (Q), macroinvertebrate density (Density; no. ind. g $AFDMr^{-1}$), functional feeding groups diversity (FFG diversity), and the proportion of FFG: shredders (Sh), scrapers (Sc), collectors- gatherers (CG), and predators (Pr).

| **Mesh bags sizes** | | **CM** | | **FM** | |
|---|---|---|---|---|---|
| **Model** | **Predictors** | **Estimate** | ***P*** | **Estimate** | ***P*** |
| **k-dd~ $NO_3$-N*Stream+Leaf** | Intercept | -7.35 | <0.001 | -7.51 | <0.001 |
| CM: Pseudo-r2= 71%; P<0.001*** | $NO_3$-N | 0.01 | 0.795 | 0.02 | 0.297 |
| FM: Pseudo-r2= 76%; P<0.001*** | Stream (OCS) | 0.13 | 0.732 | 0.72 | 0.326 |
| | Leaf (Ep) | 0.37 | 0.009 | 0.36 | <0.001 |
| | Leaf (Eg) | 0.48 | 0.001 | 0.39 | <0.001 |
| | $NO_3$-N * Stream (OCS) | -0.03 | 0.176 | -0.03 | 0.084 |
| **k-dd~ $NH_4$-N*Stream+Leaf** | Intercept | -7.24 | <0.001 | -7.33 | <0.001 |
| CM: Pseudo-r2= 82%; P<0.001*** | $NH_4$-N | 0 | 0.867 | 0.01 | 0.282 |
| FM: Pseudo-r2= 80%; P<0.001*** | System (OCS) | -0.54 | 0.005 | -0.21 | 0.13 |
| | Leaf (Ep) | 0.37 | 0.002 | 0.35 | <0.001 |
| | Leaf (Eg) | 0.48 | <0.001 | 0.39 | <0.001 |
| | $NH_4$-N * Stream (OCS) | 0.06 | 0.003 | 0.02 | 0.093 |
| **k-dd~ $PO_4$-P*Stream+Leaf** | Intercept | -7.45 | <0.001 | -7.32 | <0.001 |
| CM: Pseudo-r2= 70%; P<0.001*** | $PO_4$-P | 0.07 | 0.173 | 0.03 | 0.412 |
| FM: Pseudo-r2= 72%; P<0.001*** | Stream (OCS) | 0.18 | 0.331 | 0.06 | 0.652 |
| | Leaf (Ep) | 0.36 | 0.011 | 0.35 | 0.002 |
| | Leaf (Eg) | 0.48 | 0.001 | 0.38 | <0.001 |
| | $PO_4$-P * Stream (OCS) | -0.13 | 0.05 | -0.07 | 0.157 |
| **k-dd~ Conductivity*Stream+Leaf** | Intercept | -7.27 | <0.001 | -7.34 | <0.001 |
| CM: Pseudo-r2= 82%; P<0.001*** | Conductivity | 0 | 0.919 | 0 | 0.264 |
| FM: Pseudo-r2= 80%; P<0.001*** | Stream (OCS) | 0.83 | 0.006 | 0.52 | 0.032 |
| | Leaf (Ep) | 0.37 | 0.002 | 0.35 | <0.001 |
| | Leaf (Eg) | 0.48 | <0.001 | 0.39 | <0.001 |
| | Conductivity * Stream (OCS) | -0.01 | 0.002 | -0.005 | 0.012 |
| **k-dd~ pH*Stream+Leaf** | Intercept | -8.82 | <0.001 | -8.52 | <0.001 |
| CM: Pseudo-r2= 61%; P=0.004** | pH | 0.21 | 0.423 | 0.18 | 0.365 |
| FM: Pseudo-r2= 69%; P<0.001*** | Stream (OCS) | 3.18 | 0.323 | 1.79 | 0.42 |
| | Leaf (Ep) | 0.35 | 0.024 | 0.35 | 0.003 |
| | Leaf (Eg) | 0.47 | 0.004 | 0.38 | 0.001 |
| | pH* Stream (OCS) | -0.45 | 0.301 | -0.26 | 0.393 |
| **k-dd~ Q*Stream+Leaf** | Intercept | -7.26 | <0.001 | -7.27 | <0.001 |
| CM: Pseudo-r2= 82%; P<0.001*** | Q | -0.03 | 0.887 | 0.16 | 0.281 |
| FM: Pseudo-r2= 80%; P<0.001*** | Stream (OCS) | -0.48 | 0.002 | -0.25 | 0.024 |
| | Leaf (Ep) | 0.37 | 0.002 | 0.35 | <0.001 |
| | Leaf (Eg) | 0.47 | <0.001 | 0.39 | <0.001 |
| | Q* Stream (OCS) | 3.71 | 0.002 | 1.82 | 0.033 |
| **k-dd~ Density+Stream+Leaf** | Intercept | -7.31 | <0.001 | -7.31 | <0.001 |
| CM: Pseudo-r2= 59%; P=0.003** | Density | 0 | 0.497 | 0 | 0.196 |
| FM: Pseudo-r2= 70%; P<0.001*** | Stream (OCS) | -0.19 | 0.126 | -0.11 | 0.1 |
| | Leaf (Ep) | 0.34 | 0.03 | 0.34 | 0.002 |
| | Leaf (Eg) | 0.46 | 0.004 | 0.4 | <0.001 |
| **k-dd~ FFG diversity+Stream+Leaf** | Intercept | -7.97 | <0.001 | -7.28 | <0.001 |
| CM: Pseudo-r2= 70.5%; P<0.001*** | FFG diversity | 0.27 | 0.032 | -0.02 | 0.771 |
| FM: Pseudo-r2= 66%; P<0.001*** | Stream (OCS) | 0.11 | 0.436 | -0.11 | 0.16 |
| | Leaf (Ep) | 0.24 | 0.082 | 0.37 | 0.003 |
| | Leaf (Eg) | 0.38 | 0.007 | 0.4 | 0.003 |
| **k-dd~ Sh*Stream+Leaf** | Intercept | -7.25 | <0.001 | -7.23 | <0.001 |
| CM: Pseudo-r2= 57.6%; P=0.009** | Shredder | 0 | 0.939 | 0 | 0.84 |
| FM: Pseudo-r2= 66%; P<0.001*** | Stream (OCS) | -0.18 | 0.39 | -0.1 | 0.201 |
| | Leaf (Ep) | 0.35 | 0.035 | 0.36 | 0.002 |
| | Leaf (Eg) | 0.46 | 0.007 | 0.39 | 0.001 |
| | Shredder*Stream (OCS) | 0.02 | 0.818 | | |
| **k-dd~ Sc*Stream+Leaf** | Intercept | -7.42 | <0.001 | -7.24 | <0.001 |
| CM: Pseudo-r2= 74.6%; P<0.001*** | Scraper | 0.01 | 0.109 | 0 | 0.918 |
| FM: Pseudo-r2= 66%; P<0.001*** | Stream (OCS) | 0.17 | 0.233 | -0.1 | 0.157 |
| | Leaf (Ep) | 0.38 | 0.006 | 0.36 | 0.002 |
| | Leaf (Eg) | 0.5 | <0.001 | 0.38 | 0.002 |
| | Scraper: Stream (OCS) | -0.04 | 0.017 | | |
| **k-dd~ CG*Stream+Leaf** | Intercept | -6.54 | <0.001 | -7.27 | <0.001 |
| CM: Pseudo-r2= 60.9%; P=0.005** | CG | -0.01 | 0.326 | 0 | 0.821 |
| FM: Pseudo-r2= 67.3%; P=0.001** | Stream (OCS) | -0.32 | 0.762 | 0.33 | 0.621 |
| | Leaf (Ep) | 0.26 | 0.135 | 0.37 | 0.004 |
| | Leaf (Eg) | 0.38 | 0.032 | 0.38 | 0.004 |
| | CG: Stream (OCS) | 0 | 0.761 | -0.01 | 0.511 |
| **k-dd~ Pr*Stream+Leaf** | Intercept | -7.19 | <0.001 | -7.21 | <0.001 |
| CM: Pseudo-r2= 66%; P=0.002** | Pr | -0.01 | 0.265 | 0 | 0.694 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| FM: Pseudo-r2= 73%; P<0.001** | Stream (OCS) | 0.14 | 0.707 | 0.08 | 0.599 |
|  | Leaf (Ep) | 0.41 | 0.01 | 0.34 | 0.002 |
|  | Leaf (Eg) | 0.52 | 0.002 | 0.39 | 0.002 |
|  | Pr: Stream (OCS) | -0.06 | 0.355 | -0.03 | 0.167 |

*P values significant:* *: <0.05; **: 0.01; ***: <0.001

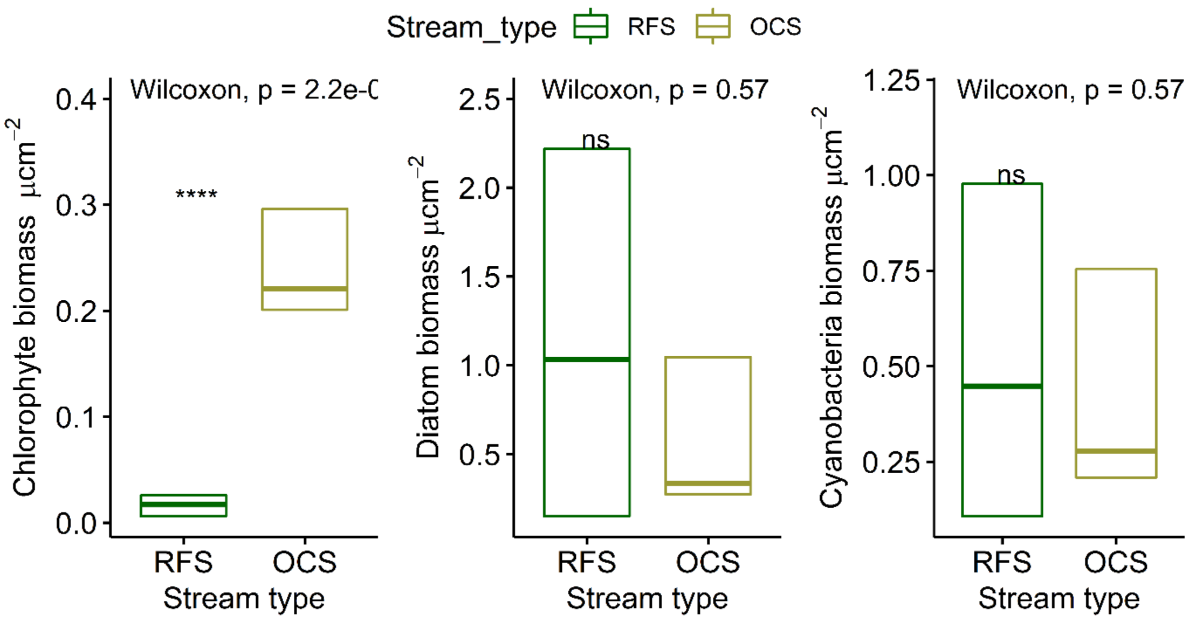

**Fig. S1**. Comparison of biomass of the components of periphyton (Chlorophytes, Diatoms, and Cyanobacteria) between stream types. Asterisk (*) indicate significant differences in the biomass of periphyton components between stream types (Wilcoxon test).

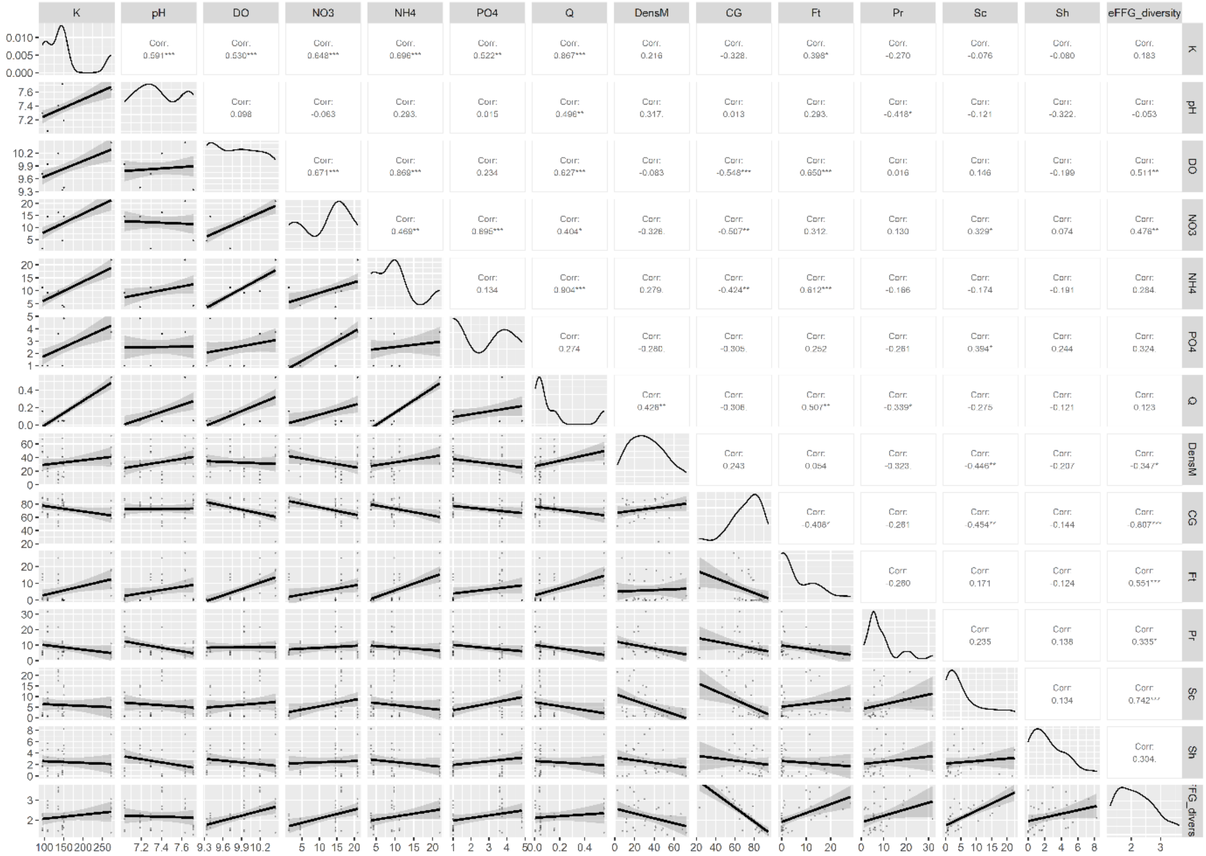

**Fig. S2**. Spearman correlations among abiotic and biotic predictors used in regression tree analyses. Variables selected for final analyses were based on high correlation ($-0.7<P<0.7$) like NH$_4$-N and PO$_4$-P, macroinvertebrate density (DensM), collectors-gatherers (CG), filters (Ft), shredders (Sh), scrapers (Sc), and predators (Pr).

# CHAPTER V: General Discussion

Our findings demonstrated that different approaches may address the effects of anthropogenic stressors on stream ecological conditions at different scales of impact. In this case, the effects span from larger spatial- basin scale associated with changes in land use, such as the afforestation of *Eucalyptus* spp., to effects at the reach scale, such as the removal of riparian forests. This thesis shows that studying macroinvertebrate communities, such as their taxonomic structure and the roles they play in ecosystems (for example, breaking down organic matter), along with environmental drivers, can help us figure out how these stressors caused by humans affect ecosystem structure and function.

By using the taxonomic structure and metrics of macroinvertebrate communities as bioindicators, in combination with water quality parameters (Chapter II), we identified the adverse effects of *Eucalyptus* afforestation in subtropical lowland streams in Uruguay. Although the stream sites varied greatly regarding geomorphological zones and the management strategies used in *Eucalyptus* plantations (such as fertilisation, pesticide use, and harvesting), we observed significant changes in both biotic and abiotic indicators. Nevertheless, the impact of these effects (whether negative, positive, or non-responsive) varies in certain instances based on the season (summer or winter), particularly regarding $NO_3$-N and $NH_4$-N water concentrations, conductivity, and suspended solids, ETP families, and collector-gatherer proportions.

Regarding the possible explanations for the observed patterns of water nutrient concentration responses in this study, we have considered the possibility that many mechanisms may be at play. In other geographical regions, such as Mediterranean streams, where *Eucalyptus* plantations are present, the rise in nutrient concentrations can be attributed to the large amount of *Eucalyptus* leaves input into the streams. This leads to higher microbial respiration and faster decomposition of the leaves, resulting in increased nutrient concentrations in the water (Canhoto et al., 2013; Manning et al., 2021). Although our analysis of the leaf litter benthic stock (Chapter III) did not reveal a substantial amount of *Eucalyptus* leaves, other studies conducted in the country have shown an increase in dissolved organic carbon (DOC) in soils that are heavily afforested (González-Sosa et al., 2024). This DOC may be entering the water bodies (Duan & Kaushal, 2013) affecting water quality mainly by lowering pH and base cation concentrations (Farley et al., 2008;

Tesón et al., 2014). This may occur by the increase of organic acid inputs through *Eucalyptus* litter decomposition in terrestrial ecosystems and root exudation promoting soil and stream water acidification as they leach into the waterways (Farley et al., 2008). Additionally, forest management practices can negatively impact water quality. In this case, clear-cut harvesting and subsequent management operations in *Eucalyptus* plantations affects the concentration and export of nutrients and suspended solids in subtropical streams (Rodrigues et al., 2019). However, changes in water nutrient concentrations during these management practices also depend on precipitations and water yield that may allow the transport of nutrients and sediments from the plantations to the streams (Rodrigues et al., 2019). The decrease in stream water volume, which is a consequence of the high evapotranspiration of *Eucalyptus* trees and groundwater removal (Calder, 1992; Allen & Chapman, 2001; Jobbágy et al., 2012; de Barros Ferraz et al., 2019), may also be associated with the increase in water nutrient concentrations, particularly in summer (Manning et al., 2021).

All these possible mechanisms by which nutrient concentrations increase with the afforestation must be tested using different approaches over a continuous temporal scale. For this, it is necessary to understand the roles of hydrology, groundwater recharge, and other processes, such as runoff rates and the dynamic of DOC as drivers involved in water quality. It is also important to consider the tree-growing stage and associated management practices, which are also related to diffuse nutrients, organic matter transport, and siltation (Rodrigues et al., 2019; Shah et al., 2022), to understand better seasonal and supra-annual variability in these impacts on stream ecosystems. Additionally, it is important to note that sites with extensive afforestation usually allow cattle to reach the water body, which could potentially enhance the impacts of *Eucalyptus* by increasing runoff and nutrient and organic matter input. Therefore, testing cattle isolation is necessary to better understand the effects of *Eucalyptus* afforestation.

Concerning the effects on macroinvertebrate metrics, we observed a direct relationship between the afforestation gradient and the changes in macroinvertebrate measurements. Nevertheless, the application of threshold indicator taxa analysis (TITAN) enabled us to determine the specific point at which the macroinvertebrate species respond to changes in their densities in the *Eucalyptus* afforestation gradient or water quality parameters, regardless of whether the changes are gradual or sudden (Baker & King, 2010). These density thresholds could serve as a foundation for establishing newly adjusted tolerance values that could be helpful to effectively biomonitoring stream ecosystems (Campos et al., 2021; Martins et al., 2021).

The taxonomic classification differs among different groups, with some classified at the genus level while others classified at coarser- subfamilies or subclass taxonomic levels. Many of the genera and families included in this study exhibited consistent and substantial responses to the afforestation gradient. Nevertheless, this categorisation may be inadequate for certain taxa, such as the Oligochaeta sub-order. A further study conducted in Uruguay has demonstrated significant variations in the responses of various Oligochaeta genera to organic pollution. Certain species have exhibited high sensitivity to higher levels of pollution (Capurro et al., 2021). A study conducted in Brazil showed that the taxonomic composition of Oligochaeta differs between areas where native forest is replaced by *Eucalyptus* plantations (Guimarães et al., 2024). Nevertheless, the taxonomy of the Oligochaeta at genus level is complex and requires specialised knowledge, which was not available for this thesis.

Other studies in intensive land uses, such as agriculture and urbanisation in Uruguay, allowed for coarser-order-level classification of macroinvertebrates was sufficient to detect the impact of these land uses (Suárez et al., 2024). In this case, a reduced richness per order (typically one genus per order) allowed to show consistent responses to ecological damage, but in our case, we found that a finer taxonomic resolution (in this case at genus level) make difficult to use Sub order or Order level to detect possible impacts. Additionally, in Uruguay, *Eucalyptus* afforestation effects on streams were found to be less harmful than these intensive land uses and could explain why we did not observe a significant change when coarser-level identification was used in some groups. Thus, a higher genus richness within each order may indicate that higher- -genus- resolution provides a better understanding of the impact of stressor effects in our system (Chalar et al., 2011; Arocena et al., 2022).

It was clear that other genera belonging to Zygoptera suborder showed varying responses along the afforestation and water quality gradients. Some genera, like *Mnesarete/Hetaerina*, were sensitive to these changes, while others, like *Acanthagrion*, were more tolerant. This highlights the importance of using a finer taxonomic resolution in diverse communities to accurately detect the impacts of human-caused stressors (de Olivera et al., 2020). We detected seasonal variation in some macroinvertebrate metric responses to afforestation. For instance, the Zygoptera suborder showed a positive response to afforestation in winter but an opposite response in summer. The development of aquatic larval stages in the winter season may explain their density increase during this period (Villalobos-Jiménez et al., 2016). Therefore, we recommend long-term evaluation of macroinvertebrate communities to understand seasonal patterns in the aquatic-terrestrial stages of insect groups. This holds significance as insects comprise most of the macroinvertebrate communities in our study.

Changes in water quality may influence macroinvertebrate metrics and community structure in response to increased afforestation. However, local physical factors, such as the availability of basal resources and the composition of the streambed substrate, can also alter macroinvertebrate communities (Buss et al., 2004). Despite the significant responses of taxonomic macroinvertebrate metrics, functional trait diversity approaches may serve as better predictors. This is because anthropogenic stressors can filter traits that tolerate habitat deterioration or those that can withstand stressful conditions, such as high flows and drier periods (Wolters et al., 2018; Forio et al., 2018). Functional trait-based approaches also reflect important ecological processes such as nutrient and carbon cycling and biomass production, which serve as proxies for ecosystem functioning (Doledec & Statzner, 2010).

In Chapter III, we detected significant responses of macroinvertebrate trait structure and functional diversity to the increase in the afforestation gradient. These responses were mediated by changes in the availability of carbon-basal resources, particularly macrophytes' biomass. These findings suggest that *Eucalyptus* afforestation replacing grassland promotes multifaceted effects on macroinvertebrates, mediated by changes in primary producers. In this case, we found that submerged and emerged (including grass) macrophytes played a significant role in our stream sites, with notable associations with macroinvertebrate traits, especially predator-feeding habits (those that feed on other invertebrates, fish, and tadpoles) and small and medium body sizes. These macrophytes have a more complex structure than other types of macrophytes (like floating), which lets them support more small macroinvertebrates and attract more predators (Wolters et al., 2018; Yofukuji et al., 2021). Consequently, the presence of complex macrophytes with greater biomass can result in a higher macroinvertebrate functional diversity. In addition, the increase of

*Eucalyptus* afforestation may promote bottom-up on macroinvertebrates by altering this basal resource.

Macrophytes not only provide shelter (Ferreiro et al., 2014; Habit & Yousuf, 2015) but also serve as a food source for macroinvertebrates (Mendonça et al., 2013; Paice et al., 2016; Feio et al., 2017; Oeding et al., 2020). Nevertheless, our study did not employ stable isotope techniques to determine if macroinvertebrates were consuming these macrophytes (Finlay, 2001; Phillips et al., 2014). Some macrophyte species, such as *Potamogeton*, may have a high nutritional value (Paice et al., 2016), but other species, like *Myriophylum* may be recalcitrant or contain secondary metabolites (Watson & Barmuta, 2011). To gain a deeper understanding of how these macrophytes function as food sources and provide shelter or support structures, future studies will require the use of stable isotope techniques and experimental methods (Watson & Barmuta, 2011; Paice et al., 2016). Mesocosms or microcosms can offer useful insights into the significance of various macrophyte growth types in prey-predator interactions (Meerhoff et al., 2003; Teixeira de Mello et al., 2016; Wolters et al., 2018; Clemente et al., 2019).

Periphyton biomass was also an important driver in shaping macroinvertebrate functional diversity, particularly in the summer season. However, *Eucalyptus* afforestation only affected periphyton biomass in winter, and this result could be more associated with temperature decreases than *Eucalyptus* effects (Rosemond, 1994). The increased activity of primary producers during warmer seasons likely drives the increased use of periphyton by macroinvertebrates (González-Bergonzoni et al., 2016; 2019). As a result, periphyton may support aquatic trophic webs by providing highly nutritious food compared to other basal resources (Lau et al., 2009). This idea could potentially reinforce the Riverine Productive Model (Thorp and Delong, 1994), as the higher nutritional values of periphyton may make small primary productivity, in terms of biomass, more significant for consumers. Also, the seasonal rise in macroinvertebrate functional diversity caused by periphyton biomass suggests that some feeding traits may be taking advantage of the resources that are available at different times of the year, which could mean that they are omnivorous or generalist. These findings support the idea that omnivory may be a strategy to overcome the high variability of basal resources and reduce intraguild competition (Cortés-Guzmán et al., 2022).

In the case of detritus and contrary to our expectations, we did not find effects mediated by replacing native leaf litter with *Eucalyptus* leaves. Other research shows that *Eucalyptus* leaves may change the communities of macroinvertebrates, especially the detritivorous shredders that eat them. This is because *Eucalyptus* leaves contain a lot of secondary metabolites that are harmful to aquatic organisms (Canhoto & Graça, 1995; Ferreira et al., 2016; 2019). However, the increase in soil impermeability may allow the input of dissolved organic carbon (DOC) and toxic compounds leached from the decomposition of *Eucalyptus* in the terrestrial environment to enter aquatic bodies through runoff (Tesón et al., 2014; González-Sosa et al., 2024). These leachates release humic substances that can impact the primary production of macrophytes, particularly those with submerged and emerging growth forms, due to reduced light irradiance in streams (Reitsema et al., 2018). *Eucalyptus* leaf leachate may also affect macroinvertebrates by reducing pH and dissolved oxygen levels (Canhoto et al., 2007; 2013).

Despite the consistency of *Eucalyptus'* effects on macroinvertebrate functional diversity in both summer and winter seasons, the importance of environmental variables and basal resources (i.e., periphyton) varied. Thus, for long-term monitoring, as we mentioned above, it is essential to also track changes in macroinvertebrate trait structure and functional diversity in response to the effects of *Eucalyptus* afforestation. We have shown that the trait approach provides an alternative mechanistic to traditional taxonomy-based descriptors (Menezes et al., 2010), as it reveals taxa's adaptations and resilience capabilities to environmental changes (Forio et al., 2018). In addition, trait-based approaches reflect important ecological processes such as nutrient and carbon cycling and biomass production, which perform as a proxy for ecosystem functioning (Doledec & Statzner, 2010). Therefore, trait-based approaches may allow a better understanding of how anthropogenic stressors, such as *Eucalyptus* afforestation, filter those traits that tolerate changes in environmental conditions—in this case, local habitat and basal resources—and thus offer better responses than taxonomic diversity (Le Provost et al., 2020; Moi et al., 2023; Paz et al., 2022).

In relation to Chapter IV, after 26 days of exposure to streams, we found no differences in the decomposition rates between open canopy streams (OCS) and riparian cover streams (RCS). During this time (the spring season), the mass loss was between 31-46%, which is higher than other studies in Uruguay. For instance, Burwood (2019), comparing afforested vs. extensive cattle systems, found that the mass loss of the same litter species ranged between 20 and 40% during 56 days of exposure during winter. In this case, the decomposition rates (k-days) were lower, with values ranging from 0.05 to 0.011 for *Eucalyptus grandis* and *Eryngium pandanifolium* compared to ours (ranging from 0.013 to 0.023). We may attribute these differences to our case's higher temperatures, which intensify the metabolic processes of the detritivore microbial and macroinvertebrate communities involved in the process (Tiegs et al., 2019; 2024; Costello et al., 2022).

A global comparison, which also includes Uruguay comparing afforested vs. extensive cattle systems, suggests that the responses to decomposition rates vary across the globe and depend on local abiotic and biotic factors (Ferreira et al., 2019). In Uruguay, they discovered significant differences in litter decomposition rates between streams flowing *Eucalyptus* afforestation and streams flowing natural grassland, but the direction of the responses (negative or positive) differed among the mesh bag sizes. For instance, microbial-mediated litter decomposition increases by 32% (ranged 13 – 54%) in fine mesh bags (FM), but overall microbial and shredder litter decomposition decreases by -36% (ranged -49 – -20%) in coarse mesh bags (CM). This suggests that shredder macroinvertebrates are important drivers of litter decomposition, but they may be affected in streams flowing through *Eucalyptus* plantations (Ferreira et al., 2019).

This thesis found no differences in the decomposition rates of CM bags. Neither the litter decomposition rates between RCS and OCS nor the comparison between *Eucalyptus* afforested systems and extensive cattle systems in Burwood's (2019) study, indicated the importance of shredder macroinvertebrates in this process. However, the global experiment (Ferreira et al., 2019) used a highly labile leaf (*Alnus*), whereas in our study and Burwood's, we used three highly recalcitrant leaves, including *Eucalyptus* leaves. In addition, our experiment did not find significant differences in decomposition rates between CM and FM bags. Thus, it is possible that leaf litter quality may obscure the effects of anthropogenic stressors and the pathways by which abiotic and biotic drivers are important in this process, especially the activity of shredder macroinvertebrates (Boyero et al., 2021). It is also important to understand the role of other functional feeding groups. For instance, we detected a positive correlation between scrapers and decomposition rates in RCS, and an indirect effect may occur via scrapers feeding on the biofilm in the leaf litter. We also recommend using stable isotopes to determine whether the biomass origin of macroinvertebrate consumers originates from leaf litter (Neres-Lima et al., 2017; Marks, 2019; Labed-Veydert et al., 2023).

Our results suggest that leaf litter identity masks the effects of riparian forest loss (or afforestation with *Eucalyptus* in Burwood, 2019) on litter decomposition rates. This is a crucial aspect to consider when evaluating leaf litter decomposition to measure functional stream integrity. This is due that leaf physico-chemical characteristics may play a more significant role in determining litter decomposition than other external drivers. On the other hand, Ferreira et al. (2019) propose that overall microbial and macroinvertebrate-mediated decomposition is more accurate for evaluating anthropogenic stressors' effects on stream ecosystem functioning, and microbial-mediated decomposition should be used when shredders are rare or absent. However, these experiments should use less recalcitrant leaf litter to understand the real participation of shredders in the decomposition process. But this aspect should be considered cautiously, as rapidly decomposing high-quality litter supports microbial rather than macroinvertebrate consumers. This is due to the fact that microorganisms compete with macroinvertebrates for carbon and other elements and may produce defensive compounds that deter macroinvertebrate feeding (Suberkropp 1992, Danger et al. 2016; Marks, 1999).

Additionally, Marks (2019) recommends abandoning the term "quality" for litter, as different microorganisms and macroconsumers may face different resource limitations, and the term "quality" does not account for these differences. In this sense, it would be interesting to move forward with future research focusing on how different litter traits influence not only the decomposition process but also the fates of the remaining organic material through food webs and ecosystems (Marks, 2019). For instance, leaf litter traits (e.g., specific leaf area) may limit microbial and macroinvertebrate colonisation. Thus, leaf litter trait diversity should also be considered when analysing biota-mediated leaf decomposition (Migliorini et al., 2018). In our systems, the remaining patch of riparian forest harbours a significant diversity of tree and shrub species, with approximately 28 species (Barrios *in preparation*). Future decomposition experiments could test these species by framing them in a gradient of physico-chemical characteristics and traits.

Experimenting with a gradient of leaf litter's physicochemical characteristics and traits could be an interesting way to test the effects of litter functional diversity on decomposition (LDED; Boyero et al., 2021). The wide range of leaf litter resources worldwide affects how detritivorous macroinvertebrates have evolved to use those resources, which in turn affects LDED (Boyero et al., 2017, 2021). For instance, a decrease in litter diversity and an increase in mean litter nutritional concentration negatively impact LDED in higher latitudes, aligning with the resource concentration hypothesis. In this case, detritivorous macroinvertebrate obtains resources from a few available but more nutritious litter types. This reduces the search and handing time, thus resulting in higher shredder-mediated decomposition rates. Conversely, positive or non-effect responses to LDED may be expected in lower latitudes. Here, the balanced diet hypothesis fits better due to higher litter diversity, where different resources are complementary because of their wide range of nutritional compositions. Detritivore shredders have a generalised diet, which is accompanied by a higher search and handling time to obtain the complete nutritional requirements to maintain their fitness (Boyero et al., 2021).

In our case, it is possible that tree species have different physicochemical and trait characteristics, and macroinvertebrate consumers complement their nutritional needs with autochthonous primary producers. Thus, it is likely that the RCC does not apply to these subtropical systems, as riparian cover streams do not necessarily rely on allochthonous leaf litter. We know very little about litter functional diversity among riparian species and how it interacts with detritivore macroinvertebrates to determine patterns in litter decomposition, and this aspect should be considered in future studies. It is possible that the importance of basal resources changes over time depending on hydrological regimes and tree species phenology (different times of leaf abscission). It is also necessary to consider other concepts, such as the RPM and RWC, considering the hydrological role in the lateral input of possible food resources, and the peaks of primary producers or leaf input (Humpries et al., 2014).

Despite the lack of differences in litter decomposition between RCS and OCS, various abiotic and biotic drivers of the litter decomposition process operate differently depending on the presence of riparian forests, particularly influencing the decomposition rate in OCS. These results partially corroborate our hypothesis suggesting that riparian cover really does act as a buffer zone in maintaining ecological processes in light of the changes in environmental conditions promoted by human stressors (Silva-Junior et al., 2014; Silva-Araujo et al., 2020; Ferreira et al., 2020; Sargac et al., 2021; Tonin et al., 2021; Boyero et al., 2021). In this case, riparian buffer regulates abiotic predictors of litter decomposition by moderating water temperatures and filtering the input of fertilisers and sediment, which are reflected in water and habitat quality (Marques et al., 2020; Sargac et al., 2021). Riparian buffer also contributes to the maintenance of macroinvertebrate community composition and determines variations in functional composition by providing food sources through the input of leaf litter supporting food webs (Moraes et al., 2014; Marques et al., 2020; Sargac et al., 2021).

In this case, we could observe in OCS that the increase in ammonium concentration and stream flow increased the decomposition rates, while nitrate, phosphate, and conductivity had opposite effects. Nutrient concentrations are crucial predictors among abiotic variables, significantly influencing decomposition rates (Boyero et al., 2016; Manning et al., 2017, 2021). However, they interact with other environmental factors (e.g., annual air temperature and hydrological variability), highlighting the complexity of these interactions (Tiegs et al., 2024). It is important to note that this research only tested linear relationships, but nonlinear relationships might reveal thresholds linked to sudden changes in decomposition rates (Tiegs et al., 2024).

## ***CONCLUSIONS***

Here, the main conclusions are presented as the main results obtained in each study in this thesis. Finally, a combination of ideas extracted from each study is presented, highlighting the potential use of bioindication using different approaches to assess stream ecosystems' ecological conditions. We present perspectives alongside these conclusions, proposing ideas and hypotheses for future research, some grounded in recent literature and some currently underway in our laboratory.

***Specific Objective 1:*** To evaluate the responses of water quality parameters and macroinvertebrate community indicators to the increase in afforested areas with *Eucalyptus* spp.

Related conclusions:

1. Eucalyptus afforestation has significant effects on water quality parameters and macroinvertebrate communities. In some cases, these effects are seasonal.

2. Water quality decreases with *Eucalyptus* afforestation, especially in the summer when water nutrient concentration increases and dissolved oxygen decreases, while conductivity and total dissolved solids increase in the winter. Higher metabolic rates of heterotrophic microorganisms and faster organic matter decomposition (especially in the form of DOC) can raise water nutrient concentrations. This is especially true when temperatures are high and dissolved oxygen levels are low.
3. Macroinvertebrate metrics and the taxon's tolerance threshold indicate negative responses to the gradient of *Eucalyptus* afforestation and the subsequent deterioration of water quality. The response of macroinvertebrate metrics, such as the EPT index, varies depending on the season, which may indicate different tolerance levels among species in these EPT families. Thus, threshold analysis was adequate to identify tolerance responses at taxa resolution.
4. Different taxa's tolerated thresholds show that a modest increase in *Eucalyptus* afforestation is sufficient to promote a decrease in the densities of the most sensitive taxa. We observed similar threshold responses under conditions of water nutrient enrichment and low oxygen.
5. Compared with other land uses in Uruguay, these effects of *Eucalyptus* afforestation are less negative than those promoted by other land uses in our country, and the presence of a buffer zone reduces the high-impact effects of this land use.

***Specific Objective 2***: To evaluate the impacts of *Eucalyptus* spp. afforestation on habitat heterogeneity and the availability of basal resources, and how these environmental changes affect functional trait structure and functional diversity of macroinvertebrate communities.

Related conclusions:

1. Functional trait-based approaches could also help to identify other pathways, in this case, at the local spatial scale, by which *Eucalyptus* afforestation affects the ecological condition of stream ecosystems.
2. The increase in *Eucalyptus* afforestation leads to indirect negative effects on macroinvertebrate functional diversity by altering the availability of basal resources, particularly the biomass of macrophytes.

3. Different macrophyte growth forms determine the structure of macroinvertebrate trait composition. In this case, submerged and emergent species filter traits related to predators and small and medium body sizes. This may be due to the higher architectural complexity of these macrophytes, which provide refuge and facilitate prey predation by retaining high prey density.
4. The buffer zone's protection allows *Eucalyptus* afforestation to occur without affecting the local variables of the physical habitat, such as substrate types, current velocity, and stream width, which also determine the trait structure and functional diversity.

***Specific Objective 3***: To analyse the effects of riparian forest loss on the abiotic (water quality and flow) and biotic (macroinvertebrates) predictors of leaf litter decomposition by comparing open-canopy streams and streams with riparian forest cover.

Related conclusions:

1. There were no significant differences in leaf dry mass between riparian forest streams (RFS) and open canopy streams (OCS) in leaf dry mass remaining after 26 days of exposure to leaf litter. However, some abiotic and biotic predictors had significant, albeit differential, effects on the decomposition process depending on the presence of riparian forests.
2. Leaf species identity was more important in determining differences between decomposition rates possibly masking the effects of anthropogenic stressors. In our case, using highly recalcitrant, low-decomposing litter makes it difficult to understand the effects of riparian removal and detritivore macroinvertebrates-mediated decomposition, as they cannot possibly use litter as a resource during the early stages of decomposition.
3. Despite these limitations, it was possible to detect the effects of some abiotic and biotic predictors on litter decomposition, as well as how these effects are dependent on the presence of riparian cover on streams.
4. Riparian cover buffers the effects of increases in water nutrient concentrations, conductivity, stream flow, and functional feeding groups, especially scrapers, suggesting indirect effects of the scraping activity on biofilm growing on litter surfaces.

## GENERAL CONCLUSION

Our study's findings demonstrate that both *Eucalyptus* afforestation and riparian cover loss had a significant and adverse effect on the ecological aspects of stream ecosystems in subtropical streams. We identified these effects by analysing taxonomic metrics, community structure, functional trait diversity, and ecological processes such as leaf litter decomposition. By examining many organisational levels, we may understand multiple ways in which stressors affect the ecological condition of stream ecosystems. This highlights the fact that:

1. The presence of extensive *Eucalyptus* afforestation cover affects water quality parameters and basal resources in streams. As a result, adverse alterations are observed in community structure, tolerance response, functional trait structure, and functional diversity.
2. Alterations in basal resources, specifically the reduction of macrophyte biomass, primarily drive the impact of *Eucalyptus* afforestation on stream ecosystem functioning. However, the introduction of *Eucalyptus* organic matter, either in the form of coarse particulate organic matter or dissolved organic carbon, into streams is likely to negatively impact the functional diversity of macroinvertebrates and their role in leaf litter breakdown.
3. To better understand the role of aquatic biota, it is necessary to include the effects of litter functional diversity in the study of the litter decomposition process. This is an important issue to consider as rates of deforestation and conversion of native forest to other land uses are high and the reduction of litter diversity may affect the offer of a balanced diet for aquatic macroinvertebrate consumers. As a consequence of litter diversity, higher microbial decomposition rates may enhance the contributions of CO2 into the atmosphere.
4. Limited research has been performed with these approaches in Uruguay. However, our findings present significant evidence on how macroinvertebrate communities respond to environmental changes resulting from human-induced stressors.

The knowledge produced by this thesis regarding the impacts of two human-caused stressors on streams will be vital for identifying novel research avenues for our ecosystems and improving our comprehension of the different pathways and mechanisms that aquatic communities and ecological processes follow to respond to environmental deterioration.

The thesis findings have the potential to serve as a tool for developing management practices aimed at preventing or mitigating the effects of the anthropogenic stressors examined in this study and possibly extending the evaluation of other land uses (e.g., agriculture and urbanisation). In our cases, possible management practices should focus on controlling the input of nutrients and possible pesticides, as well as DOC from *Eucalyptus*, by limiting activities related to fertilisation, pest control, and cattle access to the body of water. Other management and restoration activities should focus on introducing or promoting the growth of submerged and emerging macrophytes. These macrophyte growth forms can enhance macroinvertebrate functional diversity and control other ecological functions, such as sustaining clear waters and reducing carbon emissions. Other practices should focus on the conservation and restoration of the riparian forest with native species in order to preserve the diversity of allochthonous food resources that sustain aquatic food webs.

## PERSPECTIVES

In stream ecosystems, the spatial arrangement of multiple nested environmental factors determines the structure of aquatic communities (Frissell et al., 1986; Statzner et al., 1997). In other words, the potential impacts of larger-scale factors (e.g., basin-level land use changes) can influence local variables (e.g., current flow, substrate composition, and quality and quantity of basal resources) (Statzner et al., 1997). Various methods can address the responses of macroinvertebrate communities to increased anthropogenic stressors. The most basic and commonly used methods involve studying the taxonomic diversity or their structural arrangement to identify potential indicators of environmental deterioration (Domínguez et al., 2022). By using specific metrics such as the ETP index or the proportion of sensitive or tolerant families (Suárez et al., 2022) along with the identification of threshold indicator taxa (Campos et al., 2021; Martins et al., 2021), it becomes possible to identify taxa that belong to specific ecological niches. This refers to the dispersion of taxa along environmental factors affected by anthropogenic stressors. Identifying these thresholds may help delineate the conservation value of a sensitive species or predict changes in community composition (Nguyen et al., 2018). Furthermore, identifying taxa thresholds enables the implementation of more effective management strategies that aim to improve the conditions of those environmental factors (King & Baker, 2014).

Nevertheless, taxonomic-based approaches may fail to account for the various pathways that these stressors affect macroinvertebrates and their potential impact on ecosystem functioning (Jiang et al., 2021). In this scenario, employing trait-based approaches can be more useful in identifying human disruptions by assessing an organism's ability to adapt and withstand environmental changes (Meneses et al., 2010; Forio et al., 2018). In addition, the responses of traits to many stressors are more predictable and offer several advantages, including the ability to compare trait responses across different geographic regions (Dolédec & Stazner, 2008).

This study employed taxonomic and functional characteristic approaches for determining the impacts of *Eucalyptus* afforestation. It revealed that alterations in basal resources, particularly macrophytes, have the capacity to mitigate the effects of anthropogenic pressures. Future studies should prioritise using experimental methods and stable isotope techniques to investigate the potential bottom-up or top-down cascading effects on prey-predator interactions in macrophytes. The objective is to better understand the significance of various macrophyte growth forms in these interactions. Aside from macrophytes, it is important to also consider other carbon forms, such as dissolved organic carbon (DOC) and periphyton. Additionally, it is crucial to examine the interaction between DOC runoff and primary producers and their impact on macroinvertebrate communities. These effects should be investigated through experimental testing.

On the other hand, buffer areas in streams flowing through *Eucalyptus* afforestation proved to be efficient in lowering the effects of this land use via runoff. This buffer area comprised grass, herb species, and some shrubs. However, it would be interesting to test their retention capacity under both low and high flow conditions and compare it with buffer areas made up of riparian trees (Calvo, 2022), which are primarily found in patches, particularly in highland areas of the country. This, combined with these ideas and the study of hydrological patterns, may allow for the improvement of buffer areas, ameliorating the impacts of *Eucalyptus* stressors.

When studying ecological processes such as leaf litter decomposition, local environmental and biotic factors also showed a response to human-caused pressures. However, the effects of these factors may be hidden by the features of the litter itself (LeRoy et al., 2007; Ferreira et al., 2020; Tiegs et al., 2024). It is important to evaluate the use of a range of physicochemical and trait parameters for leaf species in this context. Our current research focuses on quantifying the significance of basal resources in aquatic trophic webs and these streams that still have riparian cover paths. This investigation explores|  the potential of riparian tree and shrub species to serve as basal resources within a gradient of leaf diversity. Understanding the structure of riparian areas and its significance in the biomass of the trophic web can assist in selecting suitable leaf species for future decomposition process experimentation. This will enable us to gain insights into the intricate interplay between environmental and biological factors (Costello et al., 2022; Tiegs et al., 2024).

## *BIBLIOGRAPHIC PRODUCTION*

During my PhD studies, I produced more articles and have also collaborated as a co-author on other papers and manuals, all of which are focused on the subjects of my thesis. Some of these publications are currently undergoing the editing process, while others are being prepared for publication. Here is a compilation of them.

1. **Barrios, M. C.,** Mendoza, M. D., & Montoya, J. V. (2020). Description of larva and pupa of Phylloicus cressae Prather 2003 (Trichoptera: Calamoceratidae) from a montane forest stream in the peri-urban area of Caracas, Venezuela. Zootaxa, 4768(4), 579-588. https://doi.org/10.11646/zootaxa.4768.4.8
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3. **Barrios, M.,** & Montoya, J. V. (2021). Biomass and instar estimation of larvae of Phylloicus cressae Prather, 2003 (Trichoptera, Calamoceratidae) in a montane forest stream, northcentral Venezuela. Aquatic Insects, 42(3-4), 216-229. https://doi.org/10.1080/01650424.2021.1908565
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16. Moi, D. A., Mormul, R. P., Quirino, B. A., Saito, V. S., Agostinho, A. A., Schitz, M. H., **Barrios, M.,** Figuereido, B. R. S., Romero, G. Human land-use and non-native species modify the size spectra of fish communities and jeopardize ecosystem services. *In preparation*.
17. Moi, D. A., Teixeira de Mello, F., **Barrios, M.,** et al. Human pressure and climate driving global biodiversity of freshwater invertebrates. *In preparation*.

In addition to publications, I also participated in research projects related to this thesis, one of them is currently work in process and is under my coordination:

1. **2023- current. (Responsable M. Barrios).** "Is the trophic subsistence of our main streams given by the autotropic or heterotrophic paths? case study Arroyo Maldonado. Proyecto Fondos Clemente Estable FCE_3_2022_1_172523, Agencia Nacional de Investigación e Innovación (ANII).
2. **2023- current:** Multiple stressors in the Anthropocene: effects of habitat fragmentation and climate change on planktonic and benthic pond metacommunities– TA-MetaClima Project No 871081". Financiamiento: AQUACOSM-plus – H2020-INFRIA-– Transnational Access Grant AQUACOSM-plus: Network of Leading Ecosystem Scale Experimental AQUAtic MesoCOSM Facilities Connecting Rivers, Lakes, Estuaries and Oceans in Europe and beyond - funded by the European Commission. Centre for Ecological Research in Budapest, Hungría.
3. **2022- 2023**. "Fortalecimiento de las capacidades para la gestión ambientalmente adecuada de plaguicidas incluyendo COPs" UTF/URU/035/URU. Instituciones participantes: CURE- Maldonado y CURE-Rocha, GACT (Montevideo y Paysandú), Facultad de Ciencias (Montevideo), UDELAR, DINACEA (Montevideo) Ministerio de Ambiente.
4. **2019-2020.** Indicadores ambientales multiescala de la forestación en Uruguay. Financiamiento: ANII Fondo Sectorial de Innovación Industrial ANII-FSI I 2016 1-128679. Empresas Proponentes: UPM-FO, Montes del Plata, Forestal Atlántico Sur. Ejecutante: Universidad de la República, Facultad de Ingeniería, Facultad de Agronomía, CURE, CENUR-LN. Administrador: Fundación Julio Ricaldoni. Rol: Participante, integrante del equipo colaborador.

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