Acta Limnologica Brasiliensia

Publication of the Brazilian Association of Limnology

Original Article
Acta Limnologica Brasiliensia, 2024, vol. 36, e24
https://doi.org/10.1590/S2179-975X8623
ISSN 2179-975X on-line version

Influence of eucalyptus plantations on benthic macroinvertebrate assemblages in neotropical springs

Influência de plantações de eucalipto na comunidade de macroinvertebrados bentônicos em nascentes neotropicais

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Cite as: Peixoto, S.S.J., Guimarães, L.P. and Alves, R.G. Influence of eucalyptus plantations on benthic macroinvertebrate assemblages in neotropical springs. *Acta Limnologica Brasiliensia*, 2024, vol. 36, e24. https://doi.org/10.1590/S2179-975X8623

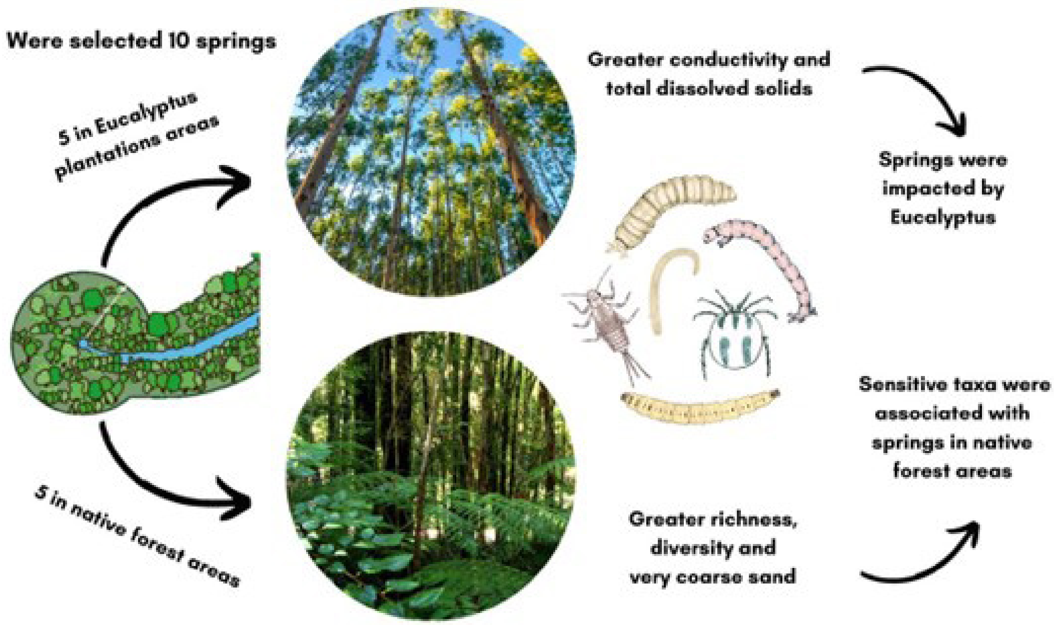
Abstract: Aim: The high sensitivity of springs to anthropic impacts is largely due to the high existing aquatic-terrestrial connectivity. The degradation of these ecosystems can be caused by land use, such as eucalyptus monoculture. The aim of this study was to test the influence of *Eucalyptus urograndis* plantations on the richness and diversity of the benthic macroinvertebrate community and on the environmental variables measured in springs in the Atlantic Forest domains. **Methods:** Ten springs (5 in native forest areas and 5 in eucalyptus area) were sampled in the dry period of 2017. The organisms were screened and identified at the family level. For each spring, dissolved oxygen, temperature, pH, turbidity, electrical conductivity, total dissolved solids, total nitrogen, nitrite, nitrate, ammonia, total phosphorus, vegetation cover, granulometric characterization and organic matter were also measured. **Results:** The t-test showed that some environmental variables differed, with the eucalyptus areas having higher values of total dissolved solids and electrical conductivity, and lower values of coarse sand. In the present study, the richness and diversity of the benthic fauna of springs were significantly lower in eucalyptus sites than in native sites. Nonetheless, the composition of the communities did not differ. The Indicator Species Analysis (IndVal) associated Acari, Hydropsychidae, Leptoceridae, Nematoda, Psychodidae, and Tipulidae with areas of native forest. **Conclusions:** Our results show that Brazilian springs are affected by eucalyptus monocultures reducing the richness and diversity of benthic macroinvertebrates and also changing water environmental properties. Thus, we verified that previous studies elsewhere in the world also apply to neotropical areas where eucalyptus is not native.

Keywords: monoculture; Brazil; aquatic insects; freshwater; anthropic impact.

Resumo: Objetivo: A alta sensibilidade das nascentes à impactos antrópicos deve-se, em grande parte, a elevada conectividade aquático-terrestre existente. A degradação desses ecossistemas pode ser causada pelo uso do solo, como por exemplo o monocultivo de eucalipto. O objetivo deste estudo foi testar a influência de plantações de *Eucalyptus urograndis* sobre a riqueza, a diversidade da comunidade de macroinvertebrados bentônicos e as variáveis ambientais aferidas em nascentes nos domínios de Floresta Atlântica. **Métodos:** Dez nascentes (5 em áreas de floresta nativa e 5 em áreas de eucalipto) foram amostradas no período seco de 2017. Os organismos foram triados e identificados ao nível de família. Para cada nascente foram mensuradas o oxigênio dissolvido, temperatura, pH, turbidez, condutividade elétrica, sólidos totais dissolvidos, nitrogênio total, nitrito, nitrato, amônia, fósforo total, cobertura vegetal, caracterização granulométrica e matéria orgânica. **Resultados:** O teste t evidenciou algumas variáveis ambientais diferiram, apresentando as áreas de eucalipto maiores valores de sólidos totais dissolvidos e condutividade elétrica e menores de areia muito grossa. No presente estudo, a riqueza e diversidade da fauna bentônica em nascentes foram significativamente menores nas áreas de eucalipto do que nas áreas de vegetação nativa. Entretanto, a composição das comunidades não diferiu. A análise de espécies indicadoras (IndVal) associou Acari, Hydropsychidae, Leptoceridae, Nematoda, Psychodidae e Tipulidae a áreas de floresta nativa. **Conclusões:** Nossos resultados mostram que nascentes em áreas de eucalipto são afetadas pela monocultura reduzindo a riqueza e diversidade de macroinvertebrados bentônicos e alterando também os parâmetros ambientais. Por fim, verificamos que estudos anteriores em outras partes do mundo também se aplicam a áreas neotropicais onde o eucalipto não é nativo.

Palavras-chave: monocultura; Brasil; insetos aquáticos; água doce; impacto antrópico.

Graphical Abstract



1. Introduction

Springs are natural discharge points from aquifers that can give origin to lakes, streams, wetlands, and geysers (Cantonati et al., 2021). They connect groundwater, surface water and the terrestrial ecosystem through spatial flows of energy, matter, and organisms (von Fumetti & Blattner, 2017). Thus, an environmental impact on springs has the potential to modify the associated ecosystem compartments. Because they receive strong influence from the aquifer, springs present unique geomorphological habitats and are distinguished from other aquatic ecosystems (Junghans et al., 2016).

In addition, they are characterized by high environmental heterogeneity through a mosaic of microhabitats (Stevens et al., 2021) that generate high biological, genetic, and functional diversity, being considered hotspots for aquatic biodiversity (Cantonati et al., 2006, 2012) and contain a high number of Red List species taxa compared to other aquatic ecosystems (Cantonati et al., 2022). Although springs are abundant around the world, they have been suffering from various anthropogenic impacts, such as habitat degradation, pollution, water flow modification, groundwater depletion, and land use (Dudgeon et al., 2006; Cantonati et al., 2020). Occupation by human populations can structurally simplify aquatic habitats and affect the quantity, distribution, biology, and/or functional characteristics of the organisms present there (Gimenez & Higuti, 2017; Vitule et al., 2012).

The replacement of native vegetation by monocultures is a widespread practice globally (Ferreira et al., 2019). Eucalyptus plantations, for example, represent 8% of all trees planted in the world (Hartley, 2002). Brazil is the largest exporter of cellulose on the world market, and Minas Gerais is the state with the largest area of trees, predominantly planted with eucalyptus (IBA, 2020). Dumnicka et al. (2007) has shown that the replacement of original forests by monocultures is usually responsible for causing changes in soil structure, which can lead to changes in the physical and chemical characteristics of freshwater environments and the fauna present there.

Eucalyptus plantations areas have less quality of organic matter, and the potential toxicity of their leaves can affect the diversity and abundance of benthic macroinvertebrates (Abelho & Graça, 1996; Larrañaga et al., 2009; Kiffer Junior et al., 2018). When analyzing the effects of eucalyptus plantations on streams at different latitudes, it was found that changes in macroinvertebrate communities that occur in temperate climate regions do not apply to other climatic zones (Ferreira et al., 2019). In subtropical streams, although the total richness and density of macroinvertebrates did not differ, the different groups of invertebrates responded differently to eucalyptus afforestation (Barrios et al., 2024). According to Amaral et al. (2021), the composition of the fauna and the taxonomic richness of Ephemeroptera, Plecoptera and Trichoptera in Neotropical springs can be negatively affected by eucalyptus plantations. However, studies evaluating the impact of eucalyptus monoculture on macroinvertebrate fauna in Neotropical springs are scarce.

In this study, the following objectives were established: (1) to investigate the structure and composition of benthic macroinvertebrate communities in neotropical springs in areas with eucalyptus plantations and in areas with native forest; (2) to define indicator taxa for each area. Our hypothesis one is that springs in eucalyptus plantation areas will present lower Shannon-Wienner index and richness of benthic macroinvertebrates. Hypothesis two is that more sensitive taxa associated with preserved environments will be indicators of springs in native forest areas.

2. Material and Methods

2.1. Study area

Ten perennial springs were selected (five in areas with *Eucalyptus urograndis* plantations and five in native forest areas), located in three rural properties (called areas A, B and C) that belong to the sub-basin of the Paraíba do Sul River in southeastern Minas Gerais, Brazil (Figure 1, Table 1).

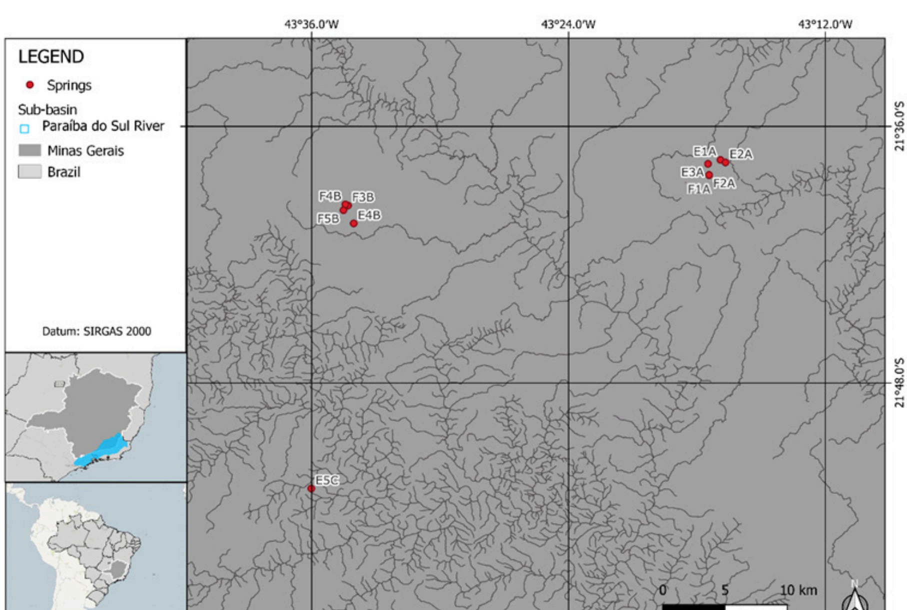


Figure 1. Springs in eucalyptus plantation areas (E1A, E2A, E3A, E4B, E5C) and in native forest areas (F1A, F2A, F3B, F4B and F5B) in rural properties in Southeast Minas Gerais, Brazil.

Table 1. Coordinates of 10 springs, 5 in native areas and 5 in eucalyptus areas, in southeastern Minas Gerais, Brazil.

Spring	Area	Coordinates
F1A	Forest	-21.6381; -43.2905
F2A	Forest	-21.6378; -43.2906
F3B	Forest	-21.6617; -43.5717
F4B	Forest	-21.6608; -43.5738
F5B	Forest	-21.6653; -43.5753
E1A	Eucalyptus	-21.6261; -43.2817
E2A	Eucalyptus	-21.6281; -43.2778
E3A	Eucalyptus	-21.6292; -43.2914
E4B	Eucalyptus	-21.6756; -43.5672
E5C	Eucalyptus	-21.8822; -43.6003

The springs, according to Brazilian environmental legislation, have their marginal edges considered permanent preservation areas - APP (Brasil, 2012). Thus, the springs studied in *Eucalyptus urograndis* areas have native vegetation margins (material available in https://doi.org/10.48331/scielodata.LRDB6H), and the native forest areas are characterized as remnants of Semideciduous Seasonal Forest, belonging to the Atlantic Forest domain. Most of the fragments in this region are in the secondary stage of regeneration (Paiva et al., 2015; Veloso et al., 1991). The regional climate, according to the Köppen classification, is Cwa (mesothermal, with a rainy and hot summer, and dry winter).

2.2. Sampling

The springs were sampled only once during the dry season in 2017, at 3 different sampling points (P1, P2 and P3), along a 5 m long transect in the eucrenal zone. Point 1, located closer to the water extrusion area (upstream); point 3 further downstream; and point 2, in an intermediate area. The three points of the same spring were considered a sampling unit. The collection of the substrate for macroinvertebrate analysis was performed by the trawling method, using a hand sampler (area 0.01 m^2; mesh 250 µm) for 15 seconds at each point, totaling a sampling effort of 45 seconds per spring. The substrate from each sampling point was stored separately in plastic bags and fixed in 85% ethyl alcohol. In the laboratory, the organisms were sorted and identified under a stereoscopic microscope. The insects were identified with the aid of specialized keys (Hamada et al., 2014; Lecci & Froehlich, 2007; Pes et al., 2005; Pinho, 2008; Segura et al., 2011; Souza et al., 2007). The identification was performed at the family level, since it is a widely used and appropriate taxonomic level to assess the quality of aquatic environments (Brito et al., 2018; Martins et al., 2023). The other groups, such as Acari, Mollusca, Crustacea, Nematoda and Oligochaeta were kept in higher taxonomic categories.

2.3. Environmental variables

Dissolved oxygen, temperature, pH, turbidity, electrical conductivity, and total dissolved solids were measured in the field using a Horiba-U10 multisensor. Water samples were collected for analysis of total nitrogen, nitrite, nitrate, ammonia, and total phosphorus (Baird et al., 2005; Wetzel & Likens, 2000). All variables were measured only once per spring. To measure the vegetation cover of each spring, photographs of the canopy were taken, 10 centimeters away from the water surface. The images were converted to black and white and analyzed using ImageJ software (Rasband, 2018). The results obtained generate an average pixel value that ranged from 0 (total cover) to 255 (total light input) and is converted into a percentage. For granulometric characterization, after being dried at room temperature and homogenized, 120g of substrate samples from each spring were processed in a mechanical shaker, using sieves of different mesh openings, for the separation of the following fractions: very coarse sand (1 mm < × < 2 mm), coarse sand (500 µm < x < 1 mm), medium sand (250 µm < × < 500 µm), fine sand (150 µm < × < 250 µm), very fine sand (75 µm < × < 150 µm) and silt and clay (<75 µm) according to the procedure recommended by the technical standard NBR 7181 (ABNT, 1984). The organic matter (OM) content was determined according to the technical standard NBR 13600 (ABNT, 1996). The environmental variables measured per spring are shown in Supplementary Material A.

2.4. Statistical analysis

Richness (number of families), abundance, Shannon-Wienner index, and Pielou's evenness (J) were calculated for each spring. To verify possible differences in environmental variables, richness, abundance, diversity (H') and evenness (J) between springs in forest and eucalyptus areas, a T-test was performed. The data were previously tested regarding the assumptions. When necessary, the data were log-transformed, and when the assumption of a normal distribution was not met, the non-parametric Wilcoxon test was performed. To verify the possible association of benthic macroinvertebrates with springs in eucalyptus and forest areas, the Indicator Species Analysis (IndVal) was performed, which considers the abundance and frequency of each taxon (Cáceres & Legendre, 2009). For this analysis, the Stats (R

Core Team, 2018) and Indicspecies (Cáceres & Legendre, 2009) packages were used. Non-metric Multidimensional Scaling (nMDS) with Bray-Curtis distance was used to order the springs according to areas, based on the abundance (log x+1) of benthic macroinvertebrates. The Analysis of Similarity (ANOSIM) was performed with the same nMDS data to verify if there was a significant difference in the fauna composition between the native forest and eucalyptus areas. The "vegan" package (Oksanen et al., 2016) was used to run the diversity indices, nMDS and ANOSIM. All analyses were performed in the program Rstudio (version 1.1.463.0) (R Core Team, 2018).

3. Results

3.1. Environmental variables

The t-test showed that the variables total dissolved solids, electrical conductivity, and very coarse sand differed significantly according to areas (Table 2).

3.2. Structure and composition of benthic macroinvertebrate fauna

We found 8474 specimens in springs in native forest areas and 5261 in eucalyptus areas, distributed in 58 taxa (Supplementary Material B). Abundance ($t = -1.05$ $df = 8$; $p = 0.163$) and evenness ($t = -1.67$; $df = 8$; $p = 0.066$) did not differ between the different areas. On the other hand, richness ($t = -1.89$; $df = 8$; $p = 0.048$) and Shannon-Wienner index (H') ($t = -2.44$; $df = 8$; $p = 0.020$) differed among treatments, being smaller in eucalyptus areas. Regarding the richness, we found that there was an overall reduction of around 28.37%.

The non-metric multidimensional scaling (nMDS) analysis (stress = 0.055), showed that there was no clear separation between the springs in native forest areas and in eucalyptus areas in relation to fauna (Figure 2), but the eucalypt sites were more different to each other than the native forest sites. This result was corroborated by ANOSIM, which showed no difference in the composition of benthic macroinvertebrate assemblages ($R = 0.092$; $p = 0.194$).

The analysis of indicator species (IndVal) showed that six taxa were associated with native forest areas (Table 3). In eucalyptus areas, no indicator taxon was evidenced.

4. Discussion

4.1. Environmental variables

Although only one measurement of the environmental variables was carried out, in our

Table 2. Mean and standard error (SE) of environmental variables of springs in forest and eucalyptus areas belonging to the Paraíba do Sul River sub-basin in southeastern Minas Gerais, Brazil.

	Forest (n= 5)		Eucalyptus (n= 5)		Results		
	Mean	SE	Mean	SE	t / W	df	p
Canopy (%)	62.60	3.04	55.00	2.28	-1.99	7.41	0.08
Altitude (m)	774.80	6.60	747.60	33.48	-0.88	4.30	0.42
Temperature (ºC)	16.72	0.24	18.08	0.57	2.16	5.58	0.07
Dissolved Oxygen (%)	5.23	0.63	3.73	0.79	-1.49	7.62	0.17
Conductivity (µs/cm)	14.52	2.34	68.92	10.80	4.92	4.37	0.01*
Total Dissolved Solids (µg/l)	11.18	1.74	51.09	7.50	5.18	4.43	0.01*
pH	6.58	0.13	6.76	0.10	0.99	7.22	0.35
Turbidity (NTU)	45.63	32.66	11.18	3.29	-1.04	4.08	0.35
Nitrate (µg/l)	233.11	34.65	438.58	145.88	1.37	4.44	0.23
Nitrite (µg/l)	1.66	0.26	1.34	0.16	-1.05	6.69	0.32
Ammonia (µg/l)	96.86	38.28	157.42	107.41	-0.03	6.72	0.97
Total Nitrogen (µg/l)	2069.99	398.71	1724.64	83.23	0.01	1.00	0.91
Dissolved Phosphorus (µg/l)	20.50	3.18	16.25	1.59	-1.19	5.88	0.27
Total Phosphorus (µg/l)	223.42	138.58	102.24	28.68	-0.85	4.34	0.43
Very Coarse Sand (%)	57.61	11.47	18.11	5.04	-3.15	5.49	0.02*
Coarse Sand (%)	10.41	4.34	22.91	8.15	1.35	6.10	0.22
Medium Sand (%)	17.07	3.90	12.29	4.76	-0.77	7.70	0.46
Fine Sand (%)	2.56	0.59	2.52	1.02	-0.03	6.44	0.97
Very Fine Sand (%)	0.95	0.38	1.21	0.56	0.53	1.00	0.46
Silt and Clay (%)	0.35	0.25	0.47	0.24	0.17	1.00	0.67
Organic Matter (%)	56.32	8.64	53.93	13.37	-0.15	6.84	0.88

t/W = test value; df = degrees of freedom; p = significance level; * = significant values.

Table 3. Indicator species analysis (IndVal) of 10 springs, 5 in native forest areas and 5 in eucalyptus areas, in southeastern Minas Gerais, Brazil.

Taxa	Area	IndVal	p-value
Nematoda	Forest	0.983	0.020
Leptoceridae	Forest	0.961	0.015
Acari	Forest	0.954	0.025
Tipulidae	Forest	0.952	0.005
Psychodidae	Forest	0.946	0.015
Hydropsychidae	Forest	0.894	0.040

p-value = significance level

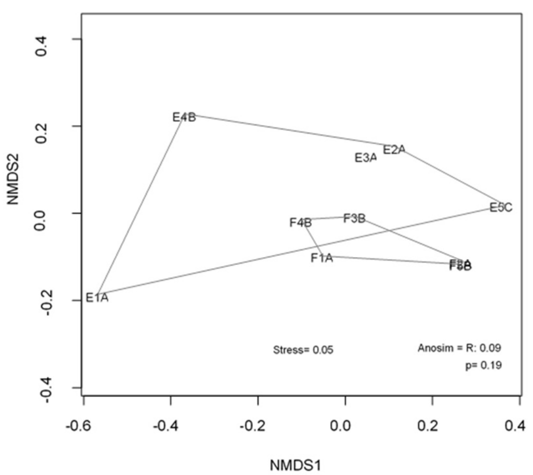


Figure 2. Similarity of benthic macroinvertebrate community composition (NMDS; Bray-Curtis similarity index) among 10 springs: 5 in eucalyptus (E1A, E2A, E3A, E4B, E5C) and 5 in forest areas (F1A, F2A, F3B, F4B and F5B) in Southeast Minas Gerais, Brazil.

study, springs in eucalyptus areas showed higher values of electrical conductivity and total dissolved solids. This shows that eucalyptus can alter environmental parameters in springs. The effect of eucalypt has been identified in streams through increased electrical conductivity and total dissolved solids (Barrios et al., 2024). Similarly, it has been found that suspended solids, nitrate, chlorides, and sulfates can be found in higher concentrations in aquatic systems that have had their native vegetation replaced by monocultures of eucalyptus (Fierro et al., 2016).

It is common for finer particle size fractions to be related to environments under the influence of eucalyptus monoculture (Amaral et al., 2020). In the present study, we found no association between the finer particle size fractions and eucalyptus areas. However, the springs in forest areas had a higher percentage of very coarse sand. It is likely that the native riparian vegetation has contributed to the maintenance of soil structure, keeping it consolidated, little exposed to erosion, in order to reduce the entry of fine particles in the springs in forest areas.

4.2. Structure and composition of benthic macroinvertebrate fauna

Springs in native forest areas had higher values of benthic macroinvertebrate richness and diversity, a fact that corroborates our first hypothesis. When studying the fauna of Ephemeroptera, Plecoptera and Trichoptera in springs located in eucalyptus areas, Amaral et al. (2021) found similar results. In contrast, faunal composition was not clearly distinguished by nMDS. However, it is noticeable that the communities in forest areas are more similar to each other than those in eucalyptus areas. The fact that springs usually present high thermal and chemical stability (Barquín & Scarsbrook, 2008; van der Kamp, 1995) leads us to assume that those in forest areas have a greater possibility of remaining stable, providing a greater similarity in faunal composition. In turn, interference from eucalyptus plantations can modify the environment and select species within communities, favoring differentiation between springs.

Springs in native forest areas showed higher richness and diversity. The composition and distribution of benthic macroinvertebrate assemblages can be influenced by substrate composition (Buss et al., 2004; von Fumetti et al., 2006); water flow (Statzner & Higler, 1986); availability of food resources (Abelho & Graça, 1996); land use; and habitat modification (Amaral et al., 2015; Ilmonen et al., 2012; Knysh et al., 2016). In streams, the impact of eucalyptus plantations on the richness and density of the benthic macroinvertebrate fauna varies according to the tolerance of the group, the local climate, and the quality of the leaf detritus (Barrios et al., 2024; Ferreira et al., 2019; Larrañaga et al., 2009).

In the same sense, the analysis of indicator species identified six groups with greater specificity in springs in forest areas and none in eucalyptus areas. Leptoceridae (Trichoptera) and Hydropsychidae (Trichoptera) are families considered sensitive to anthropogenic disturbance and are indicators of good environmental integrity (Amaral et al., 2015; Bispo et al., 2006; Cortezzi et al., 2009) and their occurrences have already been negatively related to streams that have eucalyptus plantations in their surroundings (Cordero-Rivera et al., 2017), including in the subtropical region (Barrios et al., 2024), which corroborates our hypothesis that more sensitive taxa related to preserved environments would be associated with springs in areas of native forest.

Other groups that showed a negative association to springs under eucalypt plantations were mites, nematodes, and the dipterans Tipulidae and Psychodidae. In addition, most mites have a low tolerance to electrolytes, i.e., they have a negative relationship with water conductivity (Sabatino et al., 2000). In the present work, electrical conductivity had lower values in native forest areas, which could explain the higher abundance and frequency of mites in these springs. Nematoda is a group of trophic importance in aquatic environments, as it connects the microfauna compartments to the higher trophic levels. They are able to feed on different items (bacteria, algae, fungi, protozoa, and dissolved organic matter) (Majdi & Traunspurger, 2015). However, there is almost no information on these organisms in crenal environments (Cantonati et al., 2006).

The dipterans Psychodidae and Tipulidae are detritivores/gatherers, which can be found on the banks of lotic environments with accumulation of detritus (Hamada et al., 2014), a fact that may have contributed to the higher abundance and frequency of these insects in springs in forest areas. In this sense, springs in eucalyptus areas may receive lower quality leaf resources due to the presence of oil glands, phenols, and thick leaf cuticle (Abelho & Graça, 1996) and thus, lower richness and diversity of these organisms can be expected. Tipulidae larvae reduced their consumption rates of native leaves by 50% when polyphenols and oils from *Eucalyptus globulus* were added experimentally (Canhoto & Graça, 1999) and, when fed experimentally with eucalyptus leaves from the same species abovementioned, they suffered damage to their mouth apparatus and alterations to their intestinal microbiota (Canhoto & Graça, 2006).

In conclusion, we emphasize that our hypotheses were corroborated in this study. The results obtained showed that springs in tropical areas are also negatively affected by exotic eucalyptus monocultures, with a decrease in the richness and diversity of benthic macroinvertebrates. In addition, sensitive taxa were associated with springs in native forest areas, indicating better environmental conditions in these systems. Thus, we highlight the importance of maintaining native forest for the conservation and integrity of springs and aquatic biodiversity.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Data availability

All research data analyzed in the research is available in the Dataverse of Acta Limnologica Brasiliensia in SciELO Data. Access is free. It can be accessed in https://doi.org/10.48331/scielodata.LRDB6H.

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Received: 11 September 2023
Accepted: 07 May 2024

Associate Editor: Victor Satoru Saito