



PROGRAMA DE POS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

FACULDADE UNB DE PLANALTINA– FUP/UNB

MARLON SANTOS AMÂNCIO

**AVALIAÇÃO DA DECOMPOSIÇÃO FOLIAR EM RIACHOS TROPICAIS SOB  
DIFERENTES CONDIÇÕES DE CONSERVAÇÃO**

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Ambientais da Universidade de Brasília, como requisito para obtenção do título de Mestre em Ciências Ambientais.

Orientação: Prof. Dr. José Francisco Gonçalves Junior

Coorientação: Dra. Camila Aida Campos Couto

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Brasília

2026

## Agradecimentos

Gostaria de expressar minha profunda gratidão...

Aos meus pais, Divina e Cláudio, que desde cedo ressaltaram a importância dos estudos e sempre fizeram o possível para que eu tivesse acesso a uma boa educação.

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## **Resumo**

A decomposição foliar é um processo fundamental para o funcionamento dos ecossistemas aquáticos, e alterações na integridade ambiental causadas por atividades antrópicas podem influenciar substancialmente as taxas de decomposição. Neste estudo, investigamos como a decomposição foliar varia ao longo de um gradiente contínuo de saúde dos riachos e uso do solo. Conduzimos um experimento de decomposição de serapilheira em riachos tropicais utilizando litterbags, quantificando as taxas de perda de massa e investigando suas relações com variáveis ambientais, métricas de uso do solo e componentes bióticos. A decomposição apresentou respostas predominantemente não lineares ao longo do gradiente, com as maiores taxas ocorrendo em níveis intermediários de perturbação. A biomassa fúngica contribuiu para o processo de decomposição; no entanto, a variação nas taxas ao longo do gradiente esteve principalmente associada à atividade de macroinvertebrados em litterbags de malha grossa. Além disso, as taxas de decomposição apresentaram relação positiva com a temperatura da água e a condutividade elétrica. De modo geral, nossos resultados demonstram que a decomposição da serapilheira foliar é sensível ao grau de perturbação dos riachos, sendo fortemente influenciada pelo uso do solo em escala de bacia, e destacam a importância de abordagens contínuas baseadas em gradientes para compreender as respostas ecológicas em riachos tropicais.

## **Abstract**

Leaf litter decomposition is a fundamental process in aquatic ecosystems functioning, and alterations in environmental integrity driven by anthropogenic activities can substantially influence decomposition rates. In this study, we investigated how leaf litter decomposition varies along a continuous gradient of stream health and land use. We conducted a leaf litter decomposition experiment in tropical streams using litterbags, quantifying mass loss rates and investigating their relationships with environmental variables, land-use metrics, and biotic components. Decomposition exhibited predominantly non-linear responses along

the gradient, with the highest rates occurring at intermediate levels of disturbance. Fungal biomass contributed to the decomposition process; however, variation in decomposition rates along the gradient was primarily associated with macroinvertebrate activity in coarse-mesh litterbags. Additionally, decomposition rates were positively related to water temperature, and electrical conductivity. Overall, our findings demonstrate that leaf litter decomposition is sensitive to the degree of stream disturbance, being largely driven by watershed-scale land use, and highlight the importance of continuous, gradient-based approaches for understanding ecological responses in tropical streams.

# ASSESSMENT OF LEAF LITTER DECOMPOSITION IN TROPICAL STREAMS UNDER DIFFERENT CONSERVATION CONDITIONS

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## Abstract

Leaf litter decomposition is a fundamental process in aquatic ecosystems functioning, and alterations in environmental integrity driven by anthropogenic activities can substantially influence decomposition rates. In this study, we investigated how leaf litter decomposition varies along a continuous gradient of stream health and land use. We conducted a leaf litter decomposition experiment in tropical streams using litterbags, quantifying mass loss rates and investigating their relationships with environmental variables, land-use metrics, and biotic components. Decomposition exhibited predominantly non-linear responses along the gradient, with the highest rates occurring at intermediate levels of disturbance. Fungal biomass contributed to the decomposition process; however, variation in decomposition rates along the gradient was primarily associated with macroinvertebrate activity in coarse-mesh litterbags. Additionally, decomposition rates were positively related to water temperature, and electrical conductivity. Overall, our findings demonstrate that leaf litter decomposition is sensitive to the degree of stream disturbance, being largely driven by watershed-scale land use, and highlight the importance of continuous, gradient-based approaches for understanding ecological responses in tropical streams.

## 1- Introduction

In recent decades, the expansion of urbanization into natural areas, combined with the intensification of land use for agriculture and pasture, has promoted changes in natural processes within aquatic ecosystems (Tanaka et al., 2021). These transformations include the replacement of native vegetation, increased soil impermeabilization (Fernández et al., 2021), and the simplification of riparian habitats, altering the physical and chemical structure of aquatic environments (Wen et al., 2017).

Changes such as fragmentation, vegetation loss, and intensive land use directly affect water quality and the structure of biological communities (Fernandes et al., 2021; Fierro et al., 2017). Aquatic communities, including macroinvertebrates and fungi, are sensitive to environmental degradation and tend to undergo changes in their diversity and abundance (Lima et al., 2022; Brito et al., 2020). Consequently, ecological processes such as leaf litter decomposition may be impaired, affecting the stability and functioning of aquatic ecosystems (Graça et al., 2015; Ferreira & Chauvet, 2011).

Decomposition rates in aquatic ecosystems, where leaf litter accounts for more than 60% of organic matter inputs (Esteves & Gonçalves, 2011), can be altered by environmental disturbance caused by anthropogenic activities (Ferreira et al., 2016). Agricultural expansion, the discharge of domestic and industrial effluents, and changes in riparian vegetation composition affect water quality and habitat structure (Wen et al., 2017; Ferreira et al., 2016). Nutrient enrichment, for example, can increase fungal biomass and accelerate leaf litter decomposition (Ferreira & Chauvet, 2011; Ferreira et al., 2015), whereas urbanization tends to reduce decomposition rates due to habitat fragmentation and decreased microbial activity (Martins et al., 2015). In addition, different degrees of disturbance influence decomposition in tropical streams in distinct ways (Pérez et al., 2023). These contrasting responses highlight the vulnerability of ecological processes in tropical streams to anthropogenic impacts on aquatic ecosystems (Martins et al., 2015; Pérez et al., 2023).

Several approaches have been used to characterize aquatic ecosystems based on the degree of environmental disturbance. In Brazil, current legislation

classifies water bodies into quality classes (from Special to Class 4), primarily based on physical and chemical parameters and the predominant uses of water resources (CONAMA Resolution No. 357, Brazil, 2005). At the landscape scale, land-use and land-cover indices, such as Land Use Intensity (LUI), have been applied to quantify anthropogenic pressure on watersheds and relate it to ecological responses (Rawer-Jost et al., 2024; Yang et al., 2024). More recently, Campos et al. (2024) proposed the Tropical Water Health Index (TWHI), which integrates multiple dimensions of pressure, condition and response (management) into a continuous metric, representing an important advance by allowing the detection of subtle gradients of degradation and conservation in tropical streams.

Studies comparing leaf litter decomposition rates in streams located in natural areas and in environments subjected to different types of anthropogenic pressure have been conducted over recent decades. These studies have highlighted contrasts between preserved streams and those affected by urbanization (Martins et al., 2015; Tagliaferro et al., 2022), agricultural and livestock expansion (Pérez et al., 2023), and riparian forest degradation (Silva-Araujo et al., 2020). Such comparisons have been fundamental in revealing general patterns of alteration in stream ecological functioning and in demonstrating leaf litter decomposition as a process sensitive to human interference in ecosystems. However, studies that examine leaf litter decomposition patterns along continuous gradients of environmental conditions remain scarce.

Against this background, this study aims to evaluate the sensitivity of leaf litter decomposition in Cerrado streams to gradual environmental changes along a continuous gradient of stream health, rather than relying on categorical comparisons. To address this aim, we pursued three specific objectives: (I) to examine how stream health and different land-use types affect decomposition rates; (II) to identify the limnological variables most strongly associated with decomposition; and (III) to determine which biotic factors exert the greatest influence on decomposition and how they vary along the environmental gradient. We hypothesized that: (I) decomposition rates increase with decreasing stream health; (II) water temperature is primary environmental driver of decomposition;

and (III) the colonization and activity of macroinvertebrates and microorganisms differs systematically along the stream health gradient.

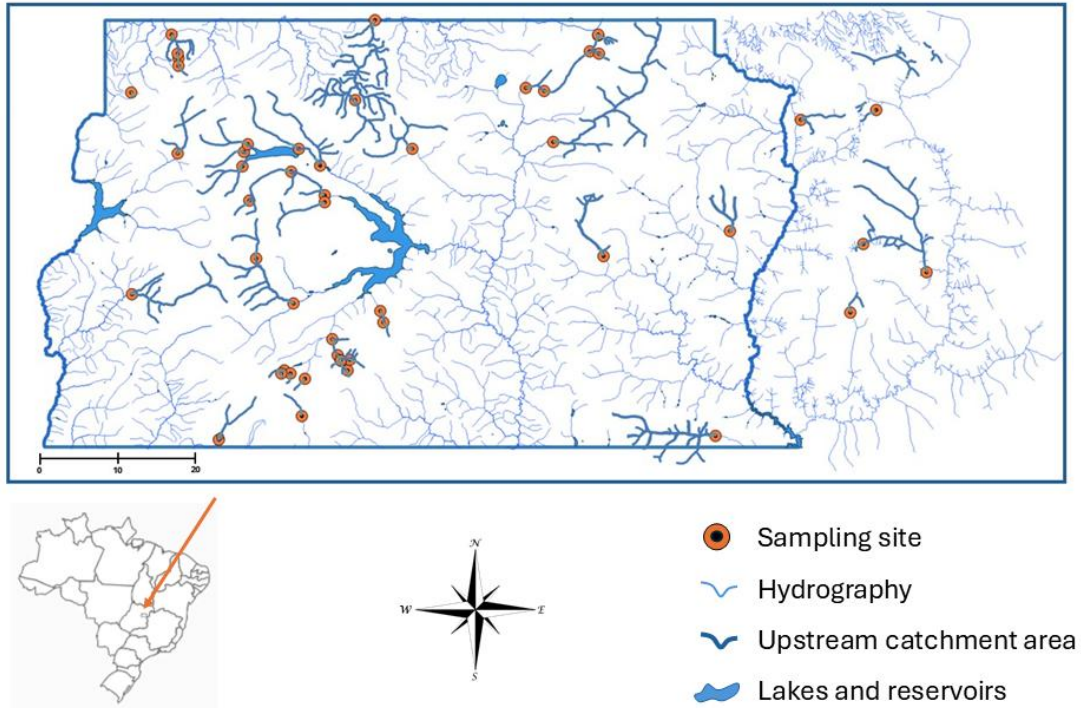
## **2- Materials and methods**

### **2.1- Study area**

The study was conducted in the Federal District and the state of Goiás (Brazil), encompassing 36 streams. Of these, seven streams had more than one sampling site, resulting in a total of 48 sampling sites (Figure 1). The analyzed streams are up to third order and belong to three different hydrographic basins: Maranhão, Paranaíba, and Preto.

The study area is located within the Cerrado biome, which is characterized by savanna vegetation, with formations ranging from open grasslands to dense riparian forests. The regional climate is seasonal, with a rainy season from October to April and a dry season from May to September. Mean annual temperature is approximately 20 °C, and annual precipitation is around 1,700 mm, with rainfall concentrated during the warmer months. Soils are generally acidic and nutrient-poor but support high plant species diversity associated with different phytophysionomies (Eiten, 1972).

The streams exhibit natural vegetation cover along their margins, ranging from 40% to 100%, with higher percentages observed in streams located within protected areas. Stream elevation ranges from 744 to 1,220 meters. These streams drain both natural areas and regions with human occupation and agricultural activities; information on land use within the drainage basins was obtained through geoprocessing analyses from Campos (2021).



**Figure 1.** Study area showing the distribution of sampling sites. Adapted from Campos (2021).

## 2.2- Stream characterization

Field collections, analyses and experiments were conducted between August and September 2018 by Campos (2021). In all streams, dissolved oxygen concentration and water temperature were measured directly in the field using portable probes. Water samples were collected for laboratory analyses, in which pH and electrical conductivity were measured using a benchtop meter (pH/conductometer, Metrohm 914), and turbidity was measured using a turbidimeter (Quimis ISO 9001, model Q279P). Current water velocity was determined using a flow meter (Swoffer Instruments, model 3000). Water samples were also collected for chromatographic analyses of cations and anions (Metrohm 930 IC). For the present study, only nitrate and phosphate concentration data were used, as they represent the main bioavailable inorganic forms of nitrogen and phosphorus directly assimilable by the microbial community.

### 2.3- Description of the Leaf Litter Decomposition Experiment

For the leaf litter decomposition experiment, portions of approximately 3g of leaves from the species *Hyeronimia alchorneoides* Allemão were weighed. Leaves were collected during the dry season, prior to the beginning of fieldwork. The leaves were placed in coarse-mesh litterbags (10 mm mesh size) and fine-mesh litterbags (0.5 mm mesh size), the latter excluding the influence of macroinvertebrates. At each sampling site, six litterbags were incubated, consisting of three coarse-mesh and three fine-mesh bags, arranged alternately so that they remained fully submerged within the streambed substrate for an incubation period of approximately 30 days, as described by Campos (2021).

### 2.4- Laboratory Procedures

#### 2.4.1- Detritus Processing

After the incubation period, the litter bags were retrieved and transported to the laboratory. Leaves were gently rinsed with distilled water to remove sediments and associated macroinvertebrates over a 0.5 mm mesh sieve, allowing their separation for subsequent identification. From each leaf sample, 10 mm diameter discs were excised and used for ash-free dry mass (AFDM) determination and for ergosterol and ATP analyses.

The set of discs designated for weighing was placed in a muffle furnace at 500 °C, where high temperatures combusted the organic compounds, leaving only inorganic material. Thus, by subtracting ash mass from total dry mass, the organic fraction of the sample (ash-free dry mass, AFDM) was obtained. The remaining leaf material was then oven-dried at 60 °C and subsequently weighed. The dry mass of the remaining leaf material was added to the mass of the excised discs, resulting in the final sample mass. Using the initial and final masses, leaf mass loss during the incubation period was calculated, and the decomposition rate constant ( $k$ ) was estimated.

#### 2.4.2- Extraction and Quantification of Ergosterol and ATP

Fungal biomass associated with the leaves was estimated based on ergosterol concentration (Gessner, 2020). For lipid extraction, three leaf discs were placed in a potassium hydroxide–methanol solution (KOH/methanol, 8 g L<sup>-1</sup>) and incubated in a water bath. Using a vacuum manifold, the extract was transferred to a solid-phase extraction (SPE) column, and ergosterol was subsequently eluted with isopropanol. Quantification was performed by chromatography using an HPLC system (DIONEX Summit P580, Sunnyvale, CA, USA), and results were expressed as µg ergosterol g<sup>-1</sup> AFDM.

The ATP extraction process begins with the homogenization of leaf discs in a mixture of sulfuric acid, oxalic acid, and HEPES solutions. The mixture is then centrifuged to separate the solids from the liquid fraction containing the extracted ATP. The resulting supernatant is filtered to remove impurities, and the pH is adjusted to 7.0–7.5. The solution is stored in vials and frozen until ATP measurement. After extraction, ATP is quantified by bioluminescence using a luminometer, and results are expressed as nmol ATP g<sup>-1</sup> AFDM (Abelho et al., 2005).

## 2.5- Sorting and Identification of Macroinvertebrates

Macroinvertebrates associated with the litterbags were preserved in glass vials containing 70% ethanol. Sorting and identification were conducted exclusively in the present study, up to the taxonomic level of Order, using the identification manual *Insetos aquáticos na Amazônia brasileira: taxonomia, biologia e ecologia* [*Aquatic insects of the Brazilian Amazon: taxonomy, biology, and ecology*].

## 2.6- Land use variables

The Land Use Index (LUI, Eq. 1, adapted from Rawer-Jost et al. 2004) were calculated for the upstream catchment.

$$\text{LUI} = 4x \% \text{CAT}_{\text{urb}} + 2x \% \text{CAT}_{\text{agr}} + \% \text{CAT}_{\text{mod}} \text{ (Eq. 1)}$$

Where LUI is the Land Use Index; CAT<sub>urb</sub>, CAT<sub>agr</sub> and CAT<sub>mod</sub> are, respectively, the percentage of urban, agricultural and pasture, and other uses (allotment, exposed soil, eucalyptus plantations) in the upstream catchment.

## 2.7- Tropical Water Health Index

To assess the influence of stream impact on leaf litter decomposition, an index that measures stream health was used (TWHI – Tropical Water Health Index). The index was constructed using three components—pressure (alterations in land use), condition (hydrology, water quality, biological assemblages and ecosystem processes), and response (water body class)—which together represent overall ecosystem health. The index assigns each stream a value ranging from 0 to 1, with values closer to 1 indicating conditions more similar to the natural state (Campos et al., 2024).

## 2.8- Data Analysis

To investigate the relationships between leaf litter decomposition and environmental gradients, Generalized Additive Models (GAMs) were fitted, with decomposition rate constant ( $k$ ) used as the response variable. Separate models were constructed to evaluate the relationship between decomposition and stream health index (TWHI), land use intensity index (LUI), and each land-use component within the upstream catchment, including natural vegetation, agriculture, urbanization, and other anthropogenic modifications. This approach allowed the identification of non-linear patterns and potential changes in response trends along the gradients.

To identify which limnological variables influence leaf litter decomposition, the Boosted Regression Trees (BRT) technique was applied. Decomposition ( $k$ , rescaled) was used as the response variable, and explanatory variables included water temperature, conductivity, current velocity, turbidity, pH, dissolved oxygen, nitrate, and phosphate. Models were fitted assuming a Gaussian distribution, with tree complexity set to 5, learning rate of 0.01, and a bag fraction of 0.75. The relative importance of each variable was obtained from its percentage contribution to the model, and the relationships between the most influential variables and decomposition were evaluated using partial dependence plots.

A Principal Component Analysis (PCA) was performed using limnological variables to visualize multivariate structure and stream ordination in the space defined by the main axes. Sampling sites were color-coded according to TWHI

intervals, allowing the identification of associations between decomposition, limnological conditions, and different levels of environmental integrity.

Linear regression models were used to assess the relationship between leaf litter decomposition and biotic factors, including EPT abundance, fungal biomass estimated by ergosterol, and bacterial activity estimated by ATP. Analysis of variance (ANOVA) was applied to compare EPT abundances among different TWHI intervals, and Generalized Additive Models (GAMs) were fitted to evaluate variation in ergosterol and ATP concentrations as a function of TWHI. All analyses were performed using R version 4.4.0 (R Core Team, 2024).

### **3- Results**

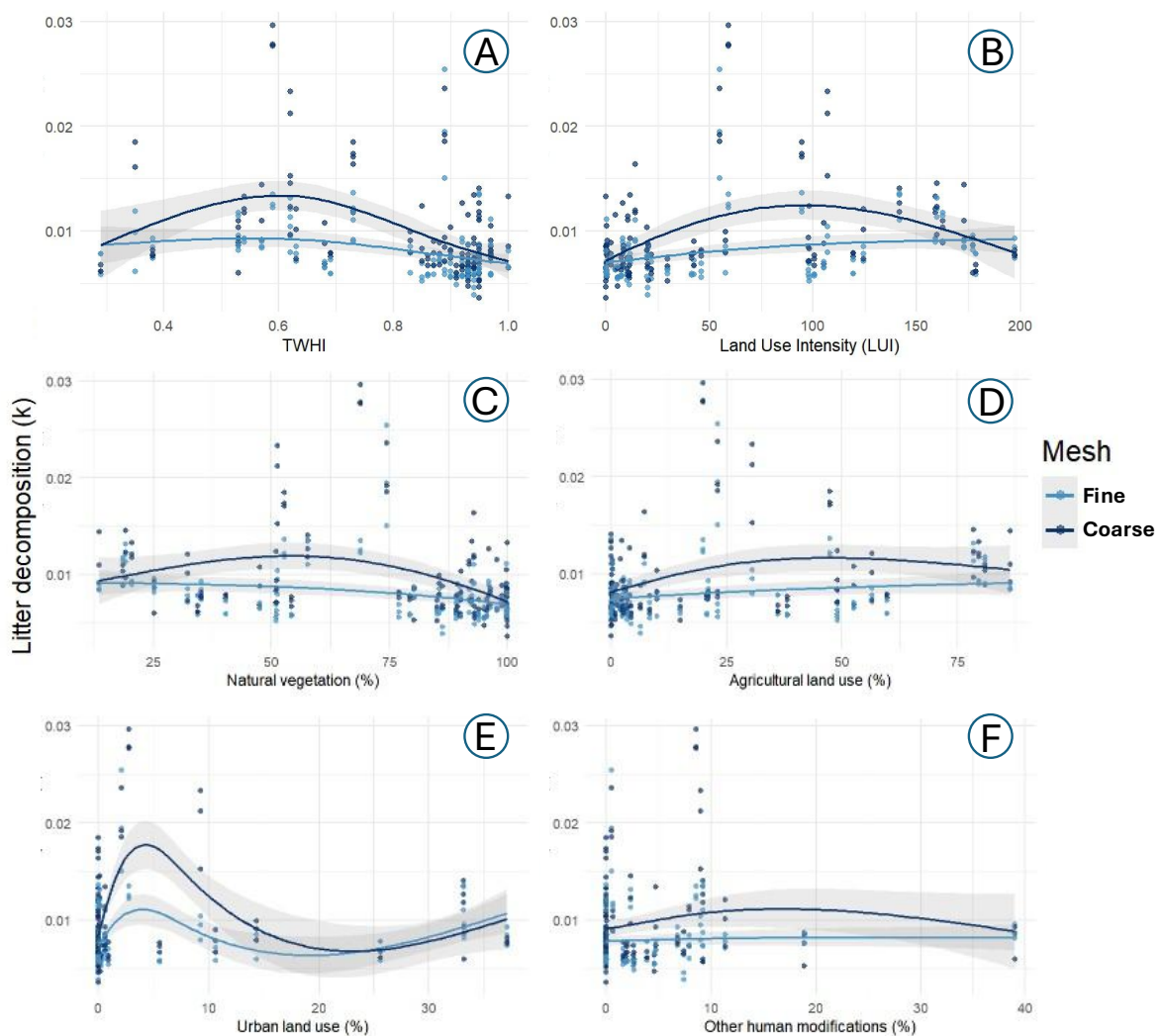
#### **3.1 – Sensitivity of leaf litter decomposition to stream health gradient (TWHI) and land use**

Leaf litter decomposition varied along the stream health gradient ( $p < 0.001$ ), with the TWHI explaining approximately 21% of the observed variation ( $R^2 = 0.21$ ). The pattern described by the model indicated a non-linear response: the lowest decomposition rates occurred in streams with both low and high TWHI values, whereas the highest rates were recorded in streams with intermediate levels of integrity. The model also showed that decomposition in coarse-mesh litterbags tended to be higher than in fine-mesh litterbags (Figure 1A).

Decomposition was affected by land use intensity (LUI;  $p < 0.001$ ; adjusted  $R^2 = 0.18$ ). The response pattern was non-linear, indicating that decomposition did not change in a strictly increasing or decreasing manner along the LUI gradient. The fitted curve showed that decomposition was highest at intermediate levels of land use intensity, with lower rates at both extremes of the gradient (Figure 1B).

Each specific land-use component exhibited distinct effects on leaf litter decomposition. Agricultural land use showed a significant effect on decomposition ( $p < 0.001$ ), explaining 13.3% of the variation and representing one of the land-use components with the highest explanatory power when analyzed independently (Figure 1D). Urbanization explained 4.93% of the

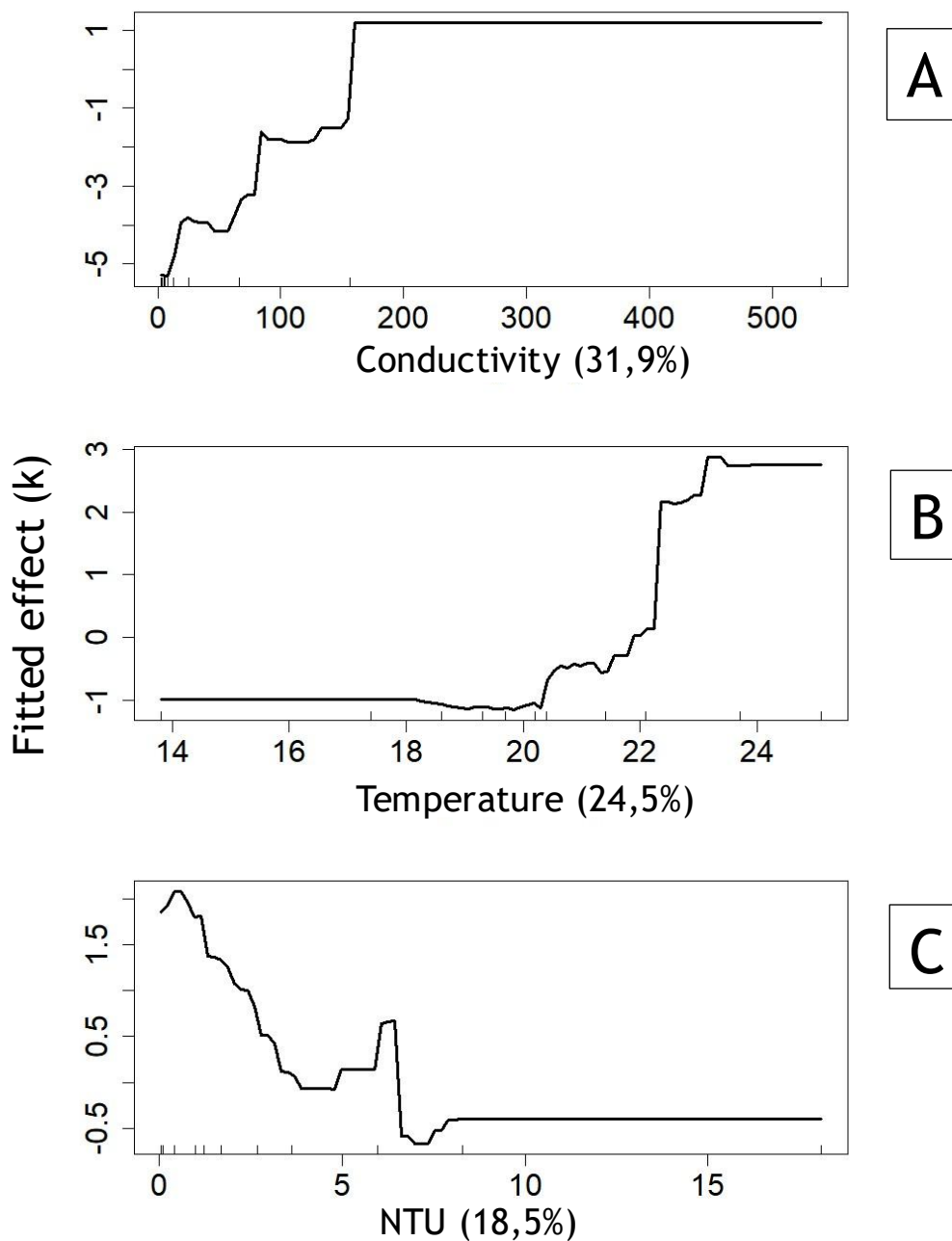
variation ( $p < 0.001$ ), with the fitted curve indicating an increase in decomposition even at low levels of urbanization (Figure 1E). Other anthropogenic modifications within the watershed explained 2.43% of the variation in decomposition ( $p < 0.001$ ) (Figure 1F). Decomposition was also modulated by the percentage of natural area ( $p < 0.001$ ), explaining 14.1% of the variation, a value similar to that observed for agricultural land use, indicating that natural land cover is a relatively strong isolated predictor (Figure 1C). The distribution of points and the fitted GAM curve exhibited a pattern opposite to that observed for LUI, reflecting the inverse relationship between these two land-use metrics.



**Figure 1.** GAM models showing the relationship between decomposition and disturbance and land-use metrics: (A) stream health index (TWHI); (B) land use intensity index (LUI); (C) percentage of natural vegetation in the watershed; (D) percentage of agricultural area; (E) percentage of urbanization; and (F) other anthropogenic modifications. Curves represent the smoothed effect of each predictor on decomposition, while points illustrate the observed data dispersion.

### 3.2 – Responses of leaf litter decomposition to water physicochemical variables

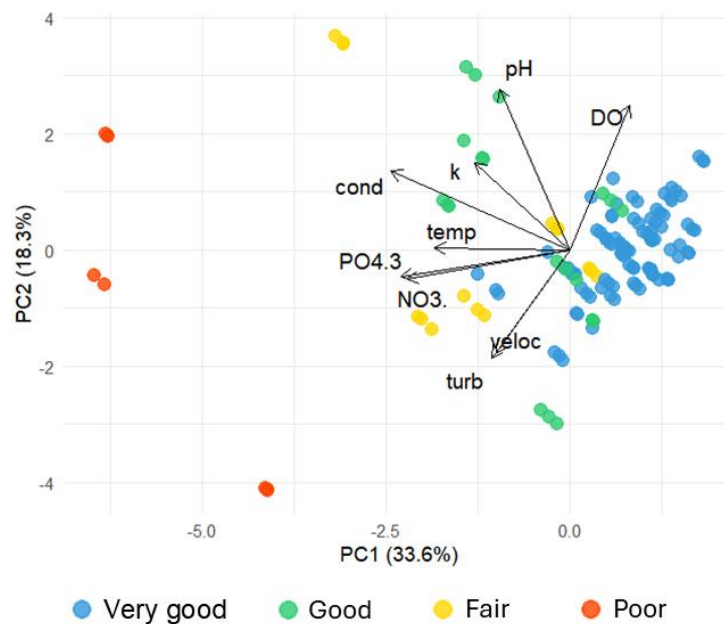
The relative importance of the variables indicated that electrical conductivity was the main predictor in the analysis (31.9%), followed by temperature (24.5%) and turbidity (18.5%). The remaining variables showed smaller effects: nitrate (9.6%), dissolved oxygen (5.0%), water velocity (4.8%), pH (2.9%), and phosphate (2.4%). The BRT analysis explained 90.7% of the variation in decomposition.



**Figure 2.** Boosted Regression Tree (BRT) analysis plots for the three most important variables. Electrical conductivity explained 31.9% of the variation in decomposition, followed by temperature (24.5%) and turbidity (18.5%).

### 3.3 – Grouping of Streams Based on Limnological Variables

The Principal Component Analysis (PCA) based on limnological variables (Figure 3) explained 51.9% of the total variation along the first two axes (PC1 = 33.6%; PC2 = 18.3%). Streams classified as optimal (blue) clustered on the right side of the ordination and were associated with higher dissolved oxygen values, in contrast to the vectors representing temperature, electrical conductivity, and nutrients. In contrast, poor-quality streams clustered toward the end of the ordination associated with higher values of these variables. Streams classified as good and moderate occupied intermediate positions, reflecting environmental conditions characterized by moderate values of these parameters.

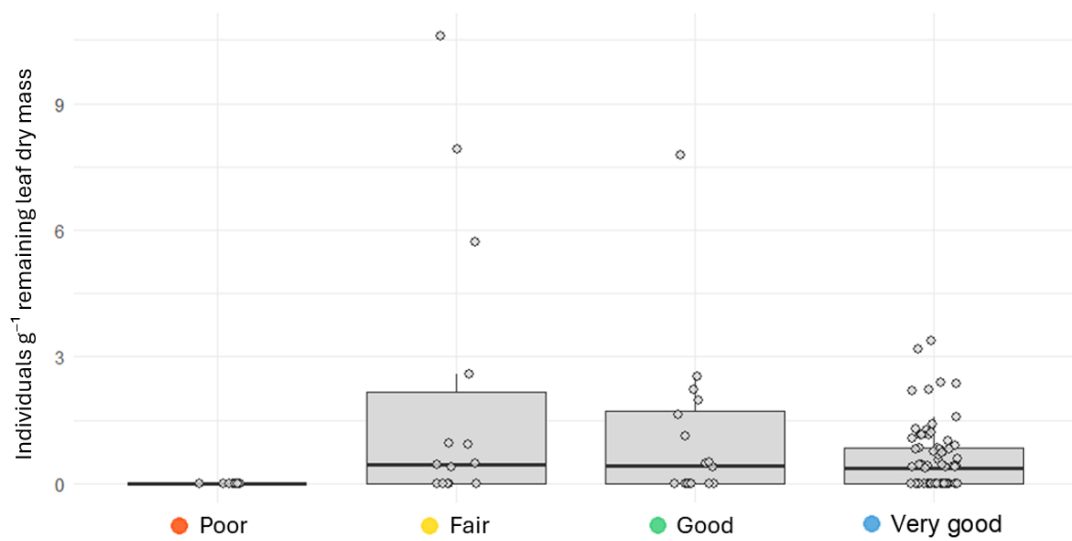


**Figure 3.** Principal Component Analysis (PCA) showing the ordination of sampling sites in relation to limnological variables. Sampling sites are classified according to Tropical Water Health Index (TWHI) intervals: Very good (0.81–1.0), Good (0.61–0.80), Fair (0.41–0.60), and Poor (0.21–0.40).

### 3.4 – Responses of leaf litter decomposition to biotic factors

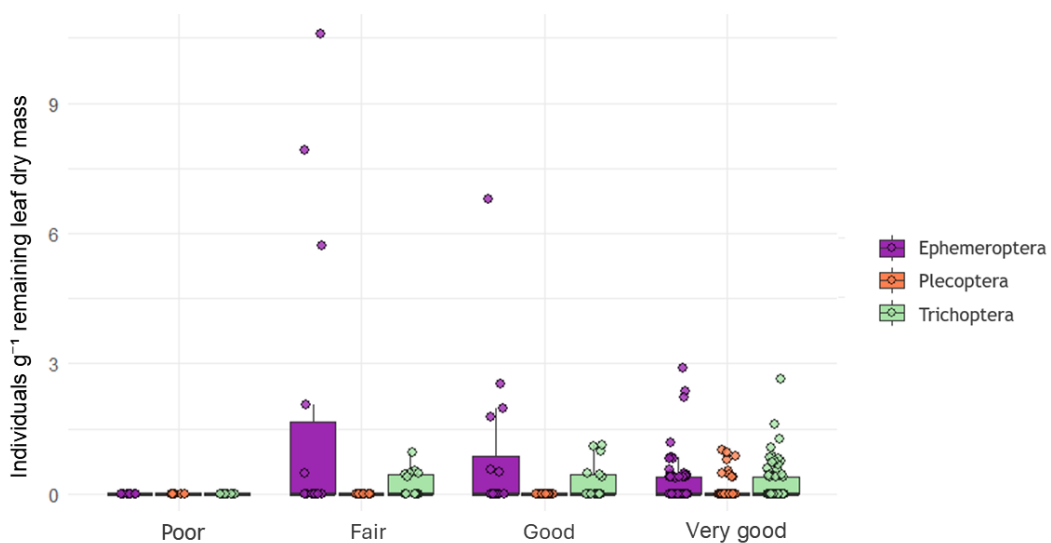
Decomposition responded to macroinvertebrate abundance, with streams exhibiting higher abundances also showing higher decomposition (linear model:  $p < 0.001$ ). EPT abundance varied along the stream health gradient (Figure 4),

with streams classified as Fair showing higher abundances than those classified as Very good ( $p > 0.001$ ) and Poor ( $p < 0.001$ ).



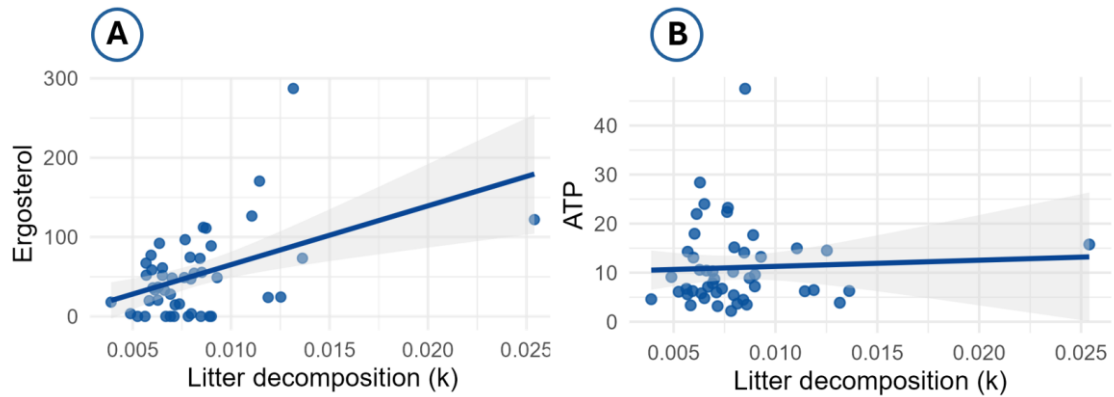
**Figure 4.** Boxplots showing the variation in the proportion of EPT (Ephemeroptera, Plecoptera, and Trichoptera) across four Tropical Water Health Index (TWHI) classes. Boxes represent the distribution of values within each class.

The relative abundance of each Order differed across the stream health gradient. Individuals of the Order *Plecoptera* were present only in streams classified as Very good. *Trichoptera* and *Ephemeroptera* were present in streams classified as Very good, Good, and Fair, with *Ephemeroptera* showing higher abundance in Fair streams. None of these orders were recorded in streams classified as Poor.



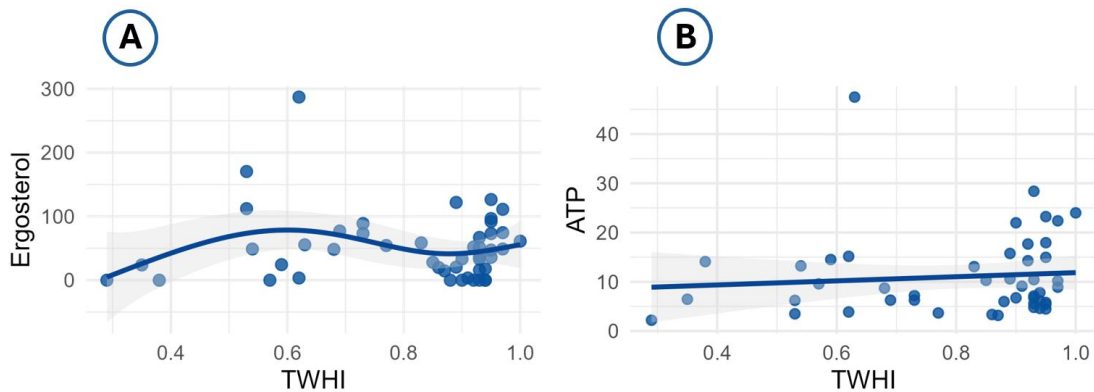
**Figure 5.** Boxplots showing the variation in the relative proportion of each order within the EPT group across the sampled streams.

Fungal biomass, estimated by ergosterol concentration, showed a positive relationship with decomposition, being a significant predictor in the analysis ( $p = 0.001$ ). Total microbial activity (ATP) was not significantly associated with decomposition ( $p = 0.611$ ). Together, these variables explained 22% of the variation in decomposition rates ( $R^2 = 0.22$ ; adjusted  $R^2 = 0.18$ ;  $p = 0.004$ ).



**Figure 6.** Relationship between leaf litter decomposition (k) and microbial descriptors. Panel (A) shows the relationship between decomposition (k) and ergosterol, while panel (B) shows the relationship between decomposition (k) and ATP.

The relationship between the stream health index (TWHI) and ergosterol and ATP was not significant ( $p = 0.129$  and  $0.533$ , respectively). Despite the lack of statistical significance, the fitted curve indicated a tendency for higher ergosterol values at intermediate TWHI levels, showing a gently unimodal response (Figure 7).



**Figure 7.** Relationship between the stream health index (TWHI) and ergosterol concentration (A) and ATP concentration (B).

## Discussion

The patterns observed along the environmental integrity gradient indicate that leaf litter decomposition does not respond linearly to environmental conditions, for either the TWHI or the land use index. The highest decomposition values occurred at intermediate positions along the disturbance gradient, a pattern that was also evident when the agricultural component was analyzed independently. This pattern, in which decomposition tends to increase with increasing disturbance, is consistent with studies reporting higher decomposition in areas with greater agricultural activity (Mancuso et al., 2023) compared to sites with lower impact intensity (Pérez et al., 2023). However, our study captured this pattern only up to a certain point along the gradient, beyond which decomposition began to decline as disturbance increased, consistent with the assumptions of the intermediate disturbance hypothesis (Tonkin et al., 2013). Moderately disturbed reaches combine higher inputs of allochthonous resources and nutrients with partial maintenance of habitat structure, favoring microbial and macroinvertebrate communities (Tian et al., 2022). Consequently, the higher decomposition observed at these intermediate levels of the gradient reflects a balance between functional stimulation and environmental limitation

The response associated with urbanization exhibited a distinct pattern. We recorded an increase in decomposition at low levels of urbanization (Yule et al., 2015); however, this effect did not persist across the entire gradient. As urbanization intensified, decomposition tended to decline, a pattern already documented in the literature through comparisons between minimally and highly urbanized areas (Martins et al., 2015; Gao et al., 2022), where more urbanized environments exhibit lower decompositional efficiency. This pattern suggests that initial increments of urban alteration may modify resource availability or habitat structure (Granados-Martínez et al., 2025), whereas higher levels of urbanization result in less favorable conditions for the communities that drive decomposition (Bohus et al., 2023).

Taxa of *Ephemeroptera* and *Trichoptera* exhibited relatively higher tolerance to environmental disturbance, being recorded both in streams with very good conditions and in streams with moderate levels of disturbance. In contrast, representatives of *Plecoptera* were restricted to streams with high environmental integrity (Nessimian et al., 2008; Faria et al., 2021). The increase in

*Ephemeroptera* individuals may be associated with community replacement by more tolerant taxa along the gradient (Bonada et al., 2006). However, in streams classified within the poorest environmental quality class, no EPT taxa were recorded, indicating that the high level of environmental degradation exceeded the tolerance limits of these disturbance-sensitive taxa, coinciding with the decline in decomposition (Faria et al., 2021; Martins et al., 2015).

Temperature showed a positive relationship with decomposition, which can be explained by increased leaching of soluble compounds at higher temperatures (Ferreira & Chauvet, 2011) and by enhanced metabolic rates of decomposer organisms (Wilmot et al., 2021). A positive relationship was observed between decomposition and electrical conductivity. Positive relationships between electrical conductivity and ecological processes in streams have been reported in the literature, for example, increases in the density of *Phylloicus* (Leite et al., 2016). In addition, electrical conductivity is closely associated with nutrient availability in the water, which can promote microbial communities and, consequently, decomposition processes (Ferreira et al., 2006). The negative correlation observed between decomposition and turbidity is associated with the fact that high turbidity values are characteristic of heavily impacted streams, particularly in landscapes subject to intensified soil erosion and increased surface runoff, which promote the transport of fine sediments into the channel. In addition, turbidity may reflect the influence of urban effluents, which simultaneously alter water quality and the physical structure of the habitat.

The positive relationship between ergosterol concentration and decomposition indicates an important contribution of fungi to the decomposition process (Baudy et al., 2021). Although previous studies have highlighted the role of bacteria in leaf litter decomposition (Pascoal & Cássio, 2004), microbial activity (ATP) was not significantly associated with decomposition in our study, suggesting that fungal biomass played a more prominent role in driving decomposition rates.

The lack of a significant relationship between TWHI and ergosterol and ATP concentrations indicates that microbial activity does not respond directly to the environmental integrity gradient. This result contrasts with the positive relationship observed between leaf litter decomposition and fungi, suggesting

that the absence of statistical significance may be associated with limitations inherent to the sampling approach. Ergosterol represents a snapshot of fungal biomass at the time of sampling (Gessner & Chauvet, 1993) and does not capture the successional dynamics of microbial communities throughout the decomposition process, which may vary across environments with different levels of integrity. Consequently, distinct environments may exhibit contrasting successional trajectories, with shifts in fungal groups over time, cumulatively influencing the metabolic activity responsible for leaf mass loss.

In this context, experiments incorporating multiple sampling times throughout leaf incubation would allow a more refined assessment of microbial dynamics, while the inclusion of approaches such as sporulation measurements (Ferreira & Chauvet, 2011) could provide additional information on community composition and metabolic activity over time under different environmental conditions. Although we found positive contributions of fungi to decomposition, the most important biological factor explaining differences in decomposition along the TWHI gradient was the proportion of EPT individuals (Oester et al., 2023).

Taken together, these results reinforce that decomposition in tropical streams responds to multiple components of the environmental gradient, including structural, biological, and physicochemical factors, and that interactions among these components determine the magnitude of organic matter processing. This complexity helps explain the non-linear patterns associated with land use and highlights the importance of considering multiple mechanisms when interpreting the ecological integrity of aquatic systems.

## **Conclusion**

Our findings demonstrate that fundamental ecological processes, such as leaf litter decomposition, are sensitive to environmental degradation, highlighting that changes in stream integrity directly affect the functioning of aquatic ecosystems. These findings underscore the need to adopt new impact assessment strategies that consider, in addition to structural indicators, the responses of ecological processes, emphasizing leaf litter decomposition as a

functional and integrative metric for more robust assessments that can inform management and conservation actions in tropical streams.

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