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Fungicide exposure reshapes gut-associated fungal communities without altering short-term feeding activity in a Neotropical shredder

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ABSTRACT

Fungicide exposure reshapes gut-associated fungal communities without altering short-term feeding activity in a Neotropical shredder.

Microbial–invertebrate interactions are central to organic matter turnover in detritus-based stream ecosystems, yet their sensitivity to agrochemical contamination remains poorly understood. We experimentally investigated how dietary fungicide exposure influences gut-associated fungal communities and feeding performance of *Phylloicus* sp., a key Neotropical shredder. Twenty larvae were maintained under laboratory conditions and fed leaf discs of *Inga vera* either untreated or conditioned with the fungicide Mancozeb (40 µg/L). Fungal morphotypes were isolated through culture-based methods and analyzed using multivariate and mixed-model approaches. Fungicide exposure significantly altered fungal community composition in the larval gut, indicating selective filtering of tolerant taxa, although fungal richness remained unchanged. Despite compositional restructuring, leaf litter consumption did not differ between treatments. These findings reveal a structural shift in gut-associated fungal assemblages without immediate functional consequences for feeding, suggesting short-term ecological buffering within shredder–microbe interactions. Our results highlight that fungicide contamination can modify microbial assembly pathways in tropical headwaters, even when detritivore performance appears stable, with potential implications for long-term ecosystem functioning under increasing agricultural intensification.

KEY WORDS: Gut microbiota, Fungicide exposure, Microbial filtering, Detritivore, Tropical streams, Community restructuring.

RESUMO

A exposição a fungicida reestrutura as comunidades fúngicas associadas ao intestino sem alterar a atividade alimentar de curto prazo em um fragmentador Neotropical.

As interações entre microrganismos e invertebrados são centrais para a ciclagem da matéria orgânica em ecossistemas lóticos baseados em detritos, porém sua sensibilidade à contaminação por agroquímicos ainda é pouco compreendida. Investigamos experimentalmente como a exposição alimentar a um fungicida influencia as comunidades fúngicas associadas ao intestino e o desempenho alimentar de Phylloicus *sp., um importante fragmentador Neotropical. Vinte larvas foram mantidas em condições laboratoriais e alimentadas com discos foliares de* Inga vera*, não tratados ou condicionados com o fungicida Mancozeb (40 µg/L). Os morfotipos fúngicos foram isolados por métodos baseados em cultivo e analisados por meio de abordagens multivariadas e modelos mistos. A exposição ao fungicida alterou significativamente a composição da comunidade fúngica no intestino das larvas, indicando uma filtragem seletiva de táxons tolerantes, embora a riqueza fúngica* *tenha permanecido inalterada. Apesar da reestruturação composicional, o consumo foliar não diferiu entre os tratamentos. Esses resultados revelam uma mudança estrutural nas assembleias fúngicas associadas ao intestino sem consequências funcionais imediatas para a alimentação, sugerindo um amortecimento ecológico de curto prazo nas interações entre fragmentadores e microrganismos. Nossos resultados destacam que a contaminação por fungicidas pode modificar as vias de montagem microbiana em riachos tropicais de cabeceira, mesmo quando o desempenho dos detritívoros aparenta estabilidade, com potenciais implicações para o funcionamento ecossistêmico em longo prazo diante da crescente intensificação agrícola.*

PALABRAS CLAVE: *microbiota intestinal, exposição a fungicidas, filtragem microbiana, detritívoro, riachos tropicais, reestruturação de comunidades.*

INTRODUCTION

Leaf litter decomposition constitutes a central ecological process in detritus-based stream ecosystems, where microbial colonization regulates the transfer of energy and nutrients across trophic levels (Graça et al., 2015, Rezende et al., 2019, Sena et al., 2024). Aquatic fungi play a preponderant role during early stages of decomposition by conditioning senescent leaves through enzymatic degradation of structural polymers, thereby increasing substrate palatability and nutritional value for detritivorous invertebrates (Graça, 2001, 2015, Biasi et al., 2019). This microbial conditioning reduces leaf toughness, enriches nitrogen content, and promotes the formation of biofilms that represent a primary food resource for shredders in headwater streams (Rezende et al., 2019, Cararo et al., 2023a, Sena et al., 2026). Consequently, the functional link between fungal assemblages and detritivore feeding activity represents a key mechanism sustaining organic matter processing and ecosystem functioning in lotic environments (Douglas, 2015, Santos et al., 2019, 2021).

Globally, shredders play a crucial role in stream ecosystems, and larvae of the order Trichoptera are widely recognized as dominant processors of coarse particulate organic matter and key indicators of environmental quality (Rezende et al., 2021, Sena et al., 2024, Durigon et al., 2025). Within this functional group, the genus *Phylloicus* Müller, 1880 (Calamoceratidae) has emerged as an excellent model for exploring these ecological interactions, particularly in the Neotropics (Rezende et al., 2021, Sena et al., 2024, Durigon et al., 2025). Like other shredders, *Phylloicus* larvae feed primarily on microbially conditioned leaf litter, selecting substrates colonized by fungal communities that enhance nutritional quality (Sena et al., 2021, Cararo et al., 2023a). During feeding, larvae continuously ingest fungal propagules and mycelial fragments (Engel & Moran, 2013, Douglas, 2015). Recent evidence indicates that these gut-associated fungi are largely transient components derived from dietary sources and therefore reflect the composition of environmental microbial assemblages (Engel & Moran, 2013, Douglas, 2015). Even so, these fungi may still contribute to host physiological and ecological functions (Wilson et al., 2014). Consequently, any shifts in leaf-associated fungal communities are expected to propagate into the gut microbiota of shredders through trophic transfer, a mechanism likely shared across diverse lotic environments (Belmont-Montefusco et al., 2020, Santos et al., 2021).

Beyond these natural ecological processes, anthropogenic disturbances increasingly disrupt microbially mediated energy pathways and nutrient dynamics in freshwater ecosystems (Kovalenko, 2019, Machado et al., 2022, Rezende et al., 2023, 2025). Among these stressors, agricultural runoff introduces pesticides and fungicides into streams, exposing non-target microbial communities to compounds specifically designed to inhibit fungal metabolism (Cornejo et al., 2021, Zubrod et al., 2019). Mancozeb, a widely used dithiocarbamate fungicide applied in preventive crop protection, represents a major ecological concern due to its broad-spectrum, multi-site toxicological action that interferes with essential metabolic pathways in fungal cells (Dall'Agnol et al., 2021, Runkle et al., 2017, Wüthrich et al., 2025, Pimentão et al., 2025). Once transported to aquatic environments, Mancozeb can alter the colonization and activity of leaf-associated fungi responsible for litter conditioning, reducing microbial biomass, modifying enzymatic processes involved in decomposition, and selecting for tolerant taxa (Casalone et al., 2010, Walia et al., 2014, Santos et al., 2021). Such alterations may disrupt gut fungal assemblages that contribute to lignocellulosic degradation, ultimately influencing feeding behavior, nutrient assimilation, and leaf consumption rates in shredders such as *Phylloicus*, thereby compromising detrital processing and trophic efficiency in stream ecosystems (Graça et al., 2015, Santos et al., 2021).

Such trophic transfer provides a mechanistic pathway linking chemical disturbance, microbial community restructuring, and detritivore performance (Wilson et al., 2014, Machado et al., 2022, Pimentão et al., 2025). However, empirical evidence connecting fungicide exposure, gut-associated fungal communities, and feeding activity remains scarce, particularly in regions where data on these interactions are underrepresented. By characterizing these fungal-insect associations, it becomes possible to elucidate their mechanistic contributions to nutrient cycling and energy flow in stream ecosystems (Wilson et al., 2014, Machado et al., 2022, Pimentão et al., 2025). Building upon these premises, the present study aimed to evaluate how dietary composition and exposure to Mancozeb-conditioned litter influence: (i) leaf consumption by *Phylloicus* larvae and (ii) the composition and richness of fungi detected within their digestive tract. We hypothesized that fungicide exposure would modify leaf-associated fungal assemblages, leading to shifts in gut fungal community composition and reduced litter consumption due to decreased microbial conditioning. By integrating microbial and trophic responses, this study clarifies how fungicide contamination propagates through detrital food webs, providing critical data to fill current knowledge gaps in ecological functioning.

MATERIALS AND METHODS

Invertebrate and leaf litter collection

Larvae of *Phylloicus* Müller, 1880 (Trichoptera: Calamoceratidae) were collected from a first-order stream (27°00′32.8″S, 52°40′55.1″W) in Chapecó, Santa Catarina State, southern Brazil. The sampling site lies within a remnant of Atlantic Forest at an elevation of 650–700 m, characterized by a seasonal deciduous forest, a typical physiognomic type of the subtropical highlands (ICMBio, 2013, Cararo et al., 2023a, Durigon et al., 2025). Specimens were captured via random active searches using aquatic entomological nets and immediately sorted in the field. To ensure experimental uniformity, only larvae with protective cases approximately 1.5 cm long were selected (Rezende et al., 2015). Larval shelters remained intact until the time of digestive tract extraction to minimize stress and maintain natural behavior (Rezende et al., 2021). A total of 20 *Phylloicus* larvae were transported to the Laboratory of Subtropical Ecosystems Studies at Unochapecó in thermal boxes to maintain ambient conditions (Fig. 1).

Leaves of *Inga vera* Willd., an abundant native species commonly found in the riparian vegetation of the Atlantic Forest (Borges et al., 2024, Pazini et al., 2025), were collected and analyzed. Senescent leaves were collected directly from individual trees using suspended nets (1 m², positioned 1.5 m above ground) to prevent contact with the soil. All collected leaves were subsequently leached in distilled water (100 g of leaves in 8 L) for seven days to remove soluble compounds. To determine chemical composition, a set of *I. vera* leaves was submitted to plant tissue analysis at the Soil Physics and Chemistry Laboratory of the University of Western Santa Catarina (UNOESC), Brazil. Average concentrations in dry leaf material were 25.2 g/kg of nitrogen, 0.32 g/kg of phosphorus, 4.29 g/kg of potassium, 14.23 g/kg of calcium, 1.12 g/kg of magnesium, and 497.18 g/kg of organic carbon, indicating relatively nutrient-rich litter compared with several woody riparian species. Plant tissue analyses followed standard procedures described by Silva (2009). Leaf samples were oven-dried at 105 °C prior to analysis. Phosphorus, potassium, calcium, and magnesium were extracted from dried material through sulfuric acid digestion (H_2SO_4 + H_2O_2). Phosphorus was determined colorimetrically using the ascorbic acid method, potassium

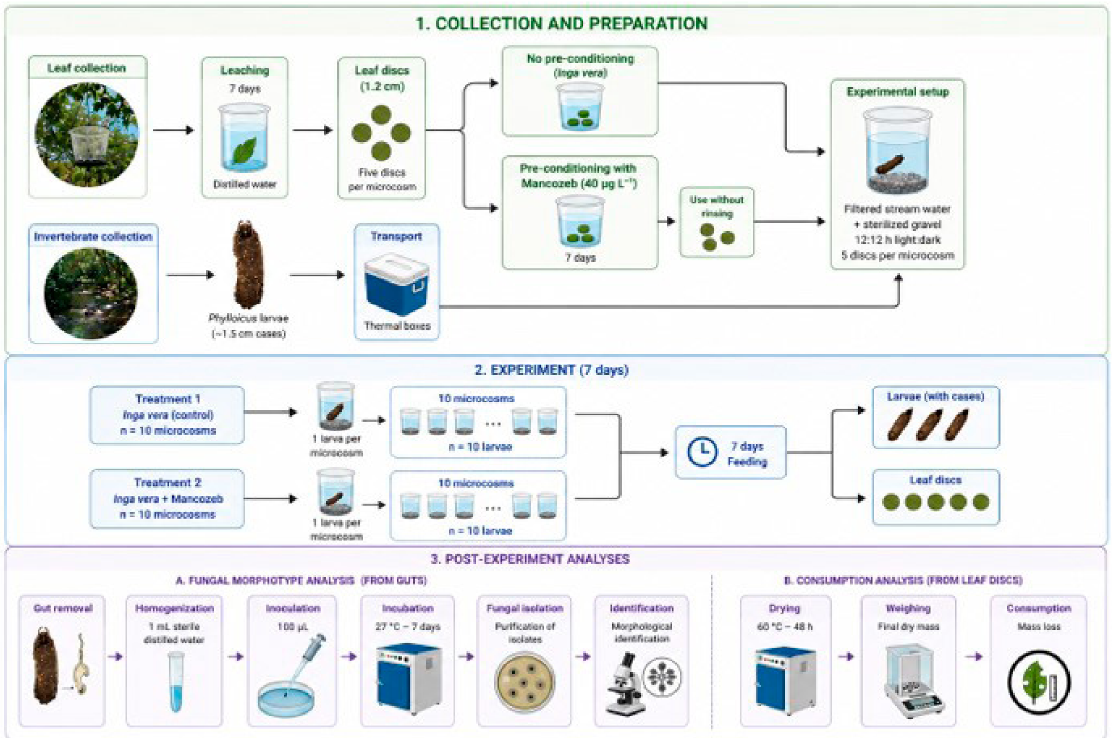

Figure 1. Experimental design showing leaf collection and preparation, establishment of control and Mancozeb-conditioned treatments (leaf discs and preconditioned leaf discs), individual exposure of *Phylloicus* larvae in microcosms, and post-experimental analyses of leaf consumption and gut-associated fungal morphotypes. *Delineamento experimental mostrando a coleta e preparação das folhas, o estabelecimento dos tratamentos controle e condicionado com Mancozeb (discos foliares e discos foliares pré-condicionados), a exposição individual de larvas de Phylloicus em microcosmos e as análises pós-experimento do consumo foliar e dos morfotipos fúngicos associados ao intestino*.

was measured using flame photometry, and calcium and magnesium were quantified by atomic absorption spectrophotometry.

Experimental design

Following leaching, litter discs (1.2 cm in diameter), excluding the midrib, were cut and used in microcosm experiments (Fig. 1). For the fungicide treatment, leaf discs were conditioned for seven days in distilled water containing Mancozeb at a nominal concentration of 40 µg/L. This concentration falls within ranges reported for agricultural runoff and experimental ecotoxicological studies assessing fungicide effects on aquatic microbial communities (Zubrod et al., 2019, Gonçalves et al., 2024). Although actual concentrations were not analytically verified, the nominal level used here represents a plausible short-term exposure scenario for headwater streams influenced by agricultural activities (Zubrod et al., 2019, Cornejo et al., 2021). After conditioning, the discs were removed from the fungicide solution and used as food resources in the feeding assays. No Mancozeb was added directly to the experimental microcosms, which contained only stream water, therefore, *Phylloicus* larvae were exposed to the fungicide exclusively through the conditioned leaf material. Leaf discs were not rinsed after conditioning to preserve fungicide residues associated with the litter surface. Sets of five leaf discs per treatment were frozen at ± 20 °C, lyophilized for 24 h, and weighed (± 0.01 mg) to determine initial dry mass (DM). Also, to correct for moisture content, additional discs were oven-dried at 60 °C for 48 h, and subsequently incinerated at 500 °C for 4 h in a muffle furnace to determine ash-free dry weight (AFDW), which was used to standardize biomass estimates across samples (Fig. 1).

Then, *Phylloicus* larvae were randomly assigned to two treatments: (i) leaf discs (n = 10), and (ii) preconditioned leaf discs (n = 10). Each larva was maintained individually in 450 mL disposable containers filled with filtered stream water and a thin layer of sterilized gravel, under a controlled 12:12 h light-dark photoperiod (Fig. 1). Each microcosm received five leaf discs corresponding to its assigned treatment (Cararo et al., 2023b, Rezende et al., 2015). Additionally, leaf discs without larvae were not included as decomposition controls, therefore, the estimated mass loss may incorporate a small contribution from microbial decomposition or handling losses in addition to actual larval consumption, which should be considered as a limitation when interpreting feeding results.

After seven days of incubation, larval cases were carefully removed, and the digestive tracts were dissected under sterile conditions (Cararo et al., 2023b, Rezende et al., 2015). Leaf discs were oven-dried at 60 °C for 48 h and weighed to determine final dry mass and estimate leaf consumption (Cararo et al., 2023b, Rezende et al., 2015). Each dissected gut was homogenized in 1 mL of sterile distilled water, and 100 µL aliquots were inoculated onto Sabouraud Dextrose Agar supplemented with chloramphenicol (0.1 mg/L) to inhibit bacterial growth. Cultures were incubated at 27 °C for seven days (Belmont-Montefusco et al., 2020). Filamentous fungal isolates were purified and identified through microculture techniques to examine reproductive structures in detail. Morphotype identification was based on microscopic and macroscopic morphological characteristics following the diagnostic criteria listed in Table S1 (supplementary information, available at https://www.limnetica.com/en/limnetica) (Dugan, 2017). It should be noted that the culture-based approach used here detects only culturable fungi and therefore underestimates total fungal diversity. Furthermore, the morphotypes described represent colony morphology units and are not equivalent to taxonomically defined species, molecular identification methods (e.g., ITS sequencing) would be required for species-level conclusions.

Data analysis

All statistical analyses were performed in R version 4.4.1 (R Core Team, 2025), using the RStudio integrated development environment (Posit team, 2024). Fungal community composition was visualized using Principal Coordinates Analysis (PCoA) based on Jaccard dissimilarity with Cailliez correction. Differences in community structure between treatments were tested using Permutational Multivariate Analysis of Variance (PERMANOVA, based on Euclidean distances with 10 000 permutations) and corroborated by a Multi-Response Permutation Procedure (MRPP) to assess within-group homogeneity. These multivariate analyses were conducted using the vegan package (Oksanen et al., 2024).

To identify fungal morphotypes significantly associated with specific treatments, we performed an Indicator Species Analysis (IndVal) using the indicspecies package (Cáceres & Legendre, 2009). This analysis identifies morphotypes that are both exclusive to and frequent within a treatment group. Additionally, we calculated the absolute occurrence (frequency) of each morphotype per treatment to assess compositional shifts in the fungal community.

Generalized Linear Models (GLMs) were applied to evaluate treatment effects on fungal richness (number of morphotypes) and leaf litter consumption. Because each microcosm housed a single individual and represented a true independent experimental unit, random factors were excluded from the models to prevent over-parameterization. For fungal richness, a Poisson distribution (canonical link = log) was used, and models were fitted with the standard glm function. Leaf consumption (expressed as proportional mass loss) was modeled using a beta distribution with a logit link function to properly account for bounded proportional data, implemented with the glmmTMB package (Brooks et al., 2017). Treatment effects for both models were evaluated using Type II Wald chi-square tests provided by the car package (Fox & Weisberg, 2019). All statistical analyses were performed in R version 4.4.1 (R Core Team, 2025).

RESULTS

Fungal morphotypes

Based on morphological analyses, a total of 20 fungal morphotypes were identified from cultures obtained from the gut of *Phylloicus* larvae (Table 1, Table S1). Cottony or floccose colony textures predominated among isolates, representing the most frequent morphological pattern observed. Several morphotypes (e.g., M3, M5, M8, M13) exhibited extensive aerial mycelium and diffused growth across the culture medium. Less common colony relief patterns included crateriform (M1), cerebriform (M11), and rugose (M12) morphologies, whereas many colonies displayed poorly defined relief characteristics.

Pigmentation patterns were generally restricted to colony surfaces, with white, gray, or orange tones commonly observed without pigment diffusion into the agar medium. Reverse pigmentation occurred in some morphotypes, particularly those exhibiting orange or dark undersides. Yeast-like colonies (M14–M19) and glabrous morphotypes represented a smaller proportion of isolates compared with filamentous cottony forms, indicating that filamentous fungi dominated the gut-associated fungal assemblage.

Fungal community

Principal Coordinates Analysis (PCoA) based on Jaccard distances illustrated the distribution of fungal morphotypes, showing partial overlap among microcosms belonging to different treatments (Fig. 2a, b). However, exposure to Mancozeb pre-conditioning significantly altered the fungal community composition in the larval gut (PERMANOVA: $F = 2.06$, $R^2 = 0.103$, $p = 0.015$, Table S2 (supplementary information,

Table 1. Morphological characterization of the 20 morphospecies cultured includes detailed descriptions of colony morphology for each type, encompassing aspects such as texture, pigmentation patterns, margin definition, colony relief, adherence to the medium, and the extent of growth observed on both the agar surface and its reverse. *Caracterização morfológica das 20 morfoespécies cultivadas, incluindo descrições detalhadas da morfologia das colônias para cada tipo, abrangendo aspectos como textura, padrões de pigmentação, definição das margens, relevo da colônia, aderência ao meio e a extensão do crescimento observada tanto na superfície do ágar quanto em seu reverso***.**

Morphospecies	Characteristics
M1	Circular, white margins, crateriform with a yellow central crater, white reverse.
M2	Dark reverse, velvety, grayish surface, circular.
M3	Completely white, well-defined margin, cottony, white reverse.
M4	Undefined but circular margin, grayish, “X” in the center, white reverse.
M5	Almost transparent but still white, whitish reverse, barely changes the medium.
M6	More yellowish center, strongly adherent to the medium, not gelatinous, lighter margins, membranous.
M7	Cottony, white hyphae, dark spores, growth covering the entire plate.
M8	Cottony, powdery, broadly white and spread out, no defined margins or reverse.
M9	Orange reverse, strongly adherent to the medium, whitish surface with orange tones, apiculate.
M10	White reverse, apiculate, velvety.
M11	Cerebriform, white reverse, very light gray surface, almost white, defined margin.
M12	Rugose, undefined margin, resembles mounds of flour, intense orange reverse, white surface.
M13	Orange reverse, undefined margins, cottony, spreads across the plate, widely dispersed white hyphae.
M14	Yeast, yellowish beads, creamy colony.
M15	Yeast, more transparent, water-like, only perceptible by its somewhat defined margin.
M16	Yeast, smooth surface, more yellowish.
M17	Glabrous, smooth surface, more turbid appearance.
M18	Yeast, orange beads, creamy.
M19	Yeast, white colony, creamy.
M20	Glabrous, strongly adherent to the medium, turns the agar orange on both surface and reverse.

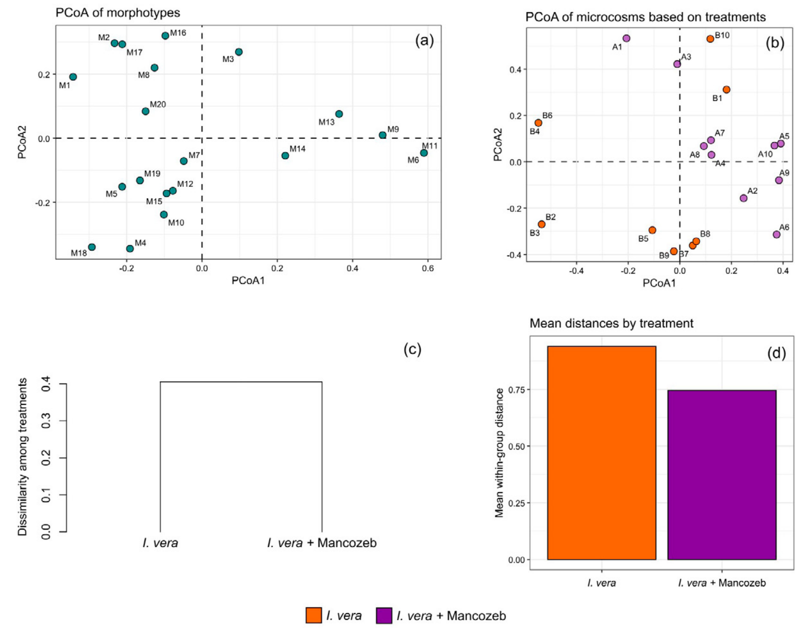

Figure 2. (A) Principal Coordinates Analysis (PCoA) based on Jaccard distances with Cailliez correction of the 20 morphotypes (M1-M20) shows the multivariate structure of trait presence/absence across microcosms. Each point represents a morphotype, illustrating their distribution in ordination space. (B) PCoA of microcosms using the same categorical morphotype matrix, with points colored by experimental treatment (leaf discs and preconditioned leaf discs). Labels correspond to individual microcosms, revealing partial overlap among treatments, consistent with weak group separation. (C) Cluster diagram derived from the meandist matrix, illustrating among-treatment dissimilarities. (D) Mean within-group distances (meandist) for each treatment. *(A) Análise de Coordenadas Principais (PCoA), baseada em distâncias de Jaccard com correção de Cailliez, dos 20 morfotipos (M1–M20), mostrando a estrutura multivariada de presença/ausência de características entre os microcosmos. Cada ponto representa um morfotipo, ilustrando sua distribuição no espaço de ordenação. (B) PCoA dos microcosmos utilizando a mesma matriz categórica de morfotipos, com os pontos coloridos de acordo com o tratamento experimental (discos foliares e discos foliares pré-condicionados). Os rótulos correspondem aos microcosmos individuais, revelando sobreposição parcial entre os tratamentos, consistente com uma fraca separação entre grupos. (C) Diagrama de agrupamento derivado da matriz meandist, ilustrando as dissimilaridades entre tratamentos. (D) Distâncias médias intragrupo (meandist) para cada tratamento.*

Table 2. Results of the Multi-Response Permutation Procedure (MRPP) assessing differences in fungal community structure between treatments. *Resultados do Procedimento de Permutação de Respostas Múltiplas (MRPP) avaliando diferenças na estrutura da comunidade fúngica entre os tratamentos.*

	A	δ	*p*-value
MRPP			
I. vera	0.034	2.187	**0.008**
I. vera + Mancozeb		1.926	

available at https://www.limnetica.com/en/limnetica)). This structural shift was corroborated by the MRPP, which indicated greater within-group homogeneity than expected by chance (A = 0.034, δ = 2.056, p = 0.008, Table 2). Furthermore, mean within-group distances (Fig. 2d) and hierarchical clustering based on treatment centroids (Fig. 2c) confirmed the separation between unconditioned leaf discs and preconditioned leaf discs treatments.

This community shift was driven by a strong indicator effect of morphotype M8, which was absent in the control group but highly frequent (Table S3, supplementary information, available at https://www.limnetica.com/en/limnetica) in the Mancozeb pre-conditioning leaves. Indicator species analysis identified M8 as a significant marker for the Mancozeb pre-conditioning treatment (IndVal = 0.89, $p < 0.001$), suggesting that this specific taxon thrives under the conditions imposed by Mancozeb. Beyond M8, minor fluctuations in the frequency of other taxa were also observed (Table S3, supplementary information, available at https://www.limnetica.com/en/limnetica), but none showed significant fidelity to a specific treatment.

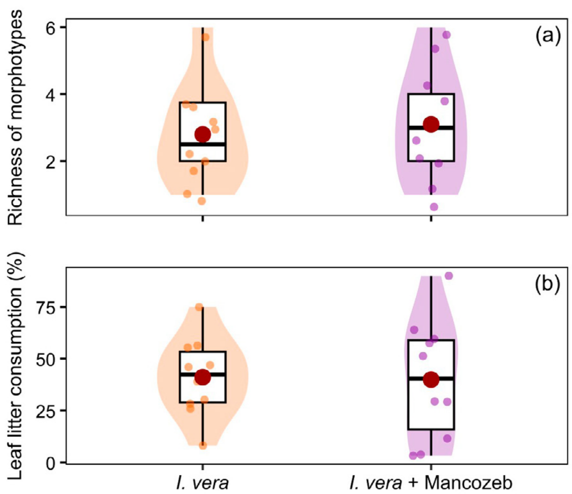

Figure 3. Fungal richness (number of morphotypes) in the gut of *Phylloicus* sp. larvae (a) and leaf litter consumption among treatments (leaf discs and preconditioned leaf discs) (b). Violin and box plots depict quartiles, with the red circle representing the mean, the bold horizontal line indicating the median, and vertical lines showing the upper and lower limits. *Riqueza fúngica (número de morfotipos) no intestino de larvas de* Phylloicus *sp. (a) e consumo de foliar entre os tratamentos (discos foliares e discos foliares pré-condicionados) (b). Os gráficos de violino e boxplot representam os quartis, com o círculo vermelho indicando a média, a linha horizontal em negrito indicando a mediana e as linhas verticais mostrando os limites superior e inferior.*

Fungal richness per larva remained constant across treatments, with individuals harboring approximately three morphotypes on average (Fig. 3a). Generalized Linear Models (GLMs) confirmed the absence of a significant treatment effect on fungal richness ($\chi^2 = 0.152$, df = 1, $p = 0.696$, Table S4, supplementary information, available at https://www.limnetica.com/en/limnetica).

Litter consumption

Leaf litter consumption varied among individuals but did not differ significantly between treatments. Mean consumption across treatments was approximately 43–45% of initial leaf mass (Fig. 3b), however, generalized linear models (GLMs) revealed no significant treatment effect on consumption ($\chi^2 = 0.176$, df = 1, p = 0.675, Table S4, supplementary information, available at https://www.limnetica.com/en/limnetica). Despite the absence of detectable differences in feeding rates, the significant shifts in fungal community composition suggest that Mancozeb exposure influenced the microbial conditioning of leaf litter without translating into measurable changes in larval consumption under the experimental conditions tested.

DISCUSSION

Fungicide-driven filtering of microbial communities

Our findings suggest that the gut-associated fungal communities in *Phylloicus* larvae respond dynamically to chemical disturbance. Although overall fungal richness did not differ among treatments, the composition of gut communities shifted markedly under Mancozeb exposure, suggesting that the fungicide acts as a selective filter favoring tolerant or resistant taxa (Gonçalves et al., 2023, Wüthrich et al., 2025). This is evidenced by the dominance of morphotype M8, which emerged as a significant indicator for fungicide-conditioned leaves, being absent in the control group. The proliferation of this opportunistic taxon under Mancozeb exposure underscores the deterministic nature of the filtering process, where sensitive taxa are likely suppressed, allowing tolerant assemblages to establish without necessarily reducing overall diversity (Baudy et al., 2021, Gonçalves et al., 2024, Wüthrich et al., 2025). This pattern aligns with previous studies indicating that agrochemical stressors may restructure microbial assemblages by favoring tolerant, resistant or opportunistic taxa (Staley et al., 2015, Zubrod et al., 2019).

Fungicides such as Mancozeb exert multi-site inhibitory effects on fungal metabolism (Dall'Agnol et al., 2021, Runkle et al., 2017), potentially suppressing sensitive taxa while allowing resistant or opportunistic taxa to persist (Zubrod et al., 2019, Staley et al., 2015), indicating deterministic filtering rather than stochastic loss of taxa. Similar restructuring patterns have been reported for leaf-associated fungal communities exposed to fungicides, where taxonomic turnover occurs without pronounced diversity decline (Gonçalves et al., 2023, Wüthrich et al., 2025).

Because gut-associated fungi in shredders are largely derived from ingested leaf microbiota (Belmont-Montefusco et al., 2020, Santos et al., 2021), the observed shifts may possibly reflect bottom-up propagation of fungicide effects from leaf-associated assemblages to the digestive tract, although this pathway was not directly measured in the present study. Thus, our findings are consistent with the hypothesis that chemical disturbance modifies fungal community composition through selective environmental filtering, even when richness remains stable. Integrating microbial and detritivore responses, therefore, provides a more mechanistic and comprehensive understanding of anthropogenic stressors on freshwater biodiversity and ecosystem processes (Cornejo et al., 2021, Konschak et al., 2021, Rezende et al., 2019).

Lack of short-term feeding response despite microbiome shifts

Contrary to our initial expectation, altered fungal community composition did not translate into reduced litter consumption. Leaf mass loss did not differ significantly between treatments, indicating functional stability in feeding performance despite microbial restructuring. This pattern highlights the importance of distinguishing structural and functional responses when evaluating agrochemical impacts on detrital food webs (Grossart et al., 2019, Baudy et al., 2021).

This decoupling between community structure and functional output has been increasingly documented in microbial ecotoxicology (Baudy et al., 2021, Gonçalves et al., 2024). One possible explanation is that functionally similar fungal morphotypes persisted under fungicide exposure, potentially maintaining digestive facilitation despite shifts in community composition (Rajeswari et al., 2021). If multiple morphotypes share similar enzymatic capacities for lignocellulosic degradation (Rajeswari et al., 2021), replacement of sensitive taxa by tolerant ones may preserve overall digestive facilitation for the host. Moreover, shredders such as *Phylloicus* exhibit behavioral and physiological plasticity in response to resource variation (Sena et al., 2020, 2021). Feeding rates may therefore be maintained through compensatory mechanisms, including increased ingestion or reliance on endogenous enzymatic activity, even when microbial assemblages shift. The relatively high nitrogen content of *I. vera* leaves may also contribute to their suitability as a food resource for shredders, as nutrient-rich substrates generally promote microbial conditioning and detritivore feeding (Sena et al., 2020, Cararo et al., 2023a).

These findings suggest that short-term fungicide exposure may alter microbial community structure without immediately impairing detritivore feeding under controlled laboratory conditions. Importantly, the absence of immediate functional effects does not preclude longer-term or cumulative consequences, particularly under chronic exposure scenarios (Konschak et al., 2021, Cornejo et al., 2021).

Stability of fungal richness under fungicide exposure

The stability of fungal richness and feeding rates may indicate that gut microbial assemblage and feeding performance in *Phylloicus* larvae were relatively resistant to short-term fungicide exposure under the experimental conditions tested. Insect gut environments impose strong physiological filters, including digestive enzymes, retention time constraints, and mechanical abrasion (Engel & Moran, 2013, Douglas, 2015), which may constrain microbial establishment and maintain a relatively stable core assemblage.

Such host-mediated filtering may explain why richness remained unchanged despite external disturbance. Similar patterns have been reported in insect-microbe systems where compositional turnover occurs within a bounded diversity framework (Belmont-Montefusco et al., 2020, dos Santos et al., 2019). This suggests that the gut microbiota of *Phylloicus* is shaped by both environmental exposure and host physiological control. Additionally, shredders in tropical streams frequently encounter variable resource quality (Rezende et al., 2019, Boyero et al., 2017, Sena et al., 2024), potentially favoring generalist digestive strategies and resilience to moderate microbial shifts. The absence of functional impairment under fungicide exposure, therefore, is consistent with short-term ecological buffering capacity within this detritivore–microbe system.

Implications for tropical stream functioning

Although litter consumption remained stable, the significant restructuring of fungal communities indicates that agrochemical contamination can modify microbial assembly pathways in detrital food webs. Because aquatic fungi regulate early stages of leaf conditioning and nutrient mobilization (Graça, 2001, Graça et al., 2015), persistent compositional shifts may alter enzymatic profiles, decomposition trajectories, and nutrient release patterns over longer timescales (Douglas, 2015).

Fungicides are increasingly recognized as an overlooked class of pollutants in freshwater ecosystems (Zubrod et al., 2019), particularly in agricultural catchments where runoff events are frequent (Dall'Agnol et al., 2021). Even when immediate functional responses are absent, compositional restructuring may reduce community resilience, alter interaction networks, and increase vulnerability to additional stressors (Kovalenko, 2019, Konschak et al., 2021). In detritus-based tropical streams, where leaf litter constitutes the primary energy input (Bambi et al., 2017, 2022), alterations in microbial assemblages can propagate through trophic levels and influence ecosystem-level processes (Rabelo et al., 2024). Our findings, therefore, suggest that fungicide contamination has the potential to reshape microbial–detritivore interactions, even when short-term feeding rates remain unchanged.

Integrating microbial community ecology with detritivore performance metrics provides a mechanistic framework for understanding how chemical disturbance propagates through tropical headwater ecosystems (Rezende et al., 2019, Cornejo et al., 2021, Sena et al., 2021). The lack of coupling between microbial structure and detritivore performance suggests that short-term changes in gut-associated fungal composition did not significantly impact litter consumption, thereby supporting short-term stability in feeding activity under our experimental conditions. Nevertheless, persistent alterations in community composition may have longer-term implications for decomposition dynamics and nutrient cycling in tropical headwater streams subjected to agricultural runoff. Indeed, predicting the full ecological consequences of fungicide contamination remains challenging due to significant knowledge gaps regarding their behavior in complex aquatic matrices and their non-target effects on ecosystem functioning (Pimentão et al., 2024). Because the experimental design here focused on a single litter species to isolate the effect of fungicide exposure, future studies should evaluate whether similar microbial restructuring occurs across different leaf types and whether fungicide effects interact with litter quality. Furthermore, evaluating longer exposure periods, enzymatic activity profiles, and field-based contamination gradients will be essential to determine whether structural microbial shifts ultimately translate into functional consequences under natural conditions.

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AUTHOR CONTRIBUTIONS

R.S.R. and R.M.O.M.: Conceptualization; V.T., G.S., R.B., W.G.B., E.C.S., C.A.L.R. and R.S.R.: Investigation; Data curation; R.S.R., G.S.: Formal analysis; Data curation; V.T. and G.S.: Writing – original draft; E.C.S., R.B., W.G.B., C.A.L.R. and R.S.R.: Writing – review & editing.

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