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**Efeitos de diferentes usos de terra sobre o grupo funcional alimentar  
fragmentador e EPT com respiração branquial**

Belém  
2026

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Dissertação de Mestrado apresentada como requisito parcial para obtenção do título de Mestre em Ecologia pelo Programa de Pós-Graduação em Ecologia da Universidade Federal do Pará - UFPA.

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia do convênio da Universidade Federal do Pará e Embrapa Amazônia Oriental, como requisito parcial para obtenção do título de Mestre em Ecologia pela Comissão Julgadora composta pelos membros:

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Dedico esse trabalho aos meus pais, por todo o apoio e suporte durante todo o meu trajeto acadêmico até aqui e à minha irmã, que nunca deixou de me orgulhar e sempre me serviu de exemplo de pesquisadora.

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## Resumo

Em riachos de pequena ordem, como os igarapés, os insetos aquáticos das ordens Ephemeroptera, Plecoptera e Trichoptera (EPT) são amplamente utilizados em estudos de monitoramento ambiental, devido à sua grande sensibilidade a estresses e importância ecológica, visto que atuam em processos ecológicos, em virtude de seus atributos biológicos (*traits*). A análise desses *traits* relaciona adaptações funcionais a características ambientais, permitindo analisar a integridade de um riacho por meio da distribuição de grupos funcionais específicos. Portanto, a presença desses insetos aquáticos é altamente influenciada por fatores externos relacionados a vegetação ao redor. Em virtude disso, o objetivo geral dessa dissertação foi analisar como tipos de usos de terra (agropecuária e monocultura de palma) influenciam na distribuição de insetos das ordens EPT, através de seus atributos funcionais. Assim, essa dissertação se dividiu em dois capítulos: o primeiro mensurou a variação da biomassa e a riqueza de fragmentadores em função dos usos, comparados a floresta e o segundo estudou a influência desses usos na riqueza de insetos com respiração branquial e na diferenciação morfológica das brânquias. O estudo foi realizado em 45 riachos de 1ª a 3ª ordem, classificados em “Floresta”, “Pasto”, “Palma” e “Mosaico”, no nordeste do Pará, de 2011 a 2017. As amostras biológicas foram coletadas em um transecto de 150 m em cada riacho. A coleta e triagem de EPT foi realizada em campo com auxílio de rede rapiché, bandejas plásticas e pinças entomológicas. A quantificação e identificação a nível de gênero, assim como a definição de insetos fragmentadores e diferenciação morfológica de insetos com respiração branquial, foi realizada em laboratório com uso de chaves taxonômicas e artigos de descrição. Para testar a hipótese de que diferentes usos de terra influenciam negativamente na distribuição e variedade de grupos funcionais de insetos aquáticos foi realizado o teste ANOVA *one-way*. As análises RLQ e *Fourth-Corner* avaliaram se as variáveis ambientais influenciaram a abundância e a distribuição de diferentes brânquias. A Análise de Redundância (RDA) foi aplicada para identificar as principais variáveis que influenciam a distribuição das ordens EPT. Os riachos florestados apresentaram maior riqueza de gênero em comparação com outros tipos de uso da terra. Portanto, as mudanças no uso da terra modificam as características dos sistemas aquáticos, influenciando a riqueza e a distribuição de insetos aquáticos. Esses resultados corroboram a premissa de que áreas mais conservadas apresentam maior diversidade de microhabitats, abrangendo diferentes nichos e requisitos fisiológicos.

## **Abstract**

In small-order streams, such as igarapés, aquatic insects of the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are widely used in environmental monitoring studies due to their high sensitivity to stressors and ecological importance, as they participate in ecological processes through their biological attributes (traits). The analysis of these traits relates functional adaptations to environmental characteristics, enabling assessment of stream integrity through the distribution of specific functional groups. Therefore, the presence of these aquatic insects is strongly influenced by external factors associated with surrounding vegetation. Accordingly, main objective of this dissertation was to analyse how different land-use types (agriculture and oil palm monoculture) influence the distribution of insects of the EPT orders through their functional attributes. Thus, this master's project was divided into two chapters: the first quantified variation in biomass and the richness of shredders as functional feeding groups according to land use, compared with forest; and the second examined the influence of these land uses on the abundance of gill-breathing insects and on the morphological differentiation of gills. The study was conducted in 45 first- to third-order streams classified as "Forest", "Pasture", "Oil Palm" and "Mosaic", in north-eastern Pará, between 2011 and 2017. Biological samples were collected along a 150 m transect in each stream. The collection and sorting of EPT were carried out in the field using a hand net, plastic trays and entomological forceps. Quantification and identification to genus level, as well as the classification of shredders and the morphological differentiation of gill-breathing insects, were performed in the laboratory using taxonomic keys. To test the hypothesis that different land uses negatively influence the distribution and diversity of functional groups of aquatic insects, a one-way ANOVA was conducted. RLQ and Fourth-Corner analyses assessed whether environmental variables influenced abundance and the distribution of different gill types. Redundancy Analysis (RDA) was applied to identify the main variables influencing the distribution of the EPT orders. Forested streams exhibited greater richness compared with other land-use types. Therefore, land-use changes modify the characteristics of aquatic systems, influencing the richness and distribution of aquatic insects. These results corroborate the premise that more conserved areas exhibit greater microhabitat diversification, encompassing a wider range of niches and physiological requirements.

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## 1. INTRODUÇÃO GERAL

Os macroinvertebrados aquáticos são amplamente utilizados na avaliação da condição ecológica em sistemas aquáticos (Cummins *et al.*, 2022). Macroinvertebrados bentônicos apresentam respostas identificáveis a impactos ambientais (Junqueira *et al.*, 2018), em virtude de sua alta especificidade e sensibilidade às características dos habitats, respondendo com mudanças na abundância e na riqueza de espécies até mesmo diante de alterações antrópicas sutis. Sua distribuição é fortemente influenciada pela disponibilidade e qualidade da matéria orgânica (Vannote *et al.*, 1980; Boyero *et al.*, 2017; Cummins *et al.*, 2022). Assim, é possível estabelecer relações de causa e efeito entre as comunidades de macroinvertebrados e tipos de uso de terra, a partir de modificações de filtros ambientais causados por estresses antropogênicos, como alterações no tipo e a quantidade de matéria orgânica e sedimentos, entre outros (Mathers *et al.*, 2017; Junqueira *et al.*, 2018).

Para realizar avaliações ou monitoramentos das condições ambientais as métricas mais fidedignas são as bióticas. Existem diferentes abordagens nesse sentido, que muitas vezes podem ser utilizadas conjuntamente com métricas abióticas e abordagens ecossistêmicas. Estudos com avaliação biológica mais antigos utilizavam a abundância de indivíduos e o número de espécies (riqueza taxonômica) para mensurar a condição ecológica de ecossistemas aquáticos (Baptista *et al.*, 2007). Atualmente se destacam o índice que quantifica a porcentagem de Ephemeroptera, Plecoptera e Trichoptera (%EPT) e o índice *Biological Monitoring Working Party* (BMWP), desenvolvido por pesquisadores do Reino Unido (Hawkes, 1998), que analisa a qualidade da água pontuando os táxons encontrados quanto a sua sensibilidade, em uma escala de 1 a 10 (Bacca *et al.*, 2023). Apesar de muito úteis, muitas vezes alterações ambientais causam um aumento da abundância ou da riqueza de espécies no ambiente, como predito pela teoria do distúrbio intermediário (Connel *et al.*, 1978), em virtude da entrada de espécies mais generalistas compensando a perda local de espécies mais especialistas, o que muitas vezes pode criar um fator de confusão nas avaliações e análises de dados de monitoramento.

Por isso, ao longo dos anos, diferentes metodologias e abordagens foram desenvolvidas a fim de avaliar e compreender o funcionamento e a importância das espécies para os processos ecossistêmicos no meio ambiente. Em destaque, há a abordagem funcional, que estuda a contribuição das espécies para o funcionamento ambiental, através de características morfológicas, fisiológicas e comportamentais que inferem sobre a funcionalidade, direta ou indiretamente (Poff *et al.*, 2006; Cianciaruso *et al.*, 2009). Além disso, o estudo de traços funcionais agiliza o desenvolvimento das pesquisas, por não necessitar de identificações a menores níveis taxonômicos, já que as características funcionais de um grupo funcional são facilmente reconhecidas (Cummins *et al.*, 2005). A avaliação da diversidade funcional de uma comunidade em um ecossistema aquático se torna fundamental uma vez que alterações na mata ciliar afetam a biomassa de macroinvertebrados e conseqüentemente, a

cadeia trófica dos riachos (Collyer, *et al.*, 2023). Como consequência, os serviços prestados no riacho são influenciados, o que altera a qualidade e o funcionamento do ambiente (Calaça & Grelle, 2016).

Grupos funcionais exercem diferentes atividades ecossistêmicas, como a decomposição da matéria orgânica e a ciclagem de nutrientes (Cianciaruso *et al.*, 2009; Bacca *et al.*, 2023). Somado a isso, sabe-se que a composição funcional de uma comunidade é fortemente influenciada por características do habitat em que estão localizadas (Shimano *et al.*, 2012) visto que, dependendo da condição ambiental, um grupo funcional pode ser mais favorecido que outro (Luiza-Andrade *et al.*, 2023). Esse tipo de relação auxilia no entendimento da influência da integridade ambiental de riachos na composição da comunidade de macroinvertebrados bentônicos.

Os insetos aquáticos são os mais indicados para estudos de avaliação da condição ecológica em riachos (Junqueira *et al.*, 2018). As ordens Ephemeroptera, Trichoptera e Plecoptera (EPT) são consideradas ótimos bioindicadores (Luiza-Andrade *et al.*, 2023), pois, além de possuírem grande similaridade no ciclo de vida e em seus nichos ecológicos, apresentam uma alta sensibilidade a mudanças ambientais (Arce *et al.*, 2023). Os organismos dessas ordens podem ser diferenciados por meio da identificação taxonômica ou de características morfofisiológicas, como comprimento, duração do ciclo de vida, estágios aquáticos, respiração, mobilidade, alimentação, entre outros (Usseglio-Polatera *et al.*, 2000).

As comunidades de EPT apresentam variação de composição de acordo com o tipo de uso do solo presente, visto que alterações na vegetação ripária desestabilizam o solo e diminuem a disponibilidade de recursos presentes (Faria *et al.*, 2021; Arce *et al.*, 2023). Dessa forma, gêneros que dependem de recursos alóctones ou que possuem estruturas mais expostas e sensíveis a alterações físico-químicas dos riachos, como brânquias, são mais influenciadas por intervenções na vegetação marginal (Mathers *et al.*, 2017; Luiza-Andrade *et al.*, 2020; McKenzie *et al.*, 2020).

Assim, o objetivo geral dessa Dissertação de Mestrado é analisar como alterações na estrutura dos riachos, provenientes de diferentes usos de terra em suas bacias de drenagem, influenciam na distribuição de insetos aquáticos em riachos amazônicos, através da mensuração de atributos funcionais das comunidades de EPT. Avaliaremos a hipótese de que alterações antrópicas no uso do solo influencia negativamente a distribuição e variedade de grupos funcionais. Para isso, esse trabalho será dividido em dois capítulos. No primeiro capítulo analisaremos como a substituição de áreas florestais por áreas de agropecuária e monocultura de palma (*Elaeis guineensis* Jacq) afetam a riqueza e a biomassa de insetos fragmentadores. No segundo, estudaremos como os diferentes usos de terra afetam a riqueza e abundância de insetos com aparelho respiratório branquial e se existem padrões de resposta a variáveis ambientais de acordo com as características branquiais.

## 2. Sessão I

### **Effects of oil palm monoculture and agricultural land use on shredder insects in eastern amazonian streams**

O Primeiro capítulo já foi publicado na revista *Environmental Monitoring and Assessment*, disponível em: <https://link.springer.com/article/10.1007/s10661-026-15076-9>

**EFFECTS OF OIL PALM MONOCULTURE AND AGRICULTURAL LAND USE  
ON SHREDDER INSECTS IN EASTERN AMAZONIAN STREAMS**

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### ***ABSTRACT***

Aquatic insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are vulnerable to changes in allochthonous inputs, mainly shredders, important to leaf litter decomposition and energy flow. This study evaluated the effects of landuse patterns (forest, pasture, oil palm, and mosaic) on the abundance, richness, biomass, and proportion of shredder EPT, as well as predictors of genus distribution. The study was conducted in northeast Pará, Brazil (2011–2017). One-way ANOVA and Redundancy Analysis (RDA) were used to test land-use effects and environmental influences on genus composition. Oil palm and forest streams presented greater abundances compared with the pasture streams. The forest treatment showed more shredder species than the pasture treatment. Biomass variability increased in pastures but decreased in oil palm areas, whereas forest streams did not differ from oil palm. The relative composition of shredders across land-use types followed similar patterns: the highest proportion was observed in forest streams, intermediate values in oil palm and mosaic, and the lowest in pasture. RDA explained 34% of the variation in genus composition, which was associated mainly with fine root cover and fast flow. *Phylloicus*, *Anacroneuria*, and *Triplectides* were negatively associated with fast flow. *Triplectides* responded positively to fine root cover, whereas *Nectopsyche* and *Fittkaulus* responded positively to fast flow. Anthropogenic alterations reduce shredder biomass and diversity, destabilizing aquatic communities. Forest conversion compromises the structure and function of Amazonian streams, reinforcing shredders as key indicators for long-term monitoring and conservation.

Keywords: EPT, land use, stream integrity, functional traits, aquatic macroinvertebrates, RDA

## Introduction

Streams are environments interconnected by a dendritic network, and in addition to their complexity and dynamism, their constant natural changes contribute to variability in habitats and resources (Stanford et al., 2017, Chapter 1, p. 3). This variability supports high biodiversity, accommodating organisms with diverse environmental requirements (Stanford et al., 2017, Chapter 1, p. 3; Peckarsky & Lamberti, 2017, Chapter 18, p. 379). Riparian vegetation structures the input of organic matter into streams through vertical input (leaf fall from riparian trees) and lateral input from the bank slope (indirect) (Pozo et al., 1997; Kochi et al., 2010; Rugenski et al., 2017, Chapter 28, p. 83). Therefore, alterations in the terrestrial environment, such as deforestation, can even indirectly influence the physical and chemical characteristics of aquatic ecosystems (Juen et al., 2016; Tchakonté et al., 2015; Valente-Neto et al., 2015). The loss of riparian vegetation can cause bank instability and siltation, affecting both physical structure and limnological conditions due to increased fine sediment input (Juen et al., 2016; Tchakonté et al., 2015).

In this context, the spatial distribution and energy content of allochthonous material are key factors structuring aquatic communities (Allen et al., 2024; Bacca et al., 2023; Luiza-Andrade et al., 2023). Considering that terrestrial and aquatic environments, although distinct, are directly interconnected through reciprocal resource exchange (Allen et al., 2024), the preservation of one should include efforts to conserve the other. The preservation of both aquatic and terrestrial ecosystems is a key objective of the United Nations (specifically, Sustainable Development Goals 6, 14, and 15), given their substantial economic, social, and environmental importance (UN, 2025).

Aquatic insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) spend their immature stages in lotic ecosystems (Morse et al., 2019; DeWalt & Ower, 2019; Reborá et al., 2019, Chapter 7, p. 139). They play a key trophic role, primarily as consumers, linking allochthonous material input with primary productivity. Some species also function as predators, occupying higher trophic positions within the ecosystem (DeWalt & Ower, 2019; Morse et al., 2019; Thorp & Covich,

2010). Shredders are potentially more sensitive to changes in land use and land cover patterns and to reductions in allochthonous resource input, reflecting their dependence on these resources for survival (Oester et al., 2023).

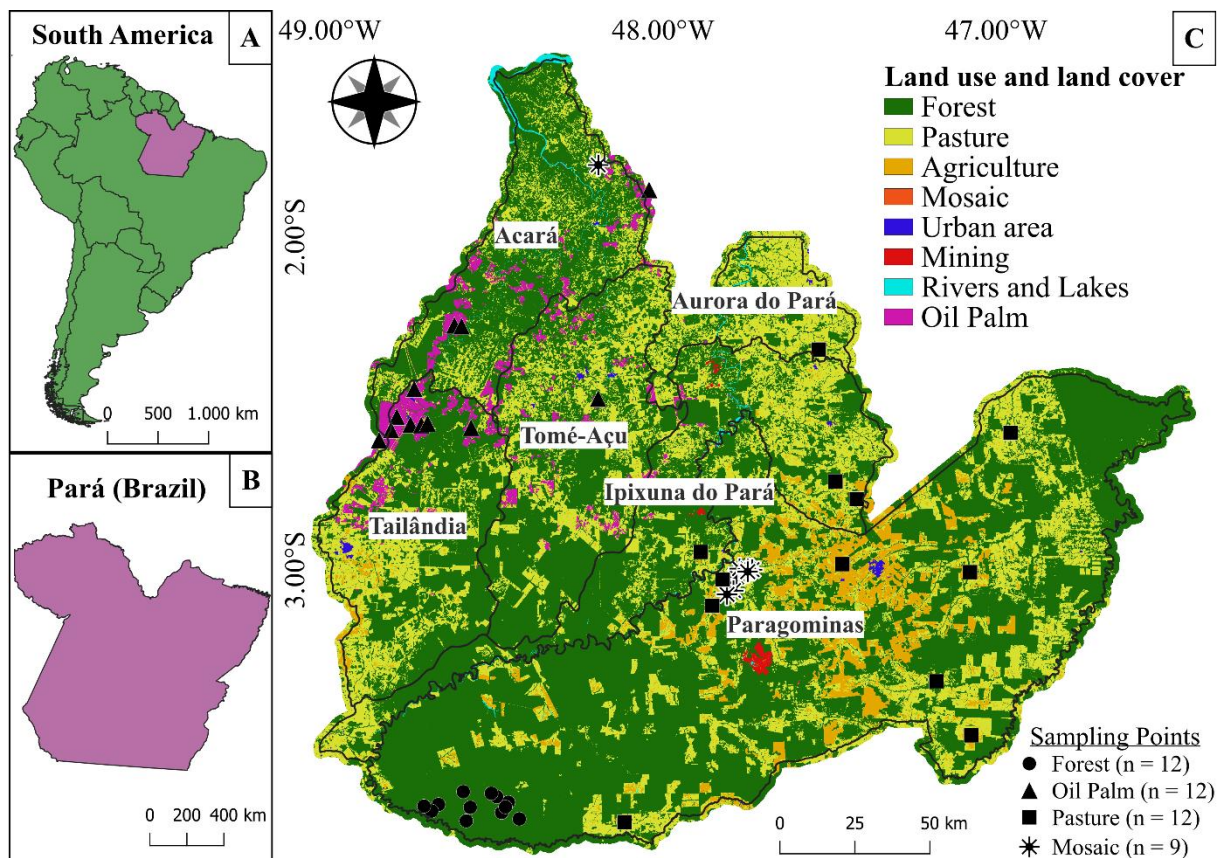
The presence of shredders is strongly influenced by external factors such as forest cover, given the positive relationship between shredder biomass and increased vegetation (Houghton, 2021; Luiza-Andrade et al., 2020). Changes in land use and land cover alter the input of allochthonous material into streams (Faria et al., 2021), thereby affecting the quality, diversity, and abundance of leaf litter (Firmino et al., 2021). Considering these shredder preferences (Gonçalves et al., 2014, Chapter 6, p. 89), the conversion of forested areas to pasture or exotic monocultures, such as oil palm, is expected to substantially influence their distribution in headwater streams. Recent research has demonstrated that shredder abundance and richness are greater in undisturbed streams, and decomposition mediated by shredders is three times higher in forested streams compared with non-forested ones (Houghton, 2021; Oester et al., 2023). However, studies in the Amazon that investigate the response of shredders to deforestation, as well as the causes of such variation, are still scarce.

In light of this, this study is aimed at evaluating how shredder distribution (abundance, richness, and biomass) responds to different land-use types, to quantify their proportional representation within the community, and to identify the environmental factors that best explain these responses. We hypothesize that the conversion of forests to anthropogenic land uses—including oil palm monocultures, pastures, and mixed-use mosaics—negatively affects shredders, resulting in reduced abundance and taxonomic richness (Faria et al., 2021; Luiza-Andrade et al., 2023). We also hypothesize that shredder biomass may remain stable or even increase in more impacted environments due to shifts in community dominance (Tonin et al., 2014; Uhler et al., 2021).

## **Materials and methods**

## Study area

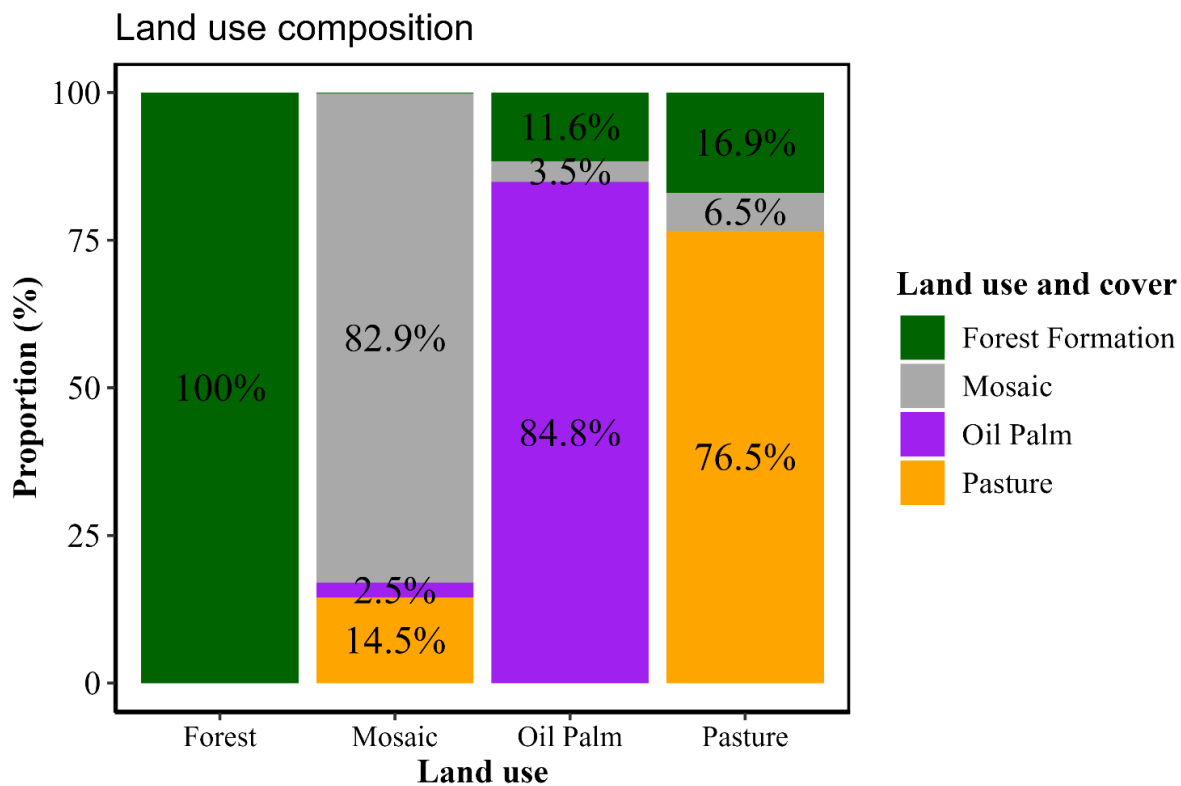
Sampling was conducted in 45 first- to third-order streams (Strahler, 1957) across the Acará and Capim river basins in northeastern Pará, Brazil (Figure 1). The sites were located within the municipalities of Tailândia, Tomé-Açu, Ipixuna do Pará, Paragominas, Acará and Aurora do Pará and were surveyed between 2011 and 2017 during the dry season, between June and November of each year. The region has a humid tropical climate, with a mean annual temperature of 26 °C and a relative humidity of 85% (Luiza-Andrade *et al.*, 2017). The natural vegetation is a dense ombrophilous forest, which is now largely degraded. Over the past 15 years, the region has lost approximately 9% of its forest cover, while soybean cultivation has expanded most rapidly, increasing by approximately 1,073% (MapBiomias, 2025).



**Fig. 1** Distribution of the 45 sampled streams in the Acará and Capim river basins in the municipalities of Tailândia, Tomé-Açu, Ipixuna do Pará, Paragominas, Acará and Aurora do Pará, Pará, Brazil

### *Sampling design*

Streams were categorized according to the dominant land-use and land-cover type (> 60%) in their catchments (Figure 2). Forest cover data were obtained from the MapBiomas platform (collection 8.1). In each region, streams classified as “Forests” were dominated by forest formations. The “Pasture” sites were dominated by livestock pasture, whereas the “Palm” sites were dominated by oil palm monoculture. The “Mosaic” sites included agricultural landscapes with mixed crop and livestock uses and less than 25% forest cover. The delineation of the sampled stream catchments was conducted via a Digital Elevation Model (DEM) from the TOPODATA project of the *Instituto Nacional de Pesquisas Espaciais* (INPE). Coordinates were obtained at section “A” of each stream, following the sampling protocol of Callisto *et al.* (2014). Catchment delineation was then processed via the “*r.watershed*” algorithm in *QGIS* (version 3.34.9).



**Fig. 2** Mean relationships among stream categories by land use within the sampled catchment

### ***Biological and Limnological Variables***

A 150 m transect was established in each stream for the collection of biological samples and the measurement of environmental variables. This transect was divided into 11 cross-sections, labelled from downstream to upstream (A-K), creating 10 longitudinal sections spaced 15 m apart (Callisto *et al.*, 2014; Luiza-Andrade *et al.*, 2017). The environmental variables were measured at the beginning of each cross-section (Callisto *et al.*, 2014). The variables assessed included the percentage of organic matter in the streambed (leaf litter, wood, macrophytes, roots, and algae), canopy cover, substrate type, riparian vegetation cover (visual estimation), human influence, and physicochemical and hydrological parameters (pH, temperature, dissolved oxygen, and flow).

Each longitudinal section was further subdivided into three 5 m segments, of which the first two were used for EPT collection. Samples were taken with an entomological drag net that was 18 cm in diameter and with 250  $\mu\text{m}$  mesh (Luiza-Andrade *et al.*, 2017; Faria *et al.*, 2017). The biological material was sorted in the field via plastic trays and entomological tweezers and subsequently preserved in 85% ethanol (Luiza-Andrade *et al.*, 2017; Faria *et al.*, 2017). Quantification and identification at the genus level were conducted in the laboratory with the taxonomic keys of Hamada *et al.* (2014) and Domínguez *et al.* (2006).

### ***Classification of functional feeding groups (FFGs)***

Ephemeroptera, Plecoptera and Trichoptera were classified into functional feeding groups (FFGs) according to the scheme proposed by Merritt & Cummins (2019). To calculate abundance, richness and biomass, as well as the relative proportion of shredders across land-use types, we followed the shredder definition of Houghton (2021) and Santos *et al.* (2024). For shredders, we

considered the main representatives of each order, selecting the taxa with the highest affinity for shredding.

### *Data analysis*

The dry biomass (mg) of shredders was estimated via allometric equations from the literature that relate body mass to body length for each EPT genus (Paciência, 2012; Dekanová *et al.*, 2022) (Table 1). Biomass estimation based on individual body size is widely used because it is fast, practical, and preserves specimens for future analyses, unlike traditional gravimetric approaches (Mährlein *et al.*, 2016). In addition, due to the influence of ethanol preservation on individual weight and the variation in storage time among samples, the indirect biomass quantification method was the most reliable option for estimating the average productivity of streams under different land-use types (Leuven *et al.*, 1985; Mährlein *et al.*, 2016). Therefore, in this study we selected the basic length-mass allometric model using constant values specifically developed for EPT insects from the Neotropical region, including the genera represented in our dataset, which provides more accurate biomass estimates than generalized or global models (Benke *et al.*, 1999; Paciência, 2012).

The dry mass (DM) of each taxon was calculated via genus-specific constants ( $a$  and  $b$ ) and the mean length of each genus ( $x$ ). The total biomass of each genus ( $bg$ ) was obtained by multiplying DM by the abundance of the respective genus ( $bgx = DM \times Nx$ ), considering the treatments separately. The total biomass per stream ( $bT$ ) was then calculated as the sum of the biomass of each genus ( $bT = \sum bgn$ ).

**Table 1.** Allometric equations used to calculate the biomass of each genus

Order	Family	Genus	a	b	Equation	Reference
<b>Ephemeroptera</b>	Leptophlebiidae	<i>Fittkaulus</i>	-5.294	2.618	$\ln(DM) = \ln(a) + b \cdot x$	
<b>Plecoptera</b>	Perlidae	<i>Anacroneuria</i>	-1.789	2.555	$\ln(x^*)$	

	Calamoceratidae	<i>Phylloicus</i>	-1.844	2.075	
<b>Trichoptera</b>	Leptoceridae	<i>Nectopsyche</i>	-1.73	2.126	(Paciência, 2012;
					Dekanová <i>et al.</i> ,
					2022)
	Leptoceridae	<i>Triplectides</i>	-1.712	2.109	

\*x = body length (cm)

To evaluate how shredders respond to different land-use types, a one-factor ANOVA was performed, with richness, abundance, and biomass considered separately as response variables. Biological data were log-transformed ( $\log(x+1)$ ) to reduce asymmetry among values (Legendre & Legendre, 2012). The assumptions of homogeneity of variances (Levene's test,  $p > 0.05$ ) and data normality (P-Plot) were verified beforehand. When significant differences were detected ( $p < 0.05$ ), Tukey's post hoc test was applied. The proportion of shredders was calculated from their abundance in each land-use type.

To identify the environmental variables influencing the distribution of shredder genera across different land-use categories, a Redundancy Analysis (RDA) was conducted with the selected environmental variables, using the *forward.sel* function ( $p\text{-value} < 0.05$ ) (Dray *et al.*, 2023). The relatively high abundances in each stream were Hellinger transformed to reduce the effect of dominance within the community, and the environmental variables were standardized via the z score method (Legendre & Legendre, 2012; Lima *et al.*, 2022). All analyses were conducted in R software (version 4.4.0) through the RStudio interface (v.2025.05.0) via the “stats” (R Core Team, 2024), “vegan” (Oksanen *et al.*, 2024), and “adespatial” packages (Dray *et al.*, 2023).

## Results

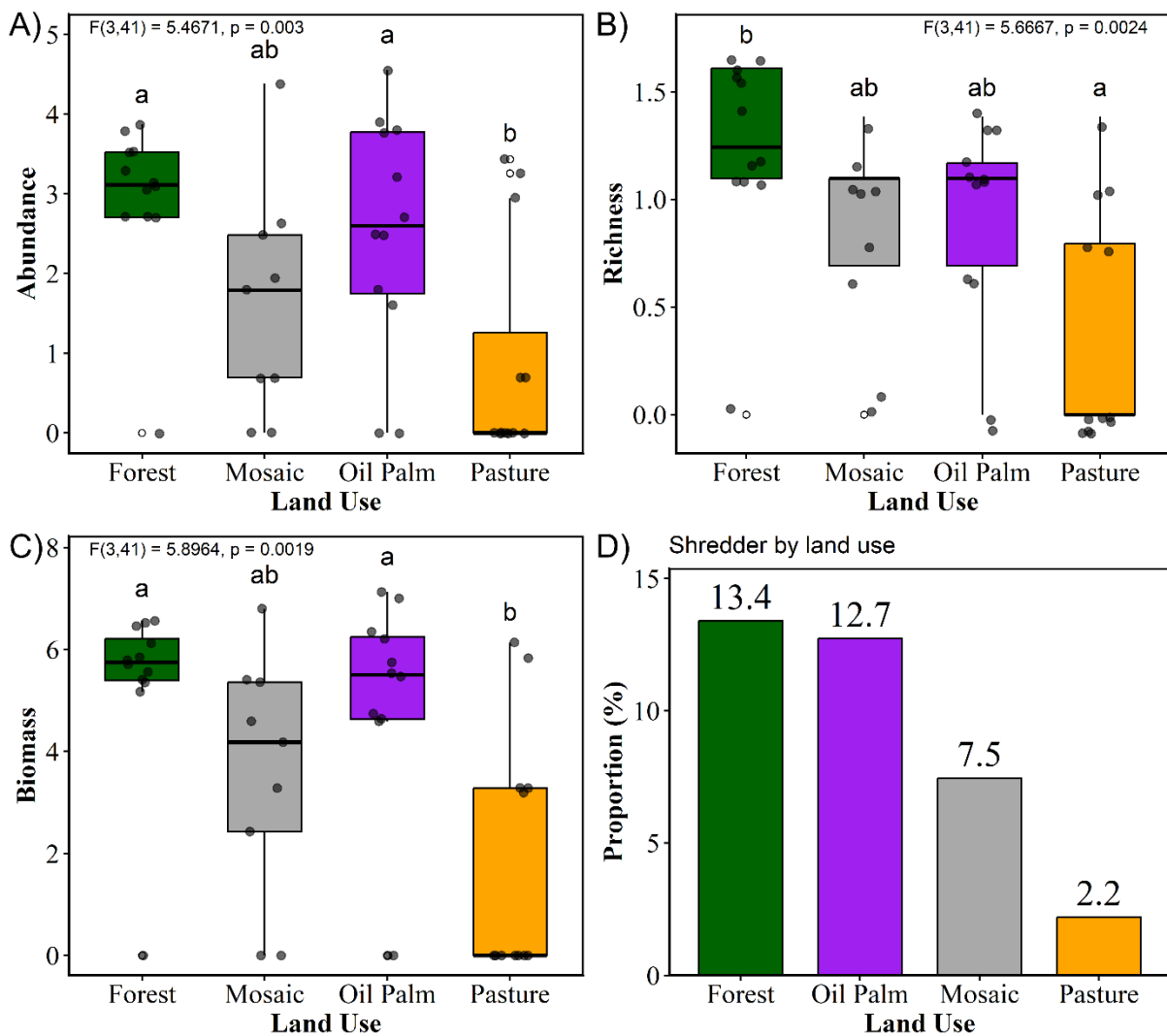
A total of 775 shredder insects belonging to five genera of the orders EPT were analysed (Table 2). We detected a significant effect of land use on shredder abundance ( $F_{(3, 41)} = 5.467$ ;  $p = 0.003$ ).

**Table 2.** Shredder abundance by dominant land use type.

GENUS	ABUNDANCE			
	OIL PALM	FOREST	MOSAIC	PASTURE
<i>Anacroneturia</i>	87	58	9	12
<i>Fittkaulus</i>	0	0	0	24
<i>Nectopsyche</i>	0	8	0	1
<i>Phylloicus</i>	130	142	87	18
<i>Triplectides</i>	80	79	20	20
<b>TOTAL</b>	<b>297</b>	<b>287</b>	<b>116</b>	<b>75</b>

We detected a significant effect of land use on shredder abundance ( $F_{(3,41)} = 5.467$ ;  $p = 0.003$ ). Compared with the pasture streams, the oil palm and forest streams presented significantly greater abundances (Figure 3a). On average, the forest streams contained 17.6 more individuals than did the pasture streams (Tukey = 0.003), whereas the oil palm streams contained 18.5 more individuals (Tukey = 0.026). No significant differences, however, were detected between forest areas and oil palm (Tukey = 0.865) or mosaic streams (Tukey = 0.128). Similarly, mosaic streams did not differ significantly from oil palm (Tukey = 0.432) and pasture (Tukey = 0.631) streams.

For richness, land-use type had a significant effect, with different land-use and cover categories influencing the richness of shredder EPT ( $F_{(3,41)} = 5.666$ ,  $p = 0.002$ ). On average, the forest treatment had two more shredder species than did the pasture treatment (Tukey = 0.001) (Figure 3b). No significant differences were observed among the other areas (Tukey<sub>forest/mosaic</sub> = 0.191; Tukey<sub>forest/oil palm</sub> = 0.394). Oil palm, mosaic, and pasture streams did not differ significantly from each other (Tukey<sub>Oil Palm/Mosaic</sub> = 0.942; Tukey<sub>Oil Palm/Pasture</sub> = 0.077; Tukey<sub>Mosaic/Pasture</sub> = 0.317).



**Fig. 3 (3a–3d)** Ecological responses of shredders in eastern Amazon streams across different land-use types. Figure 3a) Mean shredder abundance and standard deviation (SD) of shredders; Figure 3b) Mean shredder richness and standard deviation (SD) of shredders; Figure 3c) Mean dry biomass (mass - length) and standard deviation (SD) of shredders; Figure 3d) Proportion of shredders per land-use type. The land-use categories are indicated by color: forest (green), mosaic (gray), oil palm (purple), and pasture (orange). Different letters represent statistically significant differences between treatments according to Tukey's test

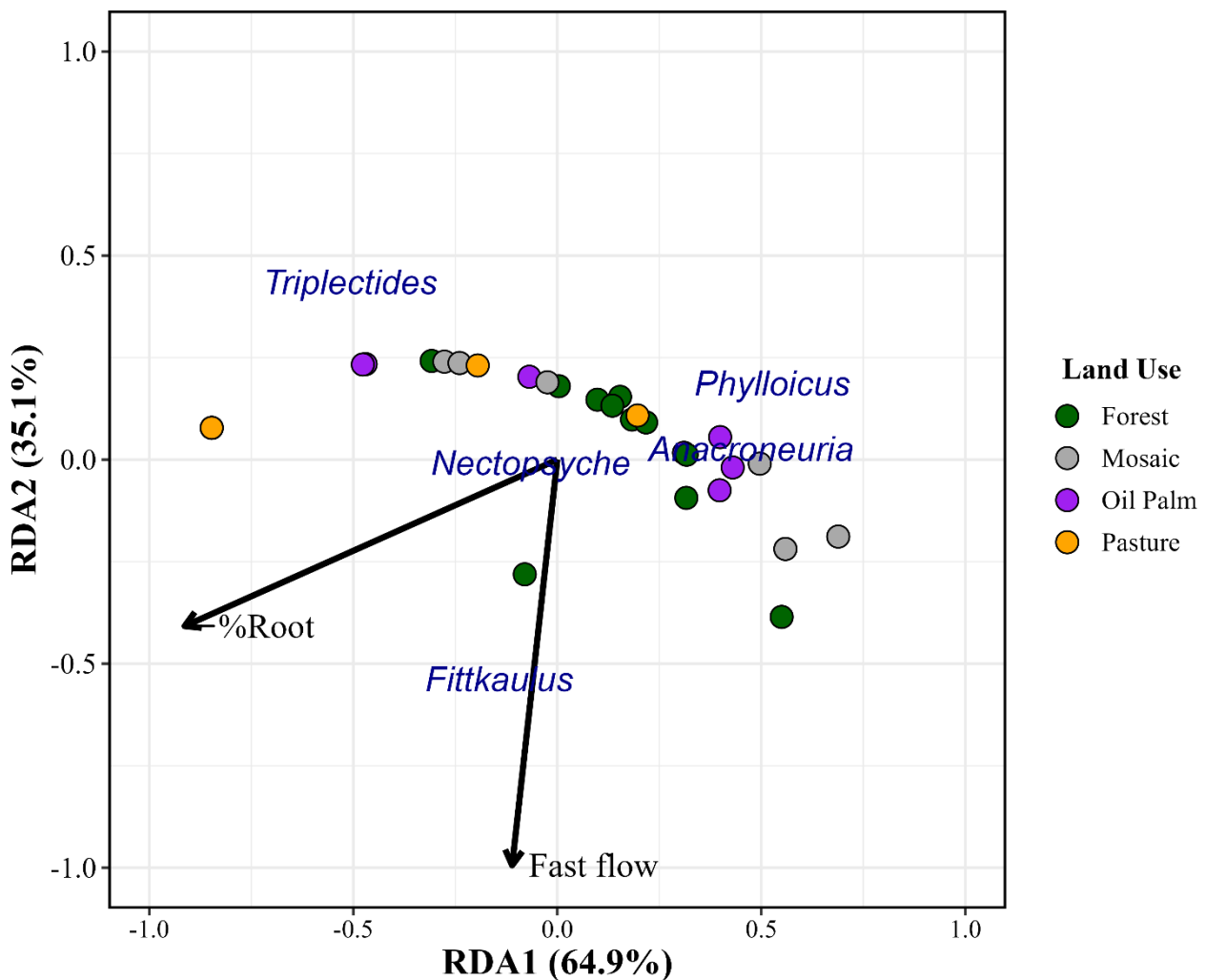
Compared with forest streams, there was significant variation in biomass among the treatments ( $F_{(3,41)} = 5.896, p = 0.002$ ) (Table 3). Forest streams did not differ from oil palm (Tukey = 0.916) or mosaic streams (Tukey = 0.272). However, pasture streams had lower biomass than did forest areas, with an average difference of 286.7 mg (Tukey = 0.002), and compared with oil palm streams, with an average difference of 303.85 mg (Tukey = 0.012) (Figure 3c). Mosaic streams

showed no significant differences from oil palm (Tukey = 0.607) or pasture (Tukey = 0.301). The relative composition of shredders across land-use types followed similar patterns: the highest proportion was observed in forest streams, intermediate values in oil palm and mosaic, and the lowest in pasture (Figure 3d).

**Table 3.** Shredder mean length and biomass per individual and per land-use type.

GENUS	Mean length (mm)	Individual biomass (mg)	OIL PALM	FOREST	MOSAIC	PASTURE
<i>Anacroneuria</i>	6.3	12.90	1122.41	748.27	116.11	154.81
<i>Fittkaulus</i>	5.5	0.61	0.00	0.00	0.00	14.61
<i>Nectopsyche</i>	7.5	8.72	0.00	69.79	0.00	8.72
<i>Phylloicus</i>	6.3	10.35	1345.42	1469.61	900.39	186.29
<i>Triplectides</i>	10.5	25.71	2057.15	2031.44	514.29	514.29
		<b>TOTAL</b>	<b>4524.97</b>	<b>4319.11</b>	<b>1530.79</b>	<b>878.73</b>

The first two axes of the RDA explained 34% of the variation in shredder composition ( $F = 9.22$ , adjusted  $R^2 = 0.34$ ,  $p = 0.001$ ) (Figure 4). Among the selected variables, only fast flow and root (%) were responsive to the distribution of shredder genera ( $p = 0.001$ ).



**Fig. 4** Relationships between environmental predictors and shredders through RDA in streams of the Capim and Acará River Basins, Pará, Brazil. The circles are colored according to the dominant land-use type

The first axis (64.9%) represented a gradient of exposed root concentration, with higher percentages of roots negatively associated with the axis and predominated by points from pasture and mosaic streams (Table 4). In contrast, forest and oil palm streams were positioned positively along the axis, indicating lower presence of exposed roots. The second axis (35.1%) was structured primarily by fast flow, which was negatively associated with the axis and especially influenced streams located in forest, oil palm and pasture catchments (Table 4).

Among shredders, *Phylloicus*, *Anacroneuria*, and *Triplectides* were negatively associated with fast flow, whereas *Nectopsyche* and *Fittkaulus* showed a positive association. In addition,

*Anacroneturia* and *Phylloicus* were more frequent in streams with a lower percentage of exposed roots, while *Triplectides* occurred in streams with higher concentrations of exposed roots.

**Table 4.** Contribution of environmental predictors to shredder abundance in streams of the Capim and Acará River Basins, Pará, Brazil (RDA axes 1 and 2).

<b>Environmental predictor</b>	<b>RDA1</b>	<b>RDA2</b>
Fast flow	-0.110	<b>-0.993</b>
%Root	<b>-0.913</b>	-0.407

## Discussion

Our hypothesis that shredder abundance, richness, and biomass are influenced by multiple land-use types was supported, as forest streams presented higher shredder metrics than did pasture streams. In headwaters (first- to third-order) or forested streams, riparian vegetation limits the primary productivity of algae and macrophytes, as the light reaching these systems is insufficient (Wallace *et al.*, 1997; Rugenski *et al.*, 2017, Chapter 28, p. 83; Graça *et al.*, 2018; Albrecht *et al.*, 2021). Consequently, allochthonous organic matter becomes the main source of energy and carbon in these ecosystems (Vannote *et al.*, 1980; Wallace *et al.*, 1997; Firmino *et al.*, 2021). The reduction of allochthonous inputs due to deforestation alters the chemical and physical characteristics of streams, negatively affecting aquatic insect communities in the eastern Amazon and resulting in the loss of more sensitive genera and a decrease in taxonomic richness (Faria *et al.*, 2021; Luiza-Andrade *et al.*, 2023). In terms of dry biomass and shredder abundance, oil palm and forest streams differed significantly from pasture streams. In pastures, the analyses confirmed a loss of diversity, abundance, and individual biomass as a consequence of converting forested land to anthropogenic uses (Sonoda, 2025).

The EPT group is widely used in biomonitoring studies because of its broad distribution in aquatic environments and its plasticity in occupying different habitats (Akamagwuna *et al.*, 2019, 2021; Brasil *et al.*, 2022; Bacca *et al.*, 2023). In addition, EPT taxa can be classified into FFGs based on morphological and behavioural traits related to feeding (Ramirez & Gutiérrez-Fonseca, 2014). The different types of organic matter consumed in streams—fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), prey, algae, and biofilms—are associated with distinct morphological adaptations, particularly in mouthpart structures (Cummins, 1973; Usseglio-Polatera *et al.*, 2000; Poff *et al.*, 2006; Gonçalves *et al.*, 2014, Chapter 6, p. 89). The principal FFGs include collectors (both gatherers and filterers), shredders, predators, and scrapers (Cummins, 1973; Usseglio-Polatera *et al.*, 2000; Poff *et al.*, 2006; Gonçalves *et al.*, 2014, Chapter 6, p. 89; Houghton, 2021).

Shredders are particularly important, as they convert CPOM into FPOM, accelerating leaf litter decomposition and making resources available to other organisms (Gonçalves *et al.*, 2014, Chapter 6, p. 89; Sena *et al.*, 2020). Their presence is influenced by leaf litter palatability, which depends on the decomposition stage, microbial conditioning, availability, and leaf quality (Kikuchi & Uieda, 2005; Silveira *et al.*, 2006; Martínez *et al.*, 2013; Moretti *et al.*, 2020). Thus, changes in the quality of leaf litter entering streams can directly affect decomposition rates and ecosystem functioning (Firmino *et al.*, 2021). Moreover, shredders are key agents of leaf litter decomposition because of their large biomass and body size and their presence is therefore proportional to the decomposition rate of stream leaf litter (Gonçalves *et al.*, 2014, Chapter 6, p. 89; Sena *et al.*, 2020). Their close relationship with environmental gradients indicates that shredder loss is associated mainly with reduced inputs from riparian vegetation due to vegetation simplification (Brand & Miserendino, 2015; Serpa *et al.*, 2020).

Compared with forested streams, agricultural streams presented the lowest shredder richness, suggesting that reduced resource availability affects organisms dependent on allochthonous energy inputs. Similarly, previous studies, such as Lima *et al.* (2022), have shown that shredders and

collectors tend to be more strongly associated with forested streams, where allochthonous resource input is higher. Furthermore, it is well established that stream integrity and water quality are directly linked to surrounding vegetation (Tanaka *et al.*, 2015). Consequently, vegetation homogenization and loss compromise stream integrity, leading to decreased aquatic community diversity and changes in microclimate, substrate and nutrient inputs (Paiva *et al.*, 2017; Arce *et al.*, 2023).

In addition to the loss of organic matter (OM), riparian vegetation can influence organism distribution. Shredders preferentially consume more palatable, high-quality resources, which are more nutritious and attractive due to their lower C:N ratios and structural components, which vary according to plant type (Martínez *et al.*, 2013; Fernandes *et al.*, 2015; Firmino *et al.*, 2021). This pattern may reflect shredder preference for native vegetation, which typically presents higher quality and palatability (Boyero *et al.*, 2012; Pelizari *et al.*, 2022) or results from the chemical composition of plants affecting microbial conditioning and influencing their feeding preference (Kikuchi & Uieda, 2005; Firmino *et al.*, 2021). In line with this, forest cover directly influences soil chemistry, providing more nutrients and microorganisms to leaves (Santorufó *et al.*, 2021). Consequently, when microbial activity is reduced, leaf conditioning is impaired, leading to slower decomposition and lower carbon and nitrogen concentrations, which classify the material as low quality (Santorufó *et al.*, 2021; Firmino *et al.*, 2021).

Furthermore, plant types may contain refractory, secondary, or toxic compounds that protect leaves against parasitism and herbivory, such as phenolic compounds (Graça *et al.*, 2001; Oliveira & Nessimian, 2010; Gonçalves *et al.*, 2014, Chapter 6, p. 89). These chemical traits inhibit microbial activity on leaves and consequently affect shredder palatability (Graça *et al.*, 2001; Oliveira & Nessimian, 2010; Kochi *et al.*, 2010; Gonçalves *et al.*, 2014, Chapter 6, p. 89; Moretti *et al.*, 2020). Therefore, shredders that select leaves according to palatability and quality may be affected in impacted streams because of the reduced availability of allochthonous resources caused by deforestation (Boyero *et al.*, 2012; Pelizari *et al.*, 2022; Firmino *et al.*, 2021).

Biomass, in addition to being important for assessing ecosystem structure and energy flow, also reflects environmental productivity (Cummins *et al.*, 2022; Lynch, 2024). Thus, although the richness of pasture streams may not differ from that of oil palm streams, they differ in terms of biomass and abundance. Moreover, a comparison of these attributes revealed that biomass variation increases in pasture streams, whereas it decreases in oil palm streams, despite abundance showing the opposite pattern. This highlights how land-use type influences environmental productivity, reflected in the allometric measures of individuals, regardless of abundance. This supports the statement by Uhler *et al.* (2021), who argued that an increase in large-bodied species may influence biomass but does not necessarily affect the total number of species. In pasture streams, although Tonin *et al.* (2014) suggest that large-bodied shredders could help sustain overall biomass even when abundance decreases, our results did not reflect this expected pattern or our initial hypothesis. Instead, the marked reduction in forest cover was accompanied by declines in both shredder abundance and biomass. The observed decline in biomass in degraded areas highlights the negative influence of land-use change on stream productivity. This result supports previous studies that associate higher biomass with intact environments, given the strong dependence of shredders on allochthonous resources (Houghton, 2021).

The impact of deforestation and alterations in riparian vegetation is reflected in the variation in biomass in pasture streams. Among the treatments, only the pasture streams presented a significant reduction in biomass, with a lower mean biomass than the forest and oil palm streams did. This finding indicates that the greatest impact on local productivity occurred in streams with relatively high deforestation rates and no forest replacement. This pattern may also explain the absence of significant differences between pasture streams and those with less than 60% forest cover, classified as mosaic. Despite the lack of differences among environments with lower vegetation cover, mosaic streams sustained sufficient biomass to show no significant differences compared with forest and oil palm streams. This pattern can be explained by the *Intermediate Disturbance Hypothesis*, proposed by Connell (1978), which states that in environments subjected to moderate levels of disturbance,

species that previously had low dispersal and growth rates are able to establish more successfully, resulting in higher diversity compared with more highly disturbed environments. Forested streams had a greater proportion of shredders, which are typically associated with greater availability of organic matter (Houghton, 2021). As primary consumers in forested streams, their higher proportion is explained by their dependence on organic matter inputs (Oliveira & Nessimian, 2010; Brasil *et al.*, 2014).

RDA revealed that the composition of the analysed genera was strongly structured along an environmental gradient represented by the percentage of exposed roots (%Root) and stream flow. The first axis represented higher percentages of root cover associated with pasture and mosaic streams, a pattern also observed by Andrade *et al.* (2022) in impacted environments. This pattern is consistent with the advance of erosive processes in human-impacted streams, where the removal of riparian vegetation favours bank instability (Santorufo *et al.*, 2021; Luiza-Andrade *et al.*, 2022). The second axis was structured by fast flow, was negatively associated with the axis and influenced mainly streams located in forest, oil palm and pasture catchments.

Although the RDA did not directly include variables related to OM quality or microbial activity, it is plausible that vegetation cover and flow dynamics indirectly influence detritus conditioning, affecting its quality and availability (Richardson & Neill, 1991; Canhoto *et al.*, 2013; Santorufo *et al.*, 2021). As stated by Rolls *et al.* (2012), slow flows associated with more stable banks favor the deposition and accumulation of particulate organic matter, creating microhabitats suitable for shredder activity. These results suggest that alterations in riparian cover and hydrological regimes influence not only the physical structure of streams but also the ecological processes underlying decomposition and energy flow in aquatic systems (Rolls *et al.*, 2012).

The response of shredders to these variables suggests distinct ecological strategies among taxa. Among shredders, *Phylloicus*, *Anacroneuria*, and *Triplectides* were negatively associated with fast flow, indicating a preference for low-current environments where detritus retention and substrate stability favour organic matter fragmentation (Rolls *et al.*, 2012). Whereas *Nectopsyche* and

*Fittkaulus* were positively associated, suggesting greater tolerance to unstable hydrodynamic conditions and, as shredders, a particular preference for more aerated streams with enhanced leaf conditioning, as observed by Canhoto *et al.* (2013). In addition, *Anacroneturia* and *Phylloicus* were more common in streams with a lower percentage of exposed roots, whereas *Triplectides* occurred in streams with higher concentrations of exposed roots, possibly exploiting these structures as refuges or zones of resource accumulation (Luiza-Andrade *et al.*, 2022). The relationships between the explanatory variables and the observed patterns align with evidence that riparian vegetation integrity directly affects the hydrological and physical conditions of lotic ecosystems (Santorufu *et al.*, 2021). In preserved forest streams, practices such as active restoration with native species contribute to increased infiltration, water retention and soil stability (Brizzi *et al.*, 2018; Rodrigues & Torres, 2023). Conversely, the removal of vegetation cover in pasture or urban land-use streams intensifies erosion and reduces the functionality of riparian zones (Gomes *et al.*, 2018).

Despite this, 66% of the variation in genera distribution remains unexplained, indicating that other factors influence shredder distribution, even indirectly. Given that the distribution of individuals was more strongly associated with streambed structure, it is plausible to suggest that such structural modification indirectly affects shredders by favouring predators – since the substrate is also used as protection from attacks (Hamada *et al.*, 2014) – or by hindering foraging, creating physical obstacles and limiting the availability of organic matter on the streambed. To further investigate these patterns, it would be useful to perform an RDA including more specific environmental variables related to streambed physical structure, or to conduct correlation analyses exploring biological relationships between shredders and their main predators.

The loss of shredder biomass and diversity highlights how anthropogenic alterations, such as deforestation, destabilize aquatic communities from the base of the food web (Yule *et al.*, 2010). As primary consumers, shredders are linked to predators (both vertebrates and invertebrates), whose presence depends on prey availability, thereby affecting higher trophic levels through a cascading effect (Oliveira & Nessimian, 2010; Brasil *et al.*, 2014). Additionally, since shredders contribute to

carbon and nutrient cycling through leaf litter decomposition in streams, their long-term loss may alter community composition or slow biogeochemical processes, such as carbon cycling (Sonoda, 2025).

## **Conclusion**

We demonstrated that the replacement of forested streams with anthropogenic land significantly reduces the abundance, richness, and biomass of shredder EPT taxa.

Our study integrated multiple biological metrics, directly linking shredder community structure with land use and land cover. Including biomass as a complementary metric revealed differences in ecological productivity that would not be evident from individual counts or species richness alone, contributing to a more robust assessment of environmental impact and energy flow. The reduction in shredder diversity, abundance and biomass compromises not only the base of the food web but also nutrient dynamics and the ecological processes associated with decomposition and carbon cycling.

Despite the ecological importance of aquatic insects, studies addressing their environmental preferences and responses to gradients of anthropogenic alterations in the Amazon remain scarce. Our results highlight the urgency of expanding research efforts and strengthening public policies aimed at conserving and monitoring aquatic ecosystems. Biological indices based on macroinvertebrates, such as shredders, appear promising for detecting environmental degradation and guiding management actions.

The preservation and restoration of riparian vegetation are fundamental to maintaining ecological functions in lotic ecosystems and achieving global sustainability targets, particularly within the framework of the UN Sustainable Development Goals. This study provides evidence reinforcing the need to protect aquatic biodiversity and associated ecosystem services, especially in regions of high environmental and ecological importance, such as the Amazon.

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**Ethics approval**

Not applicable.

**Consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Data availability**

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

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**Author contributions**

Conceptualization: Ana Beatriz Oliveira Pampolha; Methodology: Ana Beatriz Oliveira Pampolha, Josinete Sampaio Monteles, Gabriel Martins Cruz, Viviane Caetano Firmino, Leandro Juen; Investigation: Ana Beatriz Oliveira Pampolha; Formal analysis: Ana Beatriz Oliveira Pampolha; Data interpretation: Ana Beatriz Oliveira Pampolha, Josinete Sampaio Monteles, Gabriel Martins Cruz, Viviane Caetano Firmino, Leandro Juen; Preparation of figures and tables: Ana Beatriz Oliveira Pampolha, Gabriel Martins Cruz, Viviane Caetano Firmino; Writing – original draft: Ana Beatriz Oliveira Pampolha; Writing – review and editing: Josinete Sampaio Monteles, Viviane

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# 3. Sessão II

## **Influence of land use on the functional distribution of aquatic insects with gills**

A segunda sessão desta dissertação foi elaborada e formatada conforme as normas da publicação científica *River Research and Applications*, disponível em:  
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## INFLUENCE OF LAND USE ON THE FUNCTIONAL DISTRIBUTION OF AQUATIC INSECTS WITH GILLS

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## ABSTRACT

Changes and removal of riparian vegetation alter the physicochemical characteristics of streams, disrupting processes such as respiration. Insects of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are sensitive bioindicators of disturbances and exhibit variation in gill morphology. However, studies assessing the effects of deforestation on the respiratory responses of these insects remain scarce. This study aimed to quantify variation in richness and abundance of gill-breathing EPT and to evaluate the influence of environmental variables among streams under different land-use types. We sampled 45 streams in northeastern Pará State, Brazil, and used One-way ANOVA, RLQ, and Fourth-Corner to analyse the influence of land use and environmental variables on EPT gill configuration. Redundancy Analysis (RDA) was applied to identify the main variables influencing EPT distribution. Forested streams exhibited higher EPT richness. RLQ and Fourth-Corner analyses indicated that environmental variables influenced only genus composition. The RDA explained 17% of the variation, with mosaic and pasture areas showing higher indices of agricultural impact proximity, whereas forested areas exhibited greater diversity and larger mean substrate size. The absence of associations between variables and traits may indicate that multiple environmental factors influence gill distribution. Land-use types influenced substrate composition, and shelter-building genera were favoured in forested streams. In conclusion, gills are sensitive structures affected by multiple environmental factors, and substrate modifications driven by land-use changes in aquatic systems influence the richness and distribution of gill-breathing insects. These results support the premise that more conserved areas exhibit greater microhabitat diversification, encompassing different niches and physiological requirements.

Keywords: functional diversity, aquatic macroinvertebrates, substrate, environmental integrity, deforestation

## 1 Introduction

Lotic environments, such as streams, are constantly changing due to alluviation processes, mainly mediated by the surrounding landscape and moderated by the riparian vegetation (Hauer & Lamberti, 2017). The removal of this vegetation can destabilize the soil, promoting erosion altering water chemistry, reducing substrate heterogeneity and habitat quality, consequently influencing the structure of aquatic communities (Faria *et al.*, 2017; Bacca *et al.*, 2023; Cruz *et al.*, 2025).

It is well established that stream ecosystems respond directly to changes in the surrounding landscape, given the close connection between aquatic ecosystems and their adjacent landscapes (Bacca *et al.*, 2023; Cruz *et al.*, 2025). Therefore, in the long term, these changes affect hydrological processes, change flow patterns and substrate types, finally altering assemblage composition and ecological relationships (Tabacchi *et al.*, 1998; Thorp & Covich, 2010; Salles & Ferreira-Junior, 2014; Buffington & Montgomery, 2022). Thus, even land-use changes that appear to be subtle, such as the removal of riparian vegetation, can increase susceptibility to erosion and siltation, significantly compromising habitat quality and the structure of aquatic communities (Faria *et al.*, 2017; Bacca *et al.*, 2023). In addition, as riparian vegetation declines, water temperature and metabolic activity increase, raising oxygen demand and reducing its solubility (Verberk & Bilton, 2013; Faria *et al.*, 2021; Capon *et al.*, 2021).

Human activities associated with deforestation, such as agriculture and mining, promote the homogenisation of riverbeds by increasing sediment input and the burial of organic matter (Martins *et al.*, 2014; de Castro *et al.*, 2017; McKenzie *et al.*, 2020). Studies indicate that oil palm plantations are associated with increases in fine sediment and pH, leading to a loss of biological diversity and environmental integrity (Paiva *et al.*, 2017; Luiza-Andrade *et al.*, 2017). Moreover, in pasture areas this anthropogenic increase in sediment may be more detrimental than nutrient enrichment (Townsend *et al.*, 2008).

For aquatic insects, mainly individuals of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), the input of fine sediments causes abrasions to their anatomically exposed structures (Turley *et al.*, 2016; Odume, 2022). The resulting silting up of the streambed is a limiting factor for their distribution, as they depend on the substrate for oviposition, shelter, and foraging (Martins *et al.*, 2014). Furthermore, with the reduction in the input of coarse particulate organic matter (CPOM) from riparian vegetation, insect groups such as Trichoptera, which use leaves and branches as food, shelter and to build their cases, may present changes in their richness, abundance, and distribution. Among the benefits of case construction include aiding breathing through the wavelike movement of the larvae's abdomen, creating unidirectional water flow over the gills, and facilitating gas exchange. Thus, genera that depend on specific substrates, possess fragile structures, such as gills

or filtering mouthparts, or even require higher oxygen demand, are most affected (Mathers *et al.*, 2017; McKenzie *et al.*, 2020).

Within this framework, while many studies focus on general functional traits of aquatic insects, the specific role of gill respiration remains underexplored as a central functional attribute that enables insects to survive underwater, which may occur via the tegument, gills, a plastron, spiracles, or a hydrostatic vesicle (Usseglio-Polatera *et al.*, 2000; Poff *et al.*, 2006; Merritt & Cummins, 2019). Respiration is a determining factor in the maintenance of aquatic insect communities, as it depends on an adequate supply of dissolved oxygen in the water to oxygenate tissues, as well as on temperature, which influences metabolic demand (Merritt & Cummins, 2019). Therefore, environmental characteristics may shape the presence and distribution of these insects depending on their respiratory type and physiological requirements.

Among these respiratory types, gill respiration stands out as one of the most susceptible and sensitive structures to environmental changes, as it is affected by reduced dissolved oxygen and increased fine sediment input and channel substrate homogeneity (Tchakonté *et al.*, 2015). For EPT, gills serve not only for respiration but also for activities such as locomotion, filtration, and osmotic regulation (Merritt & Cummins, 2019). Owing to this functional diversity, these structures exhibit wide morphological variation and can be classified according to their shape (e.g. oval, elongated), structure (e.g. lanceolate, plate), arrangement (e.g. maxillary, thoracic), and position (e.g. lateral, dorsal) (Poff *et al.*, 2006; Domínguez *et al.*, 2006; Reynaga, 2013; Merritt & Cummins, 2019; Akamagwuna *et al.*, 2019).

This functional and anatomical diversity of gills in immature EPT stages results in differentiated responses to environmental stressors, altering the vulnerability of each species to the same impact (McKenzie *et al.*, 2020; Odume, 2022). For example, some Ephemeroptera genera are more tolerant of fine sediments because their hairy gill structures prevent sediment accumulation; however, they may still experience physical abrasion and clogging (Akamagwuna *et al.*, 2019; McKenzie *et al.*, 2020). Meanwhile, genera with larger gill surface areas, such as plate-like or lamellate gills, prefer habitats with coarse, gravelly substrates because their gills may become clogged (Akamagwuna *et al.*, 2019; McKenzie *et al.*, 2020). Conversely, genera with operculate gills are less affected, as these gills provide protection (Akamagwuna *et al.*, 2019).

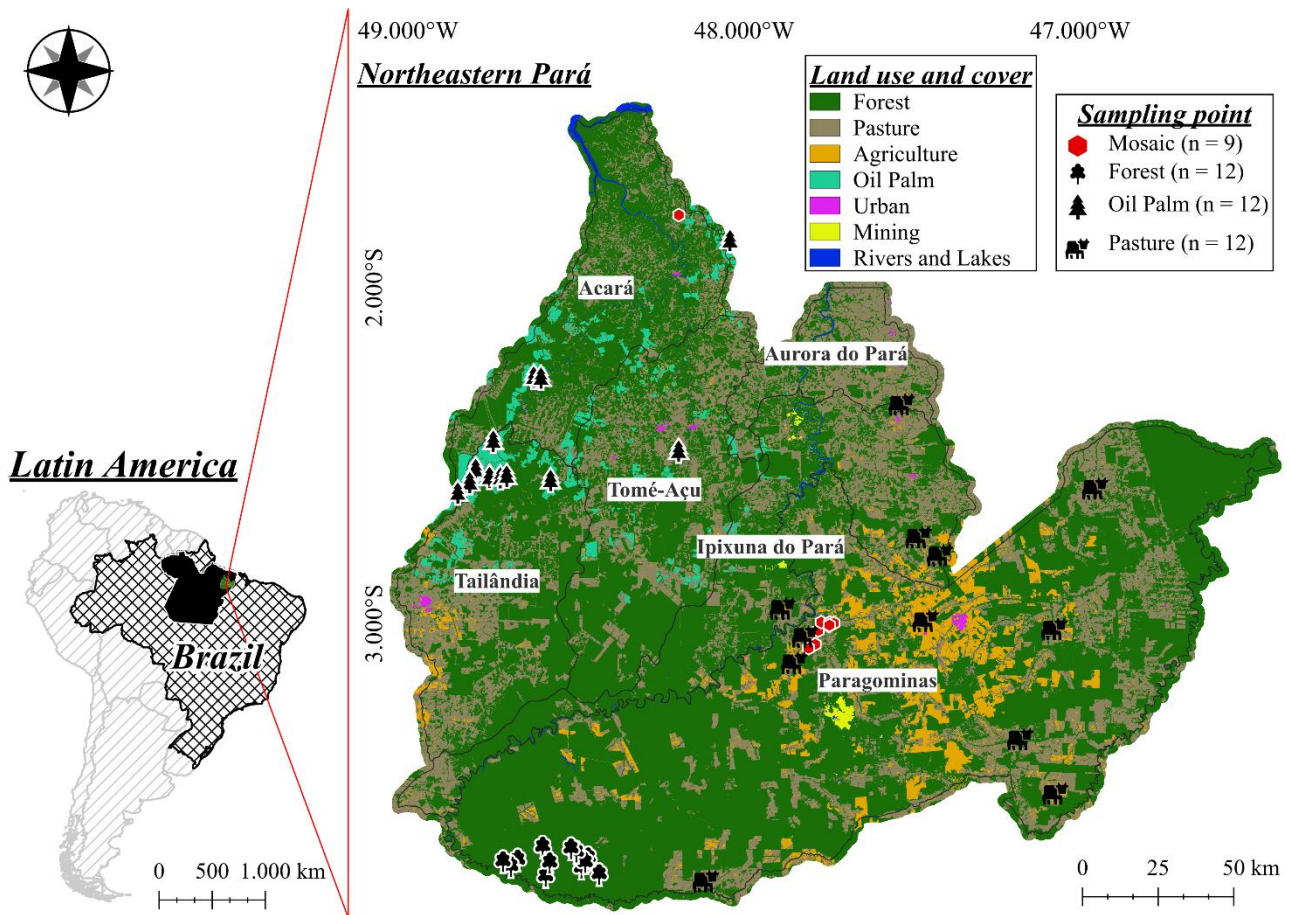
However, while many studies focus on general functional traits of aquatic insects, the specific role of gill respiration remains underexplored as a central functional attribute. Consequently, studies investigating the influence of anthropogenic alterations on the distribution of gill-bearing genera of EPT in the Amazonian streams, as well as the morphological susceptibility of these structures, remain limited.

In this context, our objective was to quantify how the richness and abundance of gill-breathing EPT larvae vary between forested streams and those embedded in oil palm, mosaic, and pasture landscapes. We also examined whether environmental variables modulate the distribution of different gill morphologies (shape and structure) present in the Amazonian EPT fauna, and whether gill types exhibit specific patterns in response to different environmental variables, in order to identify patterns potentially indicative of environmental alteration. We hypothesised that streams with different types of land use exhibit a reduced richness of gill-breathing EPT, as vegetation loss tends to intensify fine sediment deposition, reduce oxygen availability, which compromises the metabolic and respiratory efficiency of gill-bearing insects and, consequently, essential microhabitats for these organisms. Accordingly, we expected that streams in more anthropized environments would support a less diverse gill-breathing EPT fauna compared with forested streams. Our results contribute to the understanding of the interaction between gilled insects and gill morphology and land uses and habitat conditions, offering a more mechanistic way of comprehending the effects of anthropogenic alterations on aquatic biota.

## **2 Methods**

### **2.1. Study area**

For our study, 45 headwater streams (Strahler, 1957) were sampled. Each stream was sampled once during the dry season (June to November) over seven years (2011–2017). The sites are distributed across six municipalities (Tailândia, Tomé-Açu, IPIXUNA DO PARÁ, Paragominas, Acará and Aurora do Pará, in Pará State, Brazil). The region experiences a humid tropical climate, with mean annual temperatures of approximately 26 °C and a relative humidity of approximately 85% (Luiza-Andrade *et al.*, 2017). The predominant vegetation is dense ombrophiles forest, which has undergone substantial land-use change in recent decades, including forest loss and agricultural expansion (MapBiomias, 2025).



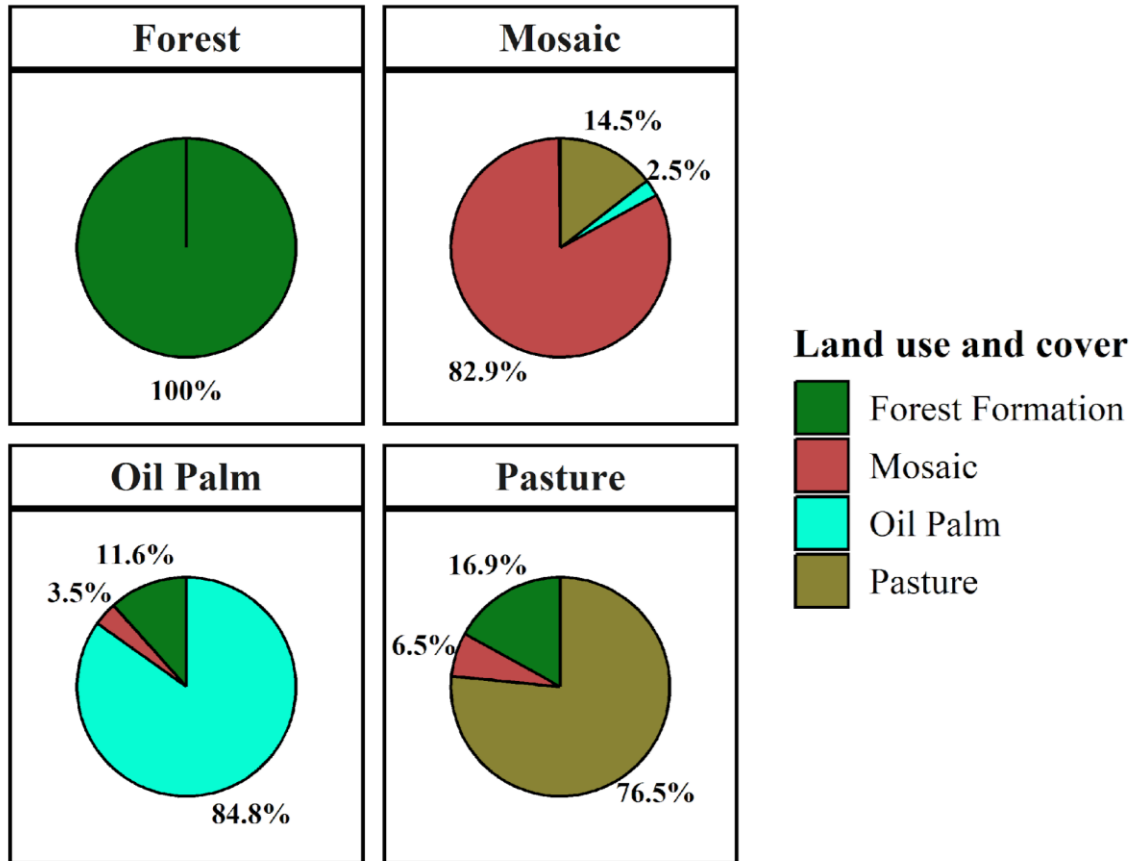
**Figure 1.** Study area showing the location of the 45 stream sampling sites in the Acará and Capim river basins, Pará State, Brazil.

## 2.2. Land use and land cover data

For our purposes, streams were categorized according to the predominance of land use and land cover (LULC) within their catchments, based on data from the MapBiomas platform (collection 8.1) (Pampolha *et al.*, 2026). Streams classified as Forest showed complete forest cover (100%), whereas those classified as Pasture were dominated by cattle ranching areas (>60%) and those classified as Oil Palm were characterized by agricultural land use with a predominance of oil palm monoculture (>60%). When no single LULC type was dominant (i.e., <60%), streams were classified as Mosaic, corresponding to agricultural landscapes with mixed crop and livestock uses and less than 25% forest cover (Figure 2).

Catchment boundaries were generated using elevation data from the TOPODATA Digital Elevation Model (DEM) provided by the *Instituto Nacional de Pesquisas Espaciais* (INPE). Delineation was carried out in *QGIS* (version 3.34.9) using the *r.watershed* algorithm, based on the geographic position of the downstream limit of each sampled stream reach.

## Land use composition



**Figure 2.** Mean percentage of land-use cover associated with each stream category within the sampled catchments.

### 2.3. Biological and Limnological Variables

Biological data collection and measurements of environmental variables were conducted along a 150 m longitudinal reach in each stream, subdivided by 11 transects established at 15 m intervals, labelled from A to K, from downstream to upstream. Consequently, within each stream reach, 10 longitudinal sections were defined, measuring 15 m each, (Callisto *et al.*, 2014; Luiza-Andrade *et al.*, 2017). Each section was then subdivided into three 5 m segments, and biological sampling was conducted in the first two segments, while environmental variables were measured at each transect, following the protocol described by Callisto *et al.* (2014) and Juen *et al.* (2025).

The metrics evaluated included substrate composition (percentage of sand, silt, macrophytes, algae, fine roots, coarse and fine litter, and large and small wood) and variation in mean substrate size per stream; physico-chemical parameters (temperature, pH and dissolved oxygen); and the agricultural impact proximity index (Agr.prox.Index) (Callisto *et al.*, 2014; Juen *et al.*, 2025). A multiparameter probe was used to measure dissolved oxygen (DO) (mg/L), temperature (°C), and pH, whereas channel substrate composition and mean substrate size variation were visually estimated, according to the protocols established by Juen *et al.* (2025). The Agr.prox. index considers the

presence and proximity of anthropogenic activities or structures, classified according to their distance from the watercourse (>10 m, <10 m, and at the stream margin) (Callisto *et al.*, 2014).

Aquatic insects were collected using an entomological hand net (rapiché) with an 18 cm diameter and 250  $\mu\text{m}$  mesh size (Luiza-Andrade *et al.*, 2017; Faria *et al.*, 2017; Juen *et al.*, 2025). EPT taxa were sorted in the field using plastic trays and entomological forceps and preserved in 85% ethanol (Luiza-Andrade *et al.*, 2017; Faria *et al.*, 2017). In the laboratory, EPT specimens were identified to genus level using taxonomic keys (Domínguez *et al.*, 2006; Hamada *et al.*, 2014).

To select insect genera with gill-based respiration, specialised literature was consulted (Merritt & Cummins, 2019; Poff *et al.*, 2006; Domínguez *et al.*, 2006), and the recent classification proposed by Santos *et al.* (2024) was adopted to identify genera occurring in the Amazon region. Based on this, a bibliographic survey of articles describing immature stages was conducted using the SciELO, Scopus and Google Scholar platforms to obtain morphological characteristics of gills in Ephemeroptera, Plecoptera and Trichoptera genera from the Amazon (Santos *et al.*, 2024), in order to classify gill characteristics such as shape, structure, arrangement and position (Table 1). Due to the scarcity of descriptions of immature stages, gill characteristics were obtained through the analysis of published descriptions and images. Additionally, for some genera, gill characteristics were inferred from species described in other regions of South America or, when necessary, at the family level.

**Table 1.** Classification of gill types of Ephemeroptera, Plecoptera, and Trichoptera genera.

Type	Classification
Shape	(Sub)triangular
	(Sub)quadrangular
	Oval
	Elongated
Structure	Lanceolate
	Plate
	Tufts
	Opercular
Arrangement	Filament
	Maxillary
	Thoracic
Position	Abdominal
	Lateral
	Dorsal
	Ventral
	Anal

## 2.4. Data analysis

In our study, each stream was considered an independent sampling unit, resulting in a total of 45 samples. To test the hypothesis that different types of LULC negatively influence the

distribution of gill-breathing taxa, a one-way ANOVA was applied. The responses of gill-breathing genera richness and abundance were evaluated separately to determine whether significant differences occurred among pasture, oil palm, and mosaic streams when compared with forested streams. The assumptions of homogeneity of variances (Levene's test,  $p > 0.05$ ) and data normality (P–P plot) of residuals were assessed after analysis. When statistically significant differences were detected ( $p$ -value  $< 0.05$ ), the model was subjected to Tukey's post hoc test. To assess whether environmental variables influence taxa abundance and the distribution of different gill configuration traits, an RLQ analysis was applied. This multivariate approach combines three separate analyses using matrices of environmental variables (R), taxa abundance (L), and trait (Q) to identify the main drivers of the separate distribution of each trait through taxa abundance, considering gill respiration as a trait subdivided into categories according to its classification (Gusmão *et al.*, 2022). Owing to structural differences in gill-type distribution among EPT orders, as gill arrangement and position are highly variable or order-specific, only gill shape and structure were selected for inclusion in the analysis (*Supplementary Table S1*).

In addition, a Fourth-Corner analysis was performed to quantify the influence of each environmental variable on gill categories. For this purpose, the False Discovery Rate (FDR) method was applied to adjust  $p$ -values for multiple testing and thus reduce the likelihood of spurious significant associations (Dray & Legendre, 2008; Dray *et al.*, 2014). The Fourth-Corner analysis was conducted using 9,999 permutations based on a Monte Carlo test, employing permutation model 6, which separately tests RLQ models 2, which associates the abundance matrix with environmental variables and 4, which associates the distribution of genera with their trait (Dray & Legendre, 2008). Subsequently, a Redundancy Analysis (RDA) was performed to identify the main variables influencing the distribution of EPT genera with gill respiration. The abundance matrix was subjected to a Hellinger transformation to reduce the effects of dominance, and environmental variables were standardised using  $z$ -scores (Legendre & Legendre, 2012; Lima *et al.*, 2022).

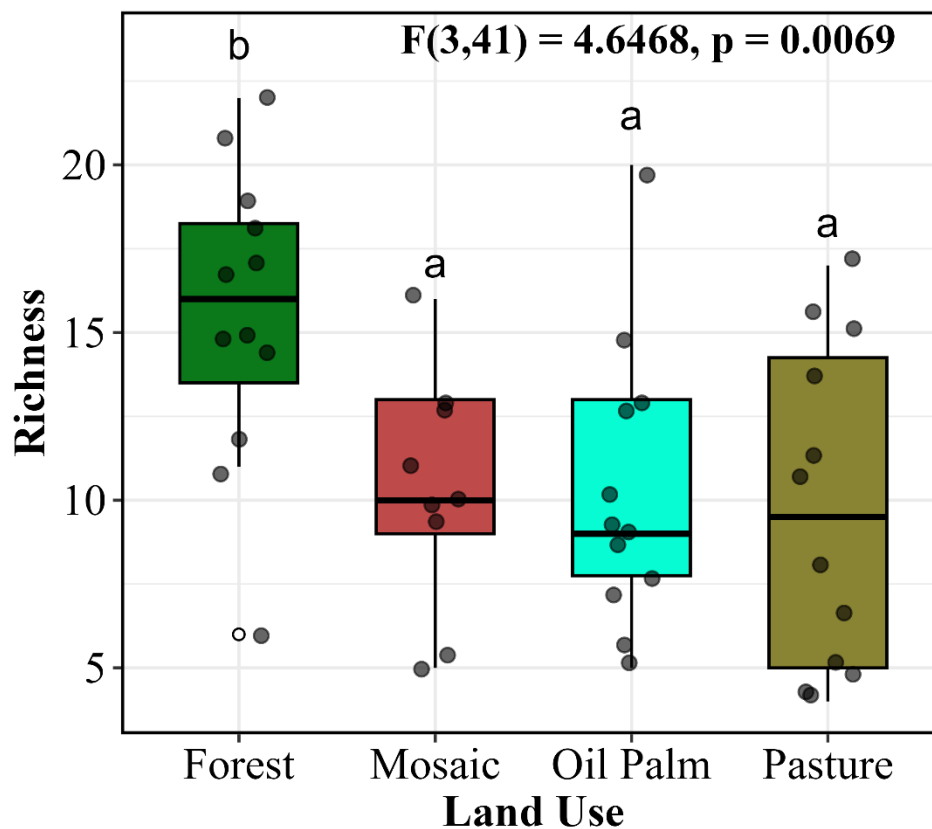
Therefore, our analytical approach was to use RDA as a complementary approach to identify the predictors most responsible for this distribution. While RLQ indicates the overall relationship between environmental predictors and the functional structure of the community (Dray & Legendre, 2008), RDA allows the identification of the genera that respond most strongly to each environmental variable and to different types of land use (Legendre & Legendre, 2012). Thus, this combined approach enables a more detailed understanding of the effects of land use on each variable, highlighting how the different LULC types affect their distribution and identifying the key variables driving this distribution.

All analyses were conducted in R software (version 4.4.0) through the RStudio interface (v. 2025.05.0), using the packages *stats* (R Core Team, 2024), *vegan* (Oksanen *et al.*, 2024),

*adespatial* (Dray *et al.*, 2025), and *ade4* (Dray & Dufour, 2007). The identification of predictor variables in the RDA was carried out using the *forward.sel* function ( $p < 0.05$ ) (Dray *et al.*, 2025).

### 3 Results

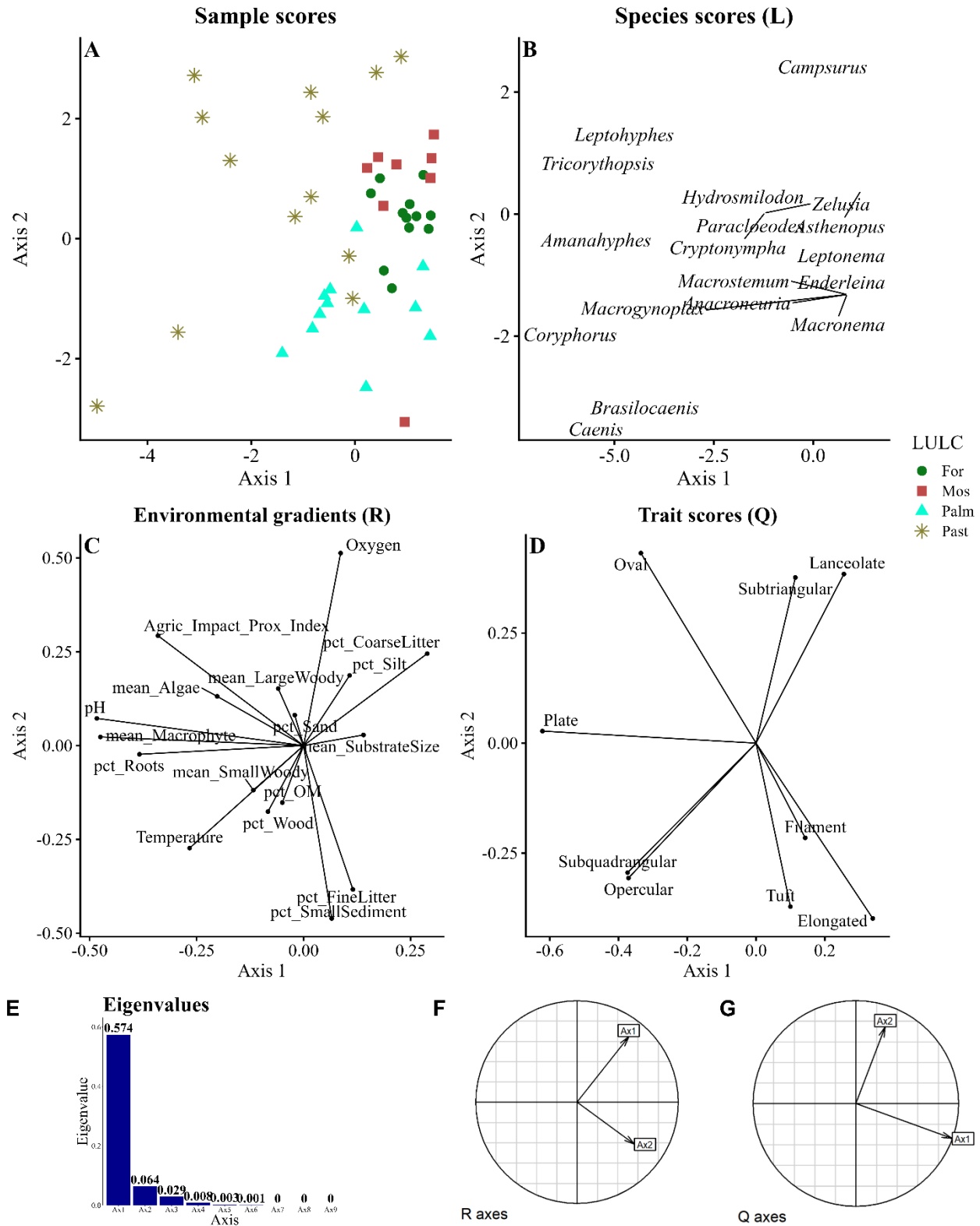
A total of 8,416 gill-breathing EPT immature were analysed, distributed across 42 genera and 13 families. The hypothesis that the richness of gill-breathing insects is affected by changes in land-use cover was supported, the results indicated that land-use treatments exerted a significant influence on the richness of gill-bearing EPT taxa ( $F(3,41) = 4.646$ ;  $p$ -value = 0.007) (Figure 3). Forested streams exhibited, on average, 5 more gill-breathing insect genera than oil palm streams (Tukey = 0.026), 5 more than mosaic streams (Tukey = 0.039), and 5 more than pasture streams (Tukey = 0.011). However, oil palm, mosaic, and pasture streams did not differ significantly from one another (Tukey<sub>palm/mosaic</sub> = 0.99; Tukey<sub>palm/pasture</sub> = 0.98; Tukey<sub>mosaic/pasture</sub> = 0.99). No significant effect of LULC on EPT abundance was detected ( $F(3,41) = 0.287$ ;  $p$ -value = 0.8), and therefore no further analyses were conducted for this variable.



**Figure 3.** Mean richness of gill-breathing insects by land-use type. Boxes indicate the interquartile range, central lines indicate the median and whiskers indicate the data range. Different lowercase letters above points indicate significant differences among treatments based on post hoc comparisons ( $p$ -value  $< 0.05$ ).

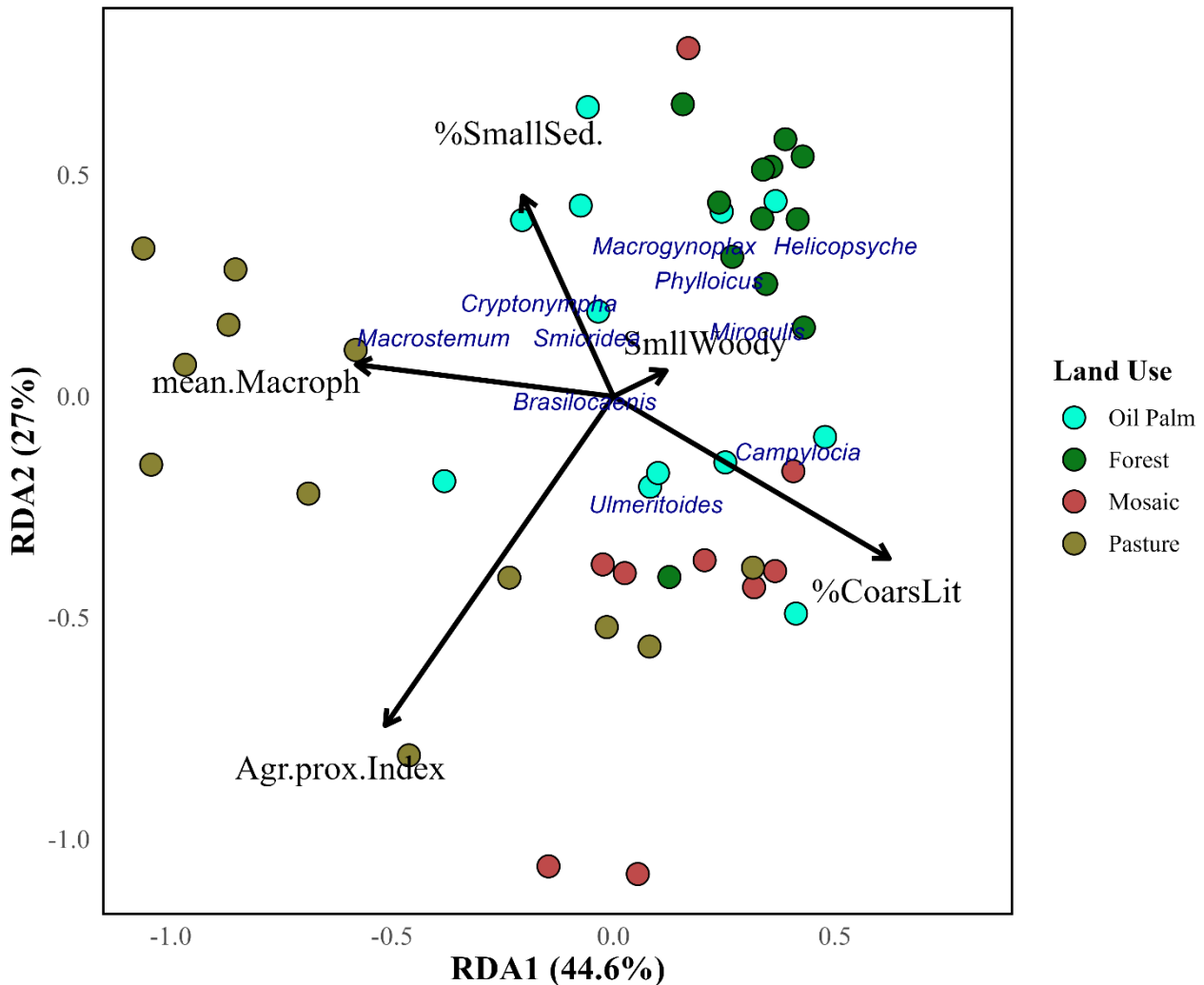
The RLQ analysis indicated that genus composition was significantly structured in relation to environmental variables (Model 2: Obs = 0.680, Std.Obs = 4.152, p-value = 0.002). On the ordination the genera *Campsurus*, *Zelus*, *Asthenopus*, *Leptonema*, *Enderleina* and *Macronema* showed positive scores along Axis 1, which was associated with forest, oil palm and mosaic land-use types and correlated with leaf banks (coarse litter), dissolved oxygen, silt percentage, degraded organic matter (fine litter), mean substrate size and small sediment percentage (Figure 4). In contrast, *Campsurus*, *Leptohyphes*, *Tricorythopsis* and *Hydrosmilodon* were positioned towards positive values of Axis 2, which was correlated with Oxygen, agricultural impact proximity index, leaf banks, silt percentage, large woody, pH and algae (Figure 4).

Regarding gill-breathing, the RLQ analysis weighted by abundance indicated a significant structure in trait composition (Model 4: Obs = 0.680; Std.Obs. = 2.389; p-value = 0.03). Tufted, filamentous, elongated, lanceolate and subtriangular gills were associated with positive scores along Axis 1, whereas subquadrangular, operculate, elongated, filamentous and tufted gills were associated with positive scores along Axis 2 (Figure 4). However, the Fourth-corner analysis did not detect significant associations between environmental variables and gill categories (environment\*trait: p-value = 0.653). Some genera and gill types exhibited positive scores along both ordination axes, reflecting their distribution.



**Figure 4 (4A–4G).** RLQ analysis. Figures 4A–4D show the contributions to axes 1 and 2 of land-use types, genera, environmental variables, and traits, respectively. Figure 4E represents the eigenvalues of each RLQ axis (*Supplementary Table S2*). Figure 4F and 4G show the contributions of axes 1 and 2 to the environmental variable (R) and trait (Q) matrices. Abbreviations of sampling units: For = Forest; Palm = Oil Palm; Mos = Mosaic; Past = Pasture.

The RDA explained 17% of the total variation in the composition of gill-breathing insects ( $F = 2.839$ , adjusted  $R^2 = 0.172$ ,  $p = 0.001$ ). Axis 1 and Axis 2 accounted for 44.6% and 27% of the variation explained by the RDA model, respectively (Figure 5).



**Figure 5.** Distribution of gill-breathing insects based on the RDA analysis in streams of the Capim and Acará river basins, Pará, Brazil. Circles are colour-coded according to the dominant land-use type. Only the ten most representative genera, that is, those with the highest contribution to the explained variation, are highlighted. Abbreviations of environmental variables: %SmallSed = percentage of small sediments; mean.Macroph = mean macrophyte cover; SmlWoody = mean small woody; %CoarsLit = percentage of leaf-litter banks; Agr.prox.Index = agricultural impact proximity index.

The first axis (44.6%) was positively related to the percentage of leaf-litter banks and mean small woody debris, with streams from forested areas being mainly concentrated, followed by oil palm and mosaic areas, whereas mean macrophyte cover was negatively related, mainly grouping pasture areas. The second axis (27%) was negatively related to the agricultural impact proximity index, mainly clustering pasture and mosaic land-use areas, while the percentage of small sediments contributed positively to the axis, grouping oil palm streams (Table 2).

**Table 2.** Contribution of each environmental predictor to axes 1 and 2 of the RDA analysis.

<b>Environmental Predictor</b>	<b>RDA1</b>	<b>RDA2</b>
Agricultural impact proximity index	-0.515	<b>-0.743</b>
Mean macrophyte	<b>-0.580</b>	0.071
% Coarse litter	<b>0.622</b>	-0.366
Mean small woody	<b>0.118</b>	0.058
% Small sediment	-0.205	<b>0.452</b>

Among the 42 genera analysed, the ten gill-breathing insects that contributed most to the formation of the axes, that is, the most representative, were *Campylocia*, *Ulmeritoides*, *Macrogynoplax*, *Helicopsyche*, *Phylloicus*, *Miroculis*, *Macrostemum*, *Cryptonympha*, *Smicridea*, and *Brasilocaenis*. A positive association with the first axis was observed for the genera *Helicopsyche*, *Phylloicus*, *Miroculis*, and *Campylocia*, which showed higher frequencies in forested streams, whereas *Macrostemum*, *Cryptonympha*, *Smicridea*, and *Brasilocaenis* were negatively associated and occurred mainly in streams within oil palm areas. The second axis was structured by a positive association of *Macrogynoplax* in forest and oil palm areas and a negative association of *Ulmeritoides*, occurring mainly in oil palm and mosaic land-use areas (Table 3).

**Table 3.** Contribution of the ten most representative genera to axes 1 and 2 of the RDA analysis.

<b>Genus</b>	<b>RDA1</b>	<b>RDA2</b>
<i>Brasilocaenis</i>	<b>-0.115</b>	-0.111
<i>Campylocia</i>	<b>0.365</b>	-0.225
<i>Cryptonympha</i>	<b>-0.208</b>	0.093
<i>Helicopsyche</i>	<b>0.191</b>	0.188
<i>Macrogynoplax</i>	0.169	<b>0.216</b>
<i>Macrostemum</i>	<b>-0.302</b>	-0.0002
<i>Miroculis</i>	<b>0.256</b>	0.057
<i>Phylloicus</i>	<b>0.163</b>	0.157
<i>Smicridea</i>	<b>-0.206</b>	0.072
<i>Ulmeritoides</i>	0.045	<b>-0.343</b>

#### 4 Discussion

Our hypothesis that areas with lower vegetation cover would exhibit reduced insect richness and abundance with gill respiration was partially supported. Forested streams showed greater taxonomic richness compared with other land-use types, whereas patterns of abundance were not clearly explained by land use. This result suggests that vegetation plays a central role in maintaining

taxonomic diversity, while abundance appears to be regulated by a broader set of interacting environmental factors, leading to less direct responses along the land-use gradient.

Forest vegetation is essential for maintaining the physical and chemical characteristics of streams, acting as the primary source of organic matter by regulating light incidence, filtering fine sediments and stabilising channel banks (Faria *et al.*, 2017; Cruz *et al.*, 2025). These processes increase habitat heterogeneity by influencing substrate composition, nutrients, pH, temperature and dissolved oxygen, which are key factors structuring aquatic insect communities (Cararo *et al.*, 2022; Buffington & Montgomery, 2022). In this context, the forested streams analysed exhibited greater substrate diversity and mean size, as well as higher dissolved oxygen concentrations, conditions favourable to the occurrence and diversification of insects with gill respiration.

The results of the species–trait–environment relationship revealed that environmental gradients associated with different land uses influenced taxonomic composition and the distribution of specific respiratory traits. Despite that, forested environments were associated with greater habitat complexity and higher oxygen availability, which may explain the higher richness of gill-breathing EPT observed in these streams, as exposed respiratory structures are favored in shaded, well-oxygenated conditions (Tchakonté *et al.*, 2015). This finding reinforces the premise that insects reflect environmental conditions through the distribution of their functional attributes as taxonomic and functional loss within EPT communities has also been reported in oil palm monoculture areas in the same region (Dray & Legendre, 2008; Luiza-Andrade *et al.*, 2017).

However, the absence of significant associations in the Fourth-corner analysis indicates that no single environmental variable independently explained the distribution of specific gill types. This suggests that the distribution of different gill types is shaped by the combined influence of multiple environmental factors rather than by isolated predictors. Such a pattern may reflect overlapping ecological responses, whereby certain traits persist across different environmental contexts due to tolerance to a wide range of conditions (Saker *et al.*, 2021; Mpopetsi & Kadye, 2024). In this sense, while RLQ describes general multivariate patterns, the Fourth-corner analysis highlights the absence of simple and direct relationships between functional traits and specific environmental variables.

Land-use types primarily associated with substrate composition, likely reflecting hydrodynamic processes related to sediment transport and the input of organic matter into streams. Together with riparian vegetation, these processes regulate the retention and export of organic and inorganic materials within aquatic habitats (Buffington & Montgomery, 2022). Preserved areas exhibited greater substrate variability and a higher diversity of plant-derived resources, highlighting the importance of riparian vegetation in shaping the organic matter present in streams (Tabacchi *et al.*, 1998). This increased habitat complexity supports the development of EPT communities by

meeting the ecological requirements of different species and expanding resource availability and refuge from predators and mechanical disturbance (Tabacchi *et al.*, 1998; Wiggins, 2007; de Castro *et al.*, 2017). Therefore, insects of the EPT orders are benefited, since Trichoptera are known for constructing different types of cases using organic and inorganic materials, which vary among genera (Wiggins, 1996, 2007; Holzenthal & Calor, 2017). In addition, these structures assist in locomotion during foraging, provide protection against predators and enhance gas exchange (Firmino *et al.*, 2024). Likewise, Ephemeroptera also use the substrate for egg attachment and resource accumulation, and some genera build shelters associated with the streambed (Domínguez *et al.*, 2006; Hamada *et al.*, 2018). Furthermore, Plecoptera use the substrate for attachment in fast-flowing areas, for feeding, for refuge, and for prey capture within interstices, reflecting their predatory habit (Heckman, 2003; Stewart, 2009; Avelino-Capistrano *et al.*, 2017). In other words, insects that use the substrate are more benefited in more heterogeneous environments, which explains the concentration of nearly half of the most representative genera in forested streams.

Contrary to expectations, oil palm areas exhibited the highest percentage of fine sediments. The replacement of forest by agricultural crops weakens the soil and promotes bank erosion, increasing suspended sediments (Zahoo & Mushtaq, 2023). Fine sediments increase turbidity and reduce light penetration into the aquatic system, impairing the development of aquatic insect communities and, together with land-use changes, exerting negative effects on taxa with gills (McKenzie *et al.*, 2020; Amaral *et al.*, 2025). Moreover, the amount of sediment input varies according to ground cover between plantations, being lower where there is greater protection by grasses and organic matter, as reported by Sahat *et al.* (2016), which may explain why pasture areas did not show higher values, as grasses used for cattle grazing may contribute to soil protection.

Despite this, the higher mean abundance of macrophytes in pasture streams suggests a possible onset of eutrophication, as cattle waste increases nitrogen levels and nutrient enrichment of the streambed stimulates macrophyte biomass growth (Skovsholt *et al.*, 2024; Jones *et al.*, 2019). An increase in macrophyte abundance within streams reduces dissolved oxygen and pH, negatively affecting insects with gills, as variation in these conditions may alter respiration rates and cause respiratory stress (Merritt & Cummins, 2019; Skovsholt *et al.*, 2024).

Environmental variables influence the abundance of EPT taxa with gill respiration. The genera *Helicopsyche*, *Phylloicus*, *Miroculis*, *Macrogynoplax* and *Campylocia* were positively associated with axis 1, which represented forested streams with greater substrate diversity, which supports different ecological strategies and increases species richness. *Helicopsyche* (Trichoptera: Helicopsychidae) builds cases using sand grains and silk (Hamada *et al.*, 2014); *Phylloicus* (Trichoptera: Calamoceratidae) uses leaves, leaf litter and woody debris for case construction and oviposition (Hamada *et al.*, 2014; Holzenthal & Calor, 2017). *Macrogynoplax* (Plecoptera: Perlidae)

is sensitive to anthropogenic stress and to sedimentation in streams and uses trunks, stones, and leaves for egg-laying (Hamada *et al.*, 2014). *Miroculis* (Ephemeroptera: Leptophlebiidae) scrapes the substrate and prefers environments with higher substrate diversity (Hamada *et al.*, 2014; Carvalho *et al.*, 2025), whereas *Campylocia* (Ephemeroptera: Euthyplociidae) is associated with leaf accumulation, living beneath rocks and leaves (Crisci-Bispo *et al.*, 2007; Hamada *et al.*, 2014).

In contrast, *Macrostemum*, *Cryptonympha* and *Smicridea* were negatively associated with first axis, being concentrated in oil palm streams characterised by a higher proportion of small and medium sediments and macrophytes. *Macrostemum* and *Smicridea* (Trichoptera: Hydropsychidae) construct silk retreats and, together with *Cryptonympha* (Ephemeroptera: Baetidae), exhibit a filter-feeding habit, which is favoured by higher sediment concentrations in streams (Hamada *et al.*, 2014; Santos *et al.*, 2024). This feeding strategy, considered generalist, is common in agricultural areas where algal and macrophyte abundance is greater (Marques *et al.*, 2021; Santos *et al.*, 2024; Skovsholt *et al.*, 2024).

*Ulmeritoides* (Ephemeroptera: Leptophlebiidae) was negatively associated with axis 2, which represented streams with a higher proportion of leaf banks. It is regarded as a specialist genus, sensitive to environmental changes, and is typically found in streams with greater diversity of leaf litter and woody substrates (Carvalho *et al.*, 2025).

Finally, we observed that case-building genera and those dependent on the substrate for protection and gas exchange, as well as genera described as more sensitive to environmental disturbance, were more frequent in forested streams. This pattern highlights that land-use change is associated taxonomic and functional losses in aquatic insect communities. Such losses may have broader ecological implications, particularly through the food web, as other organisms, especially predators, may be affected by the resulting reduction in resource availability, potentially altering trophic interactions and energy flow within aquatic ecosystems (Sánchez-Hernández, 2023). Furthermore, land use modifies the physical characteristics of streams, potentially resulting in pronounced biological degradation or even irreversible hydrological changes (McKenzie *et al.*, 2020).

The hypothesis that forested areas would exhibit greater diversity of genera with gills was supported. Forested streams showed higher substrate diversity and a greater diversity of insects with gill respiration. However, variation in abundance could not be clearly confirmed across treatments. This study sought to integrate different statistical approaches to improve understanding of the effects of land use on EPT insect communities, particularly those with gills. RLQ analysis allowed us to examine how predictors were structured within the studied sampling space and, together with RDA, to identify the most responsive environmental variables.

These findings support the premise that more conserved areas contain greater substrate diversity, accommodating different niches and physiological requirements of EPT insects.

Furthermore, the distribution of gill types appears to be influenced by multiple environmental predictors acting jointly, as individual variables were weak in explaining distribution patterns. Further analyses are needed to determine which specific environmental predictors, acting individually or in combination, most strongly influence each gill type.

Land-use changes, particularly those that contribute to deforestation (i.e., agricultural activities), affect local aquatic communities both directly and indirectly through increased fine sediment input, reduced variability of organic matter and chemical alterations within streams. Such alterations silt up streambeds and negatively affect aquatic insects with gill respiration, creating microhabitats that favour certain genera over others according to their ecological strategies. This channel simplification threatens aquatic macroinvertebrate life, given the fundamental importance of habitat heterogeneity for community maintenance, as well as other trophic groups, which may be affected through cascading effects. Moreover, in the long term, these alterations may also compromise the environmental and physical integrity of streams. Therefore, further studies and management strategies are needed to minimise or prevent the negative impacts of land-use change on streambeds to promote the conservation of Amazonian aquatic biodiversity.

## 5 Ethics and Integrity statements

**Data Availability:** The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

**Ethics and Permit Approvals:** Not applicable

**Use of Artificial Intelligence:** The authors acknowledge the use of ChatGPT (OpenAI, GPT-5 model; accessed January 2026) to assist with the translation of portions of the manuscript into English and with language editing for clarity and readability. The AI tool was used solely to support language refinement and did not generate original scientific content, restructure arguments, or influence data interpretation. All AI-assisted text was carefully reviewed, revised, and validated by the authors, who take full responsibility for the final content of the manuscript.

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#### 4. CONCLUSÃO GERAL

Nossa hipótese de que alterações antrópicas no uso do solo influencia negativamente a distribuição e a diversidade de grupos funcionais de insetos aquáticos foi corroborada. A conversão de riachos florestados modifica o aporte de matéria orgânica e o leito dos riachos, entre outros fatores, impactando negativamente a distribuição de EPT fragmentadores e de EPT com respiração branquial. Nesse contexto, riachos florestais apresentaram melhores condições para ambos os atributos funcionais avaliados, evidenciado pela maior riqueza e distribuição de EPT. Atividades agropecuárias afetam diretamente a comunidade aquática local ao reduzir o aporte de matéria orgânica e homogeneizar o leito dos riachos. Também, a perda de fragmentadores compromete processos ecológicos, como a decomposição foliar e a ciclagem de nutrientes, enquanto a perda de ambos altera a cadeia alimentar, podendo impactar negativamente o ecossistema aquático como um todo, através do efeito cascata.

Nosso estudo integrou diferentes métricas biológicas e abordagens estatísticas, estabelecendo uma relação direta entre a estrutura da comunidade e o uso da terra. A análise da biomassa evidenciou diferenças na produtividade ecológica, indetectável pelas outras métricas. Adicionalmente, a aplicação da RDA, complementar à análise de RLQ, contribuiu para uma melhor visualização da distribuição dos componentes bióticos e abióticos no espaço amostral.

Esse estudo demonstrou a importância de fragmentadores para serviços ecológicos na comunidade aquática e como eles respondem diante de alterações antrópicas. Em conjunto, o estudo também ajudou a organizar e caracterizar informações morfológicas de brânquias em insetos aquáticos amazônicos e entender sua resposta ecológica diante de alterações no uso da terra. Dessa forma, nossos resultados evidenciam a necessidade urgente de ampliar os esforços de pesquisa e fortalecer políticas públicas voltadas à conservação e ao monitoramento dos ecossistemas aquáticos na Amazônia.

Apesar disso, ainda são escassos os estudos que investiguem como a comunidade de insetos das ordens EPT se estrutura sob pressões antrópicas, especificamente seus mecanismos para reduzir os impactos sofridos. Em adição, carecem os planos de ação alternativas que minimizem ou evitem os impactos das mudanças no uso da terra sobre o canal e leito dos riachos. Assim, espera-se que esse estudo auxilie futuras pesquisas e estudos ecológicos que visem a preservação dos riachos, assim como a resposta ecológica de insetos das ordens EPT diante de perturbações ambientais.

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## 6. CRONOGRAMA

Ano/Trimestre	2024				2025				2026
	1º	2º	3º	4º	1º	2º	3º	4º	1º
Revisão bibliográfica	X	X	X	X	X	X	X	X	X
Disciplinas	X	X	X	X	X	X			
Escrita do projeto	X	X	X	X	X	X	X	X	X
Análise de dados				X	X	X	X	X	

Seminário PPGECO				X				X	
Qualificação					X				
Submissão capítulo 1								X	
Submissão capítulo 2									X
Defesa									X

## 7. ATIVIDADES COMPLEMENTARES

Ao longo do mestrado além das minhas atividades relacionadas a elaboração da dissertação e de fazer as disciplinas do Programa de Pós-graduação, realizei diversas atividades que também foram fundamentais para o meu aprendizado, as quais listo abaixo.

1. Realização do curso online “Dengue: conheça mais sobre os mosquitos”, da Escola Superior do instituto Butantan;
2. Participação na 76ª Reunião Anual da Sociedade Brasileira para o Progresso da Ciência;
3. Participação e apresentação de trabalhos no XXIX Congresso Brasileiro de Entomologia e XIII Congresso Latino-Americano;
4. Participação no Seminário das Pós-graduações em Biodiversidade – PPGZOO e PPGECO;
5. Realização do curso online “Fundamentos e Práticas de Conservação de Solo e Água”, da Empresa Brasileira de Pesquisa Agropecuária;
6. Participação no V Encontro de Entomologia e Conservação da Biodiversidade;
7. Participação no II simpósio PPGECEB (UEMA) – "Ecossistemas em foco: desafios e soluções para a conservação integrativa";
8. Participação e apresentação de trabalhos no VII Simpósio de Insetos Aquáticos Neotropicais;
9. Realização do curso online “Introdução à Ciência de Dados - Estatística Essencial”, da Escola Nacional de Administração Pública;
10. Monitoria de aula da disciplina “Estatística Aplicada a Estudos Ecológicos” da turma de 2025.1 do programa de pós-graduação em Ecologia (PPGECEB) e Zoologia (PPGZOO) da Universidade Federal do Pará;
11. Participação da expedição de coleta de dados socioambientais na terra indígena Panará (TI Panará);
12. Realização do curso online “Curso Básico sobre Lei Geral de Proteção de Dados” da Universidade de São Paulo;
13. Realização do curso online “Curso Básico de Integridade em Pesquisa” da Universidade de São Paulo;
14. Participação na Primeira Jornada de Biotecnologia Ambiental

15. Publicação do primeiro capítulo da dissertação
16. Submissão do segundo capítulo da dissertação
17. Elaboração de um artigo paralelo, ainda em desenvolvimento, durante a 1ª Oficina de Discussão e elaboração de Artigos, organizada e ministrada pelo Laboratório de Ecologia e Conservação.

## 8. APÊNDICE

**Supplementary Table S1.** Extracted morphological data on gills of immature Ephemeroptera, Plecoptera, and Trichoptera (EPT).

ORDER	Family	Genera	Shape				Structure					Position			A/T segment	Observation	
			S T	S Q	O	E	L	P	T	O	F	M	T	A			
EPHEMER OPTERA	Baetidae	<i>Americabaetis</i>			1		1							1		II - VII	
		<i>Adebrotus</i>			1		1							1		I - VII	
		<i>Aturbina</i>			1		1							1		I - VII	
		<i>Apobaetis</i>				1		1						1		I - VII	
		<i>Callibaetis</i>			1		1							1		I - VII	
		<i>Callibaetoides</i>			1		1							1		I - VII	
		<i>Camelobaetidius</i>			1		1							1		I - VII	
		<i>Cloeodes</i>			1		1							1		I - VII	
		<i>Cryptonympha</i>					1		1					1		II - VII	
		<i>Guajirolus</i>			1		1							1		I - VII	
		<i>Harpagobaeis</i>			1		1							1		I - VII	
		<i>Paracloeodes</i>					1		1					1		I - VII	
		<i>Rivudiva</i>			1		1							1		I - VII	
		<i>Spiritiops</i>					1		1					1		I - VII	
		<i>Tomedontus</i>					1		1					1		II - VII	
		<i>Waltzoyphius</i>					1		1					1		I - VII	
		<i>Zelusia</i>					1		1					1		II - VII	
		Caenidae	<i>Brasilocanis</i>	1		1		1		1	1			1		I - VI	<b>Family-level description only Family-level description only No data available for other gills</b>
			<i>Alloretochus</i>	1		1		1		1	1			1		I - VI	
			<i>Caenis</i>	1		1		1		1	1			1		I - VI	
		Coryphoridae	<i>Coryphorus</i>	1				1		1				1		II - V	
		Eutyplocidae	<i>Campylocia</i>			1		1						1		II - VII	<b>Gill I vestigial, bifurcated</b>
		Ephemeridae	<i>Hexagenia</i>			1		1						1		II - VII	<b>Gill I reduced</b>
		Leptophlebiidae	<i>Askola</i>			1		1						1		I - VII	
			<i>Fittkaulus</i>			1		1						1		I - VII	
			<i>Farrodes</i>			1		1						1		I - VII	
			<i>Hagenulopsis</i>			1		1						1		I - VII	

	<i>Hydrosmilodon</i>	1	1	1	1	I - VII	Gill VII occasionally vestigial	
	<i>Microphlebia</i>	1	1	1	1	I - VII	Structure not reported	
	<i>Miroculis</i>	1	1	1	1	I - VII		
	<i>Needhamella</i>	1	1	1	1	I - VII	Gill VII reduced	
	<i>Paramaka</i>	1	1	1	1	I - VII	Gill VII vestigial	
	<i>Simothraulopsis</i>	1	1	1	1	I - VII	Shape not reported	
	<i>Thraulodes</i>	1	1	1	1	I - VII		
	<i>Terpides</i>	1	1	1	1	I - VII		
	<i>Tikuna</i>	1	1	1	1	I - VII	Shape not reported	
	<i>Ulmeritoides</i>	1	1	1	1	I - VII	Shape and structure not reported	
Leptohyphidae	<i>Amanahyphes</i>	1	1	1	1	1	II - V	
	<i>Leptohyphes</i>	1	1	1	1	1	II - VI	
	<i>Macunahyphes</i>	1	1	1	1	1	II - VI	
	<i>Tricorythodes</i>	1	1	1	1	1	II - VI	
	<i>Tricorythopsis</i>	1	1	1	1	1	II - VI	Shape not reported
Oligoneuriidae	<i>Fittkauneria</i>	1	1	1	1	1	II - VII	
	<i>Homoeoneuria</i>	1	1	1	1	1	II - VII	
	<i>Oligoneuria</i>	1	1	1	1	1	I - VII	Family-level description only
Polymitaeridae	<i>Asthenopuss</i>	1	1	1	1	1	I - VII	Gill I reduced
	<i>Campsurus</i>	1	1	1	1	1	I - VII	
	<i>Hubbardipes</i>	1	1	1	1	1	I - VII	
	<i>Tortopsis</i>	1	1	1	1	1	I - VII	
	<i>Tortopus</i>	1	1	1	1	1	I - VII	
PERLIDAE	<i>Anacroneuria</i>	1	1	1	1	1	ASC[1]; PSC[1]; AT[2,3]; PT[3]	
	<i>Enderleina</i>	1	1	1	1	1	ASC [1,2,3]; PSC [1,2,3]; AT [2,3]; PT [3]; anal	
	<i>Macrogynoplax</i>	1	1	1	1	1	ASC [1,2,3]; PSC [1,2,3]; AT [2,3]; anal	
TRICHOPTERA	Calamoceratidae	<i>Phylloicus</i>	1	1	1	1	I - VIII	
Helicopsycheidae	<i>Helicopsycha</i>	1	1	1	1	1	II - IV	Shape and structure not reported
Hydropsychidae	<i>Blepharopus</i>	1	1	1	1	1	TII - TIII; I - VII, anal	
	<i>Centromacronema</i>	1	1	1	1	1	TIII; I - VIII	
	<i>Leptonema</i>	1	1	1	1	1	TII - TIII; I - VII, anal	
	<i>Macrostemum</i>	1	1	1	1	1	TIII; I - VII	
	<i>Macronema</i>	1	1	1	1	1	TIII; I - VII	

	<i>Pseudomacronema</i>					No information available for larval stage
	<i>Plectromacronema</i>	1	1	1	1	I - VIII
	<i>Synoestropsis</i>	1	1	1	1 1	TII - TIII; I - VIII
	<i>Smicridea</i>	1		1	1 1	TII - TIII; I - VII
Leptoceridae	<i>Amazonatolica</i>					Absence of gills reported
	<i>Nectopsyche</i>	1		1	1 1	TIII; II - VIII
	<i>Oecetis</i>	1		1	1	II - IV
	<i>Triplectides</i>	1		1	1	II - VIII
Odontoceridae	<i>Marilia</i>	1		1	1	II - VIII

**Abbreviations of gill configurations.** ST = (Sub)triangular; SQ = (Sub)quadrangular; O = Oval; E = Elongated; L = Lanceolate; P = Plate; T = Tufts; O = Opercular; F = Filament; M = Maxillary; T = Thoracic; A = Abdominal; AT = Anterior Metathoracic gills (gills located on the anterior margin of the second [AT2] or third [AT3] thoracic segments); ASC = Anterior Pronotal Supracoxal (gills located anteriorly and dorsal to the first [ASC1], second [ASC2], or third [ASC3] coxa); PSC = Posterior Pronotal Supracoxal (gills located posteriorly and dorsal to the first [PSC1], second [PSC2], or third [PSC3] coxa); PT = Posterior Metathoracic (gills located on the posterior margin of the third [PT3] thoracic segment); for Trichoptera: T = Thorax; TI = Pronotum; TII = Mesonotum; TIII = Metanotum.

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**Supplementary Table S2.** RLQ analysis: eigenvalues and inertia explained by each axis.

<b>Axis</b>	<b>Eigenvalues</b>	<b>Projected inertia (%)</b>	<b>Cumulative projected inertia (%)</b>
1	0.574325	84.3789	84.38
2	0.063884	9.3858	93.76
3	0.028982	4.2579	98.02
4	0.008259	1.2135	99.24
5	0.003231	0.4746	99.71
<b>Total inertia</b>	0.6807	-	-