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PRIMARY RESEARCH PAPER

Decomposition of green and senescent leaves in Amazonian streams: nutrients, toughness, and decomposers biomass

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Abstract Studies on leaf decomposition in streams usually focus on senescent leaves; however, in the Amazon, green leaves are a crucial source of allochthonous material, linked to the rainy season and strong winds. We assessed the decomposition of green and senescent leaves in Amazonian streams by using 16 plant species. We hypothesized that green leaves would exhibit higher decomposition, fungal biomass, and shredder biomass due to the remobilization of nutrients during leaf senescence and lower toughness. Multivariate analyses segregated the plant species into two groups corresponding to green and senescent leaves associated with distinct litter traits. Green leaves had higher concentrations of carbon, nitrogen, phosphorus, potassium, sulfur, tannin, and cellulose, whereas senescent leaves had higher values of toughness, specific leaf mass, lignin, C:N, C:P, and N:P. Polyphenol, calcium, and magnesium concentrations varied widely between and within species, but were similar between green and senescent leaves. The leaf breakdown rate was 1.7 times faster in the green leaves than in the senescent leaves. Fungal biomass was similar between green and senescent leaves on average. However, the shredder biomass was 5.9 times higher in green leaves than in senescent leaves. Our findings highlight the difference between green and senescent decomposition patterns in Amazonian streams.

Keywords Aquatic insects · Fungal biomass · Leaf litter traits · Shredder biomass · Plant phenology · Freshwater ecosystem

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Introduction

Leaves from riparian vegetation are the primary energy source in the food web of forested streams owing to the limitation of primary production by canopy shading (Bega et al., 2024). The decomposition of leaf litter in these systems is an integrative process involving biotic and abiotic components, including leaching of soluble compounds, physical abrasion by water, microbial conditioning (i.e., bacteria and fungi), and breakdown by invertebrates (Benfield et al., 2017). Additionally, the decomposition rate is affected by the chemical and physical characteristics of leaf litter (Jabiol et al., 2019) and can be specific (Guo et al., 2024). Generally, leaf litter with high concentrations of nutrients (e.g., nitrogen and phosphorus), low concentrations of structural compounds (e.g., lignin and cellulose), and secondary compounds (e.g., tannins and phenols) decompose quickly (Graça et al., 2001; Rabelo et al., 2024).

Studies of leaf decomposition in streams from both temperate and tropical regions usually employ senescent leaves (e.g., Lopes et al., 2015; Gonçalves Jr. et al., 2017; Jabiol et al., 2019). However, green leaves are available year-round and can be an important component of allochthonous matter input into tropical streams, mainly during tropical storms (Camacho et al., 2009; Boyero et al., 2012; Tonin et al., 2021). For instance, in two Mexican forests, green leaves accounted for 95% of litter input during a hailstorm and represented 40% of the monthly litter fall (Williams-Linera et al., 2023). Green leaves have also been used in aquatic and terrestrial decomposition experiments for logistical reasons (Guo et al., 2024). Several studies on leaf decomposition in neotropical streams have employed green leaves (Landeiro et al., 2010; Lopes et al., 2015; Martins et al., 2015; Gonçalves Jr. et al., 2017; Alves et al., 2021) .

The input of green leaves into aquatic systems can be influenced by climatic events (e.g., storms and floods; Wantzen et al., 2008; Kassa et al., 2022), tree characteristics (e.g., deciduous and evergreen species; López et al., 2001), terrestrial insect herbivory (Kochi et al., 2004), and seasons (Kochi et al., 2010). In the Amazon Forest, evergreen trees predominate, continuously shedding leaves year-round, which ensures a relatively constant input of leaves into the streams. However, green leaf fall is associated with heavy rainfall during the rainy season (Hayashi et al., 2012; Alves et al., 2021) and strong winds (up to 18 m s^{-1}) during the transition from the dry to the rainy season (Mendonça et al., 2023; Emmert et al., 2024). In addition, climate change is increasing the frequency of extreme events, such as storms, which may lead to a higher input of green leaves into Amazonian streams (Urquiza-Muñoz et al., 2024).

Green leaves generally have higher nitrogen and phosphorus concentrations than senescent leaves (Graça, 2001; Kochi et al., 2010; Alves et al., 2021) because of the remobilization of nutrients by trees during senescence (Fonte & Schowalter, 2004; Zhang et al., 2008). The higher nutritional value and softer tissues of green leaves make them an important food resource (Kochi & Kagaya, 2005; Graça et al., 2016). Some studies have shown that shredder invertebrates have greater growth and faster development in the presence of both green and senescent leaves than when only senescent leaves are available (Kochi & Kagaya, 2005; Kochi et al., 2010). Green leaves can also serve as complementary sources when senescent leaves are scarce (Kochi & Kagaya, 2005). Additionally, some shredder species have been observed to prefer green to senescent leaves (Stout et al., 1985; Yeates & Barmuta, 1999). On the other hand, green leaves have higher phenols concentrations than senescent leaves, which can delay colonization by microbes and shredders (Leff & Vaun McArthur, 1990; Kochi & Kagaya, 2005; Kochi et al., 2010). Some studies have found an increase in fungal and shredder biomass over time in green leaves owing to the leaching of soluble compounds, such as phenols, carbohydrates, and amino acids (Leff & Vaun McArthur, 1990; Martins et al., 2017a).

Studies evaluating the decomposition of green and senescent leaves in streams have reported faster decomposition rates in green leaves. For instance, in urban Amazonian streams, the green leaves of *Mabea speciosa* Müller Argoviensis decompose faster than the senescent leaves of *Coussapoa trinervia* Spruce ex Mildbr. (Martins et al., 2015). Another study conducted in Amazonian streams found that green leaves decomposed up to 3.5 times faster than senescent leaves of three plant species (Alves et al., 2021). Moreover, although green leaves generally decompose faster than senescent leaves, the rate of decomposition varies among species depending on their specific traits (Guo et al., 2024). On the other hand, the fast decomposition of both green and senescent leaves of *Acer mono* Maxim., and *Alnus hirsuta* Turcz. was observed in a Japanese stream, with no significant differences in the decomposition of either leaf type (Kochi & Yanai, 2006).

The input of green leaves significantly affects litter quality and nutrient availability, which in turn influence the functioning of tropical stream ecosystems. However, few studies have evaluated the effects of green and senescent leaves on fungal and shredder biomass in tropical streams and those that have often focused on a limited number of plant species. Therefore, we conducted a comprehensive study to assess the decomposition of green and senescent leaves from 16 plant species in the Amazonian streams. We hypothesized that green leaves would exhibit higher decomposition rates and greater fungal and shredder biomass than senescent leaves because of their higher nutrient concentrations and lower toughness. In addition, we used random forest models to determine the relative importance of leaf traits, fungal biomass, and shredder biomass in predicting the decomposition rates.

Methods

Study area

The experimental study was conducted during the rainy season (December/2012 to January/2013) in three low-order streams (1st and 2nd) in the Reserva Florestal Ducke (RFD) in Manaus, Brazil (02°55′ 03°01 s – 59°53′ 59°59’ W). These streams are characterized by dense and closed riparian vegetation, with beds covered with sand, roots, and leaf litter. The water in these streams were acidic ($\text{pH} = 4.6 \pm 0.1$ standard deviation; One-way ANOVA: $F_{2,6} = 1.37$; $P = 0.323$), well-oxygenated (6.8 ± 0.4 mg L^{-1}; $F_{2,6} = 0.93$; $P = 0.447$) and had low water velocity (0.3 ± 0.1 m s^{-1}; $F_{2,6} = 1.76$; $P = 0.250$). The average water temperature was 24.9 ± 0.2ºC ($F_{2,6} = 1.39$; $P = 0.319$), with low electrical conductivity (12.6 ± 2.5μS cm^{-1}; $F_{2,6} = 2.10$; $P = 0.204$), and low concentrations of nitrogen (0.3 ± 0.1 mg L^{-1}) and phosphorous (0.03 ± 0.01 mg L^{-1}; Martins et al., 2015).

Leaf litter

We used green and senescent leaves from 16 tree plant species (see Table S1) spanning 15 families and 16 genera that represent a broad spectrum of functional traits. These Amazonian plant species are commonly found in the riparian zone of the studied region and present variations in leaf chemistry (e.g., nitrogen, phosphorus, and lignin content) and differences in physical properties (e.g., toughness, specific leaf mass, and secondary compounds), which affect decomposition rates (Graça et al., 2016). Only *Carapa guianensis* Aubl., *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., and *Lecythis pisonis* Cambess. were deciduous species, whereas all other species were evergreen.

Senescent leaves were collected directly from the forest floor in the RFD and the *campus* of the Instituto Nacional de Pesquisas da Amazônia (INPA, Manaus, Brazil), ensuring that only recently abscised leaves were selected. To prevent contamination by soil particles or prior fungal activity, we selected only intact, non-fragmented leaves from the top layer of leaf litter, avoiding direct contact with the ground. Green leaves were collected directly from plants in the same area. We then air-dried the leaves (10 days) until the start of the experiment. To estimate the initial mass of the leaves, air-dried leaves (ADM) and oven-dried leaves (ODM) were used to determine the final mass. A correction factor (CF) was used to estimate the oven-dried initial mass (ODIM; $\text{ODIM} = \text{ADM}_{\text{initial}} \times \text{CF}$). For each plant taxon, CF was determined as the mean ODM/mean ADM, using leaves that were not incubated in the stream. CF values ranged from 0.262 for green leaves of *Cecropia hololeuca* Miq. (Urticaceae) to 0.978 for senescent leaves of *Ocotea nigrescens* Vicent. (Lauraceae).

The chemical and physical characteristics of leaves were determined using samples that were not incubated in the stream. For each treatment, a single composite sample comprising multiple leaves was used to represent the intraspecific variability (Sena et al. 2023). Specific leaf mass (SLM; dry mass/leaf-disk area; mg cm^{-2}) was calculated by cutting 10 leaf disks ($\text{diameter} = 1.8$ cm) with a cork-borer, oven-drying them (45 °C for 48 h), and weighing them on an accurate balance (10 μg). Leaf toughness (mg) was determined using a penetrometer in 10 different leaves for each treatment (Graça & Zimmer, 2020). Organic carbon (%; C) was obtained using the loss-on-ignition method (450 °C for 4 h; Flindt et al., 2020). Nitrogen (%; N) was determined using the Kjeldahl method (Flindt et al., 2020). Phosphorus (%; P) was determined using the molybdate-ascorbic acid method (Flindt et al., 2020). Calcium (%; Ca), Magnesium (%; Mg), Potassium (%; K) and Sulfur (%; S) were determined using the nitric-perchloric acid method (Malavolta et al., 1997). Polyphenols (%) and tannins (%) were measured using a spectrophotometer and the Folin–Ciocalteu (Bärlocher & Graça, 2020) and radial diffusion methods (Graça & Bärlocher, 2020), respectively. The lignin (%) and cellulose (%) contents were measured gravimetrically using acetone and sulfuric acid (Gessner, 2020a).

Experimental design and procedures

We built 864 litter bags (10 × 20 cm) with coarse mesh (10 × 10 mm openings), each containing 1.95 ± 0.25 g of air-dried leaf litter. In each of the three streams, we selected a 150 m reach for the experiment, where 288 litter bags (18 for each of the 16 plant taxa) were incubated in pool habitats and spaced at a minimum of 30 cm. Six litter bags for each plant species (96 bags) were removed after 15, 30, and 60 days. After removal, the litter bags were placed in plastic bags and transported to the laboratory in ice coolers. The litter bags were stored in a refrigerator (4 °C) until processing. The remaining material in the litter bags was washed with distilled water and sieved (mesh opening = 0.12 mm) to separate the invertebrates and leaf litter fragments.

To estimate shredder biomass, we oven-dried (60 °C for 48 h) the larvae of two large and abundant caddisflies, *Triplectides* (Leptoceridae) and *Phylloicus* (Calamoceratidae), and weighed them (balance accuracy = 10 µg). Due to limited information on functional feeding groups in the Neotropics, we conservatively included only these two Trichoptera genera as shredders (Landeiro et al., 2010; Martins et al., 2015). Shredder biomass was assigned to each plant species based on the average value recorded in 18 litter bags (3 streams × 3 removal dates × 2 litter bags) during the leaf litter decomposition process.

From each litter bag, we cut 10 leaf disks (diameter = 18 mm) to determine the ash-free dry mass (AFDM; five disks) and estimated fungal biomass (ergosterol concentration). Five disks were used to determine the AFDM. These disks were oven-dried (60 °C – 72 h) and subsequently combusted in a muffle furnace (500 °C – 4 h). The remaining leaf litter material (RLM) from each litter bag was oven-dried (60 °C – 72 h) and weighed (balance accuracy = 0.001 g). In addition, we estimated the dry mass of the two sets of removed leaf disks (RDM) based on the dry mass of the leaf disks used to obtain AFDM. The final dry mass was obtained by adding RLM and RDM. We calculated the leaf litter breakdown rate (k) using a negative exponential model of the percentage of mass loss over time (Petersen & Cummins, 1974). Moreover, we have included $k_{\text{degree-days}}$ in the supplementary material (Tab. S1).

Fungal biomass was estimated by quantifying the ergosterol concentration in five leaf-disks. The disks were initially frozen at -20 °C and later subjected to ergosterol extraction using methanol and potassium hydroxide at 80 °C for 30 min. The extract was then purified using solid-phase extraction cartridges and the ergosterol retained on the cartridge columns was eluted with isopropanol. Quantification was performed using high-performance liquid chromatography (HPLC). The final ergosterol concentrations were adjusted based on the ash-free dry mass (AFDM) of the disks (Gessner, 2020b). For each plant taxon, fungal biomass was calculated as the average value from 18 litter bags (3 streams × 3 removal dates × 2 litter bags) throughout the leaf litter decomposition process. Fungal biomass could not be obtained from green leaves of *Buchenavia huberi* Ducke and *Tapirira guianensis* Aubl., nor from senescent leaves of *Clusia* sp., *Eperua glabriflora* (Ducke) R.S. Cowan, *Ficus* sp., *Hevea brasiliensis*, *Licania* sp., and *Tapirira guianensis*. These seven species were excluded from the statistical analysis to evaluate differences in fungal biomass between green and senescent leaves.

Data analyses

We analyzed the physical and chemical traits of green and senescent plant taxa using principal coordinate analysis (PCoA; Euclidian distance; ‘stats’ R package) by applying standardization to the data (*scale* function). PCoA was used to identify the major axes of variation in leaf traits, allowing us to assess whether green and senescent leaves form distinct groups. This supports our hypothesis that differences in decomposition rates and decomposer biomass are driven by the chemical and physical properties of leaves. To assess trait changes during senescence, we quantified both the intensity and direction of change in the multivariate space (see Appendix). Intensity was measured as the Euclidean distance between the PCoA coordinates of a species in the green and senescent states. The direction was evaluated by calculating the angle (in radians) between the vectors representing these states in the PCoA space, indicating a shift in the trait composition. Additionally, we used Permutational Multivariate Analysis of Variance (PERMANOVA; function *adonis2*, 'vegan' R package; Oksanen et al., 2024) to test for differences in trait composition between the green and senescent leaves.

For each plant species, we calculated the difference (Δ) between the green and senescent leaves for leaf traits, decomposition rate (k), shredder biomass, and fungal biomass. Pearson's correlation analysis (function *cor.test*,'stats' R package) was used to assess the relationship between the intensity and direction of trait changes and Δk, Δshredder biomass, and Δfungal biomass. Additionally, we used Pearson's correlation to examine the relationships between k, shredder biomass, and fungal biomass in the green and senescent leaves. To evaluate the homogeneity of leaf traits between green and senescent leaves, we conducted dispersion tests (function *betadisper*, 'vegan' R package) to determine whether trait variability differed between the two leaf conditions.

Random forest models were used to assess the relative contributions of leaf traits to k, shredder biomass, and fungal biomass. Additional models were developed by incorporating biotic variables (shredder biomass and/or fungal biomass) and the remaining leaf mass. All models also included categorical predictors for leaf condition (green or senescent) and plant species identity. Random forest is an ensemble learning method that combines multiple decision trees to generate robust predictions and capture complex nonlinear relationships between predictors and responses (Siroky 2009).

We performed a paired t-test to verify whether the difference between the means of the green and senescent leaves was statistically significant. Assumptions of normality and homogeneity of the residuals of the adjusted models were confirmed. We used Raincloud plots for repeated measures, as they visually represent the raw data, probability density, and key summary statistics, such as the median (Allen et al., 2021). Plots were constructed using the "Raincloudplots" R package (Allen et al., 2021).

Results

Leaf species

The 16 plant species exhibited high variability in their chemical and physical traits. *Tapirira guianensis* showed high nutrient content (N and P) and low toughness, structural (cellulose and lignin), and secondary compounds (tannins and polyphenols), resulting in a high decomposition rate. In contrast, *Clusia* sp., *Carapa guianensis*, and *Eperua glabriflora* had low nutrient content and high toughness, and structural and secondary compounds, resulting in slow decomposition (Tab. S1).

Leaf characteristic

The first two components of the PCoA retained 57% of the variability in litter traits (PC1 = 40% and PC2 = 17%, Fig. 1A). For most plant species, the senescence process was associated with an increase in SLM, leaf toughness, lignin, N:P, C:N, C:P, Ca, and Mg, whereas the levels of P, N, K, C, tannins, and polyphenols decreased (Fig. 1A). However, *Helicostylis tomentosa* (Poepp. & Endl.) Rusby and *Ocotea nigrescens* exhibited the opposite trend, showing contrasting changes in leaf traits between the green and senescent conditions. PERMANOVA for differences in traits between green and senescent leaves was significant ($F_{1,30} = 23.08$; $P = 0.001$). Among the analyzed species, *Ficus* sp. exhibited the least variation in leaf traits during senescence, whereas *Carapa guianensis* and *Cecropia hololeuca* showed the most pronounced changes. The homogeneity of leaf traits was similar in senescent (3.26) and green leaves (3.32; $F = 0.02$, $P = 0.892$). The intensity of trait change was primarily correlated with the delta values (the difference between senescent and green leaves) of SLM ($r = -0.77$; $p < 0.001$), C:P ($r = -0.75$; $p < 0.001$), toughness ($r = -0.72$; $P = 0.001$), N:P ($r = -0.68$; $P = 0.004$), S ($r = -0.58$; $P = 0.018$), P ($r = 0.62$; $P = 0.010$), and N ($r = 0.58$; $P = 0.019$), and directional changes were mainly associated with the

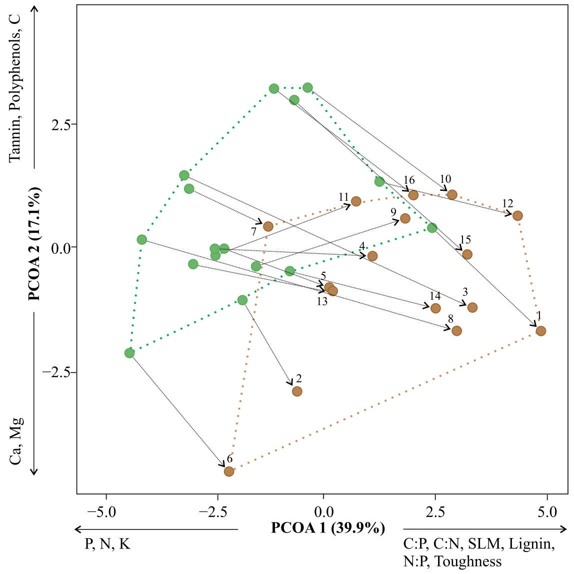

Fig. 1 Principal Coordinates Analysis (PCoA) biplot of leaf characteristics. Green = green leaves; Brown = senescent leaves. 1- *Clusia* sp.; 2- *Eugenia stipitata* McVaugh; 3- *Carapa guianensis*; 4- *Dipteryx odorata* (Aubl.) Willd.; 5- *Hevea brasiliensis*; 6- *Tapirira guianensis;* 7- *Ficus* sp.; 8- *Cecropia hololeuca;* 9- *Helicostylis tomentosa*; 10- *Croton lanjouwensis* Jabl.; 11- *Ocotea nigrescens*; 12- *Eperua glabriflora*; 13- *Licania* sp.; 14- *Davilla rugosa* Poir.; 15- *Lecythis pisonis*; 16- *Buchenavia huberi*

delta values of polyphenols (r = −0.54; P = 0.030), Mg (r = 0.72; P = 0.002), and Ca (r = 0.61; P = 0.012). Plant species characterized by higher levels of nutrient content (N, P, S, Mg, and Ca) and lower values of structural traits (SLM and toughness) and secondary compounds (polyphenols) showed more pronounced changes during the senescence process.

Assessment of individual litter traits revealed that green leaves had higher values of C, N, P, K, sulfur, tannin, and cellulose (Table 1; Fig. 2). In contrast, senescent leaves had higher values for toughness, SLM, lignin, C:N, C:P, and N:P. Although polyphenol, calcium, and magnesium concentrations varied widely between and within species, these traits were similar in both green and senescent leaves (Table 1; Fig. 2).

Leaf litter decomposition

The overall decomposition rate of green leaves was 1.7 times faster than that of senescent leaves (Table 2; Fig. 3A). For green leaves, the decomposition rate ranged from -0.0376 d^{-1} for *Tapirira guianensis* to -0.0051 d^{-1} for *Eperua glabriflora*. The maximum (−0.0036 d^{-1}; *Eperua glabriflora*) and minimum (−0.0414 d^{-1}; *Tapirira guianensis*) values for the decomposition rate of senescent leaves were recorded in the same species as those for green leaves. After

Table 1 Summary of paired t-tests for chemical and physical characteristics of green and senescent leaves incubated in Amazon streams. C – Carbon; N – Nitrogen; P – Phosphorus; SLM—Specific leaf mass

Response variables	Leaf litter		t	Df	*p*-value
	Green	Senescent			
Phosphorus (%)	0.12 ± 0.05	0.04 ± 0.02	6.92	15	**< 0.001**
Potassium (%)	0.94 ± 0.59	0.34 ± 0.37	6.37	15	**< 0.001**
Nitrogen (%)	2.24 ± 0.72	1.37 ± 0.40	4.96	15	**< 0.001**
Tannin (%)	5.46 ± 3.18	2.97 ± 1.55	3.93	15	**0.001**
Sulfur (%)	0.27 ± 0.21	0.23 ± 0.22	2.84	15	**0.012**
Carbon (%)	42.98 ± 2.18	42.25 ± 2.78	2.65	15	**0.018**
Cellulose (%)	29.95 ± 5.17	26.03 ± 5.40	2.24	15	**0.041**
C:P	448.47 ± 248.96	1608.70 ± 762.29	7.09	15	**< 0.001**
Lignin (%)	28.56 ± 8.39	42.96 ± 9.73	6.08	15	**< 0.001**
N:P	20.18 ± 5.49	47.28 ± 18.94	5.94	15	**< 0.001**
C:N	21.54 ± 8.33	34.26 ± 14.02	4.96	15	**< 0.001**
Toughness (mg)	264.02 ± 167.40	396.59 ± 246.50	2.6	15	**0.020**
SLM (mg cm^{-2})	0.008 ± 0.004	0.011 ± 0.005	2.42	15	**0.028**
Calcium (%)	1.45 ± 1.00	1.82 ± 1.33	2.07	15	**0.056**
Polyphenols (%)	13.41 ± 12.15	8.14 ± 5.26	1.70	15	0.110
Magnesium (%)	0.17 ± 0.08	0.17 ± 0.12	0.06	15	**0.953**

Bold values indicate statistically significant differences (*p* 0.05)

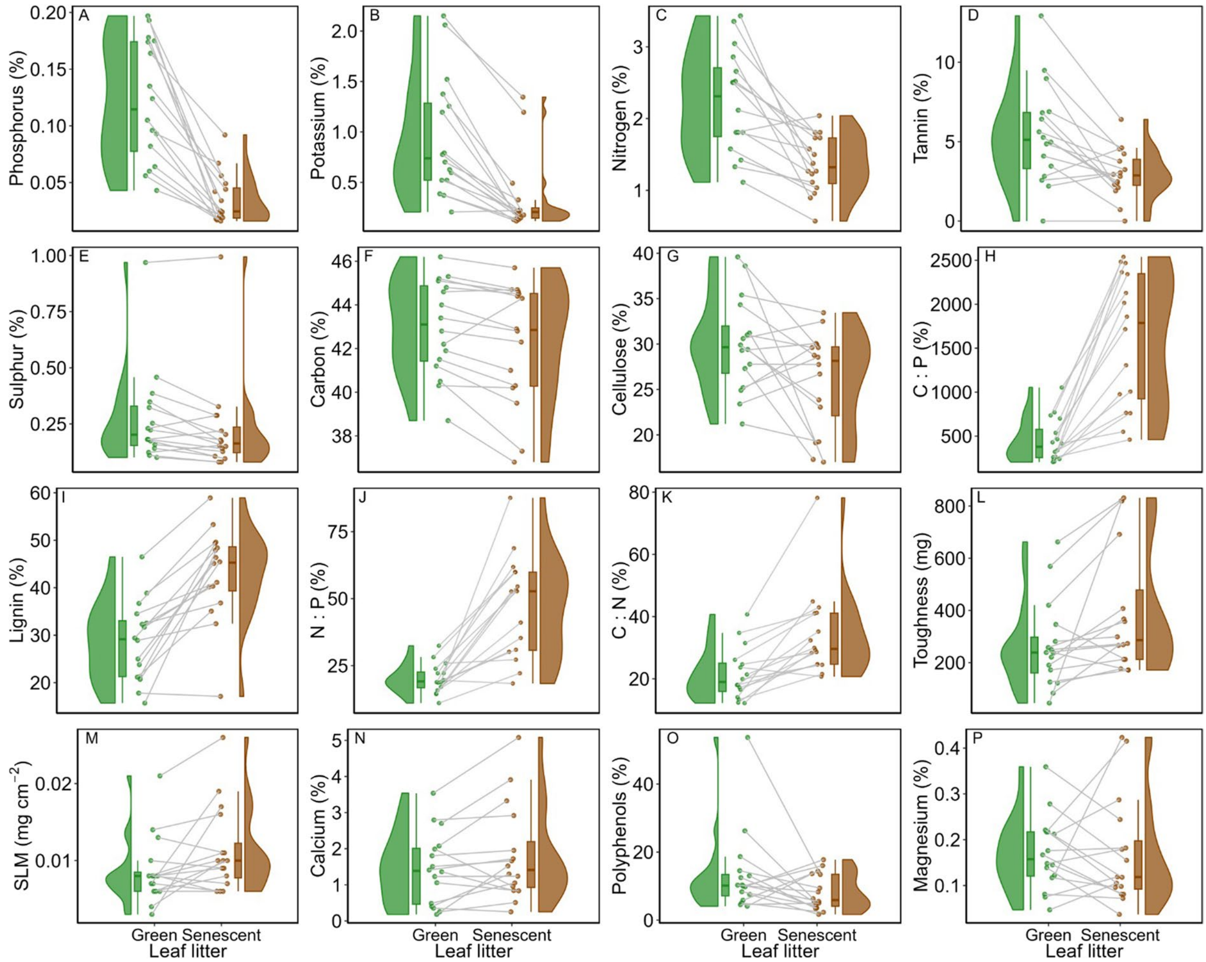

Fig. 2 Raincloud plots for the chemical and physical characteristics of green and senescent leaves from 16 plant taxa in the Amazon Forest

Table 2 Summary of paired t-tests for leaf breakdown rates, fungal and shredder biomass, of green and senescent leaves incubated in Amazon streams

Response variables	Leaf litter		t	df	p-value
	Green	Senescent			
Leaf-litter decomposition (d^{-1})	-0.018 ± 0.010	-0.011 ± 0.009	4.38	15	**< 0.001**
Remaining leaf mass (%)	40.94 ± 17.41	59.66 ± 16.97	4.79	15	**< 0.001**
Fungal biomass (μg g^{-1})*	325.58 ± 263.24	307.63 ± 136.98	0.26	8	0.804
Shredders biomass (mg)	17.67 ± 17.70	3.02 ± 6.00	3.03	15	**0.008**

[*] We calculated the mean, standard deviation, and t-test, excluding plant taxa with fungal biomass in only one leaf type

Bold values indicate statistically significant differences (*p* 0.05)

60 days, the remaining mass of green leaves was approximately 41%, compared with 60% for senescent leaves (Table 2; Fig. 3B). Decomposition rates of senescent and green leaves were strongly correlated ($r = 0.75$; $p = 0.001$). The Δk showed no significant correlation with the intensity ($r = 0.23$; $p = 0.393$)

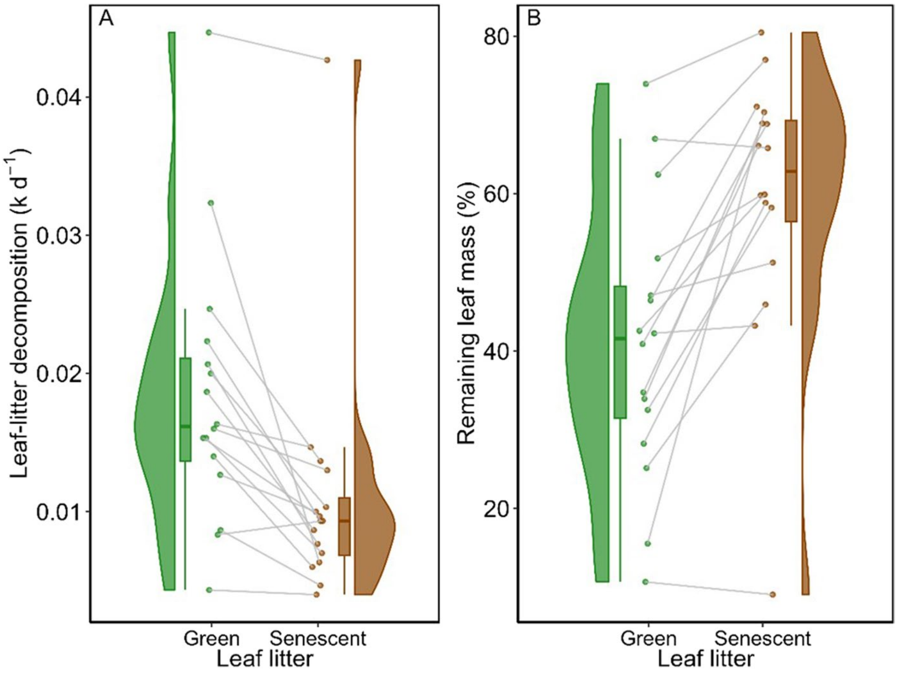

Fig. 3 Raincloud plots for repeated measurements of leaf litter decomposition rates (A) and remaining leaf mass (B) of green and senescent leaves from 16 plant taxa in the Amazon streams

and a moderate but not significant correlation with the direction change ($r = 0.46$; $p = 0.073$) of leaf traits during senescence. This suggests that the specific direction of trait changes, such as shifts in nutrient content and/or secondary compounds, rather than the overall magnitude of change, may better explain differences in decomposition during senescence. Random forest models explained 50.4% of the variance in leaf breakdown rates, based on leaf traits alone. Decomposition was negatively influenced by lignin, C:N, SLM, C:P, and toughness (Table S2), whereas it was positively associated with potassium, N, P, and N:P. Species identity also plays a significant role in regulating decomposition dynamics. When shredder biomass was included in the model, the explained variance increased slightly to 52.9%, and with the addition of both shredder and fungal biomass, it increased to 53.1%. The key leaf traits influencing decomposition remained consistent across all models, whereas the importance of species identity declined when biological factors were included.

Fungal and shredder biomasses

Fungal biomass ranged from 73.32 µg g^{-1} in green leaves of *Ocotea nigrescens* to 829.63 µg g^{-1} in green leaves of *Helicostylis tomentosa*. However, the average fungal biomass was similar in the green and senescent leaves (Table 2; Fig. 4). The fungal biomass of senescent and green leaves showed a moderate but not significant correlation ($r = 0.61$; $p = 0.079$). The Δfungal biomass showed no significant correlation with the intensity ($r = 0.06$; $p = 0.882$) and a moderate correlation with the direction change ($r = 0.67$; $p = 0.047$) of leaf traits during senescence. Similar to Δk, the differences in fungal biomass between green and senescent leaves were better explained by the direction rather than the intensity of trait changes. The random forest models explained 7.2% of the variance in fungal biomass based on leaf traits alone. Fungal biomass was mainly influenced by tannin, calcium, polyphenols, C:N, N, and P (Table S2). Including the remaining leaf mass in the model did not increase the explained variance (7.2%). With the addition of both the remaining leaf mass and shredder biomass, the explained variance increased slightly to 10.2%. The key leaf traits that influenced decomposition remained consistent across all the models. In the final model, the remaining leaf mass moderately influenced fungal biomass, whereas shredder biomass and species identity played a minimal role.

Shredder biomass ranged from 0 mg in the green leaves of *Tapirira guianensis* and *Buchenavia huberi* and senescent leaves of *Dipteryx*

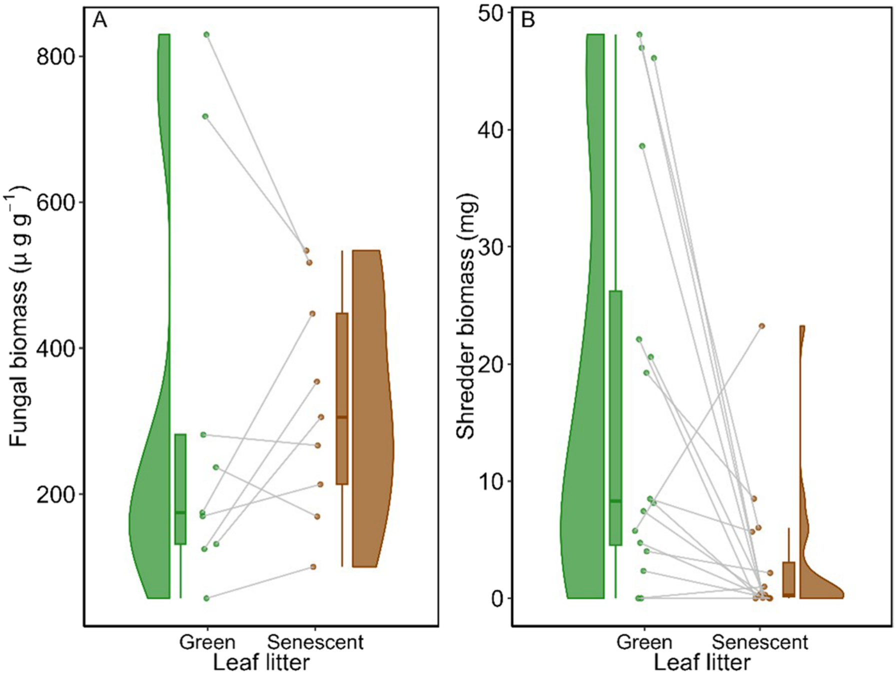

Fig. 4 Raincloud plots for fungal biomass (A) and shredder biomass (B) of green and senescent leaves from 16 plant taxa incubated in three Amazon streams

odorata and *Carapa guianensis* to 48.12 mg in the green leaves of *Dipteryx odorata*. Overall, the average shredder biomass was 5.9 times higher in the green leaves than in the senescent leaves (Table 2; Fig. 3). The shredder biomass of the senescent and green leaves showed no significant correlated ($r = -0.11$; $p = 0.675$). The Δshredder biomass showed no significant correlation with both the intensity ($r = -0.05$; $p = 0.867$) and direction change ($r = -0.02$; $p = 0.944$) of leaf traits during senescence. In contrast to Δfungal biomass and Δk, changes in shredder biomass were not explained by leaf trait variation. This suggests a weaker dependence on leaf chemistry and may indicate the influence of other factors such as habitat structure, microbial conditioning, or species-specific preferences. Random forest models explained -6.2% of the variance in shredder biomass based on leaf traits alone, indicating that the model performs worse than the null model. When the remaining leaf mass was included in the model, the explained variance did not improve (explained variance = −7.7%). However, when the remaining leaf mass and fungal biomass were added to the model, the explained variance increased by 12.3%. In the final model, shredder biomass was influenced mainly by C, sulfur, remaining leaf mass, and leaf condition (green or senescent; Table S2). Species identity and fungal biomass were not found to be significant predictors.

Discussion

Green leaves had higher concentrations of nutrients (N and P) and lower toughness and SLM, resulting in faster decomposition than senescent leaves. As expected, nutrient-rich and softer leaves are more palatable to decomposers (microorganisms and shredders), resulting in higher leaf breakdown rates (Fonte & Schowalter, 2004; Graça & Canhoto, 2006; Kochi & Yanai, 2006; Alves et al., 2021). Furthermore, the strong correlation between decomposition rates and the direction of trait changes during senescence suggests that polyphenols, Mg, and Ca play important roles in the decomposition process, even though they were not selected in the random forest models. Although polyphenols act as chemical defenses and can initially inhibit decomposer colonization, their high hydrophilicity may enhance leaf decomposition through leaching (Gonçalves Jr. et al., 2017). In contrast, leaves with high Ca and Mg concentrations generally presented higher decomposition rates, likely because of their positive influence on microbial activity and leaf palatability (Schneider et al., 2012).

In all decomposition models, the inclusion of decomposer biomass (shredders and/or fungi) had minimal influence on the explained variance, indicating that leaf traits were the primary drivers of decomposition across the 16 plant species studied. Similarly, Gonçalves Jr. et al. (2017) found that leaf litter quality was the main factor controlling decomposition in Amazonian streams, regardless of whether the leaves were exotic or native. Although species identity played a variable role in decomposition rates, its influence diminished when biological factors were included in the models, further highlighting leaf traits as key determinants of decomposition in the studied streams. Generally, functional traits have a stronger influence on ecosystem processes than species identity, which can be confounded by high intraspecific trait variability (Lecerf & Chauvet, 2008; López-Rojo et al., 2021). The high importance of leaf traits suggests that shifts in riparian vegetation composition could influence organic matter processing in streams, thereby influencing nutrient cycling and energy flow (Gonçalves Jr. et al., 2017; Rezende et al., 2025).

The strong correlation between senescent and green leaf decomposition rates indicated that species-specific decomposition patterns remained consistent across leaf conditions. This suggests that both leaf conditions may serve as complementary resources within detritus-based food webs, providing diverse substrates for decomposers. A similar pattern has been observed for deciduous and herbaceous species in China (Guo et al., 2024). However, unlike our results, their study reported a weak correlation between green and senescent leaf decomposition rates in evergreen species.

Fungal biomass did not differ between green and senescent leaves and showed a moderate correlation between leaf conditions, indicating that hyphomycete colonization patterns are maintained regardless of leaf stage. Random forest models identified tannins, Ca, polyphenols, C:N, and N as the key predictors of fungal biomass. Low concentrations of macro-and micronutrients can limit the growth and reproduction of aquatic hyphomycetes (Sridhar & Bärlocher, 2011; Simões et al., 2021; Alonso et al., 2024), resulting in low fungal biomass in nutrient-poor detritus. In contrast, although tannins and polyphenols can initially inhibit fungal colonization, their negative effects decrease over time (Gonçalves Jr. et al., 2017). The remaining leaf mass also influenced fungal biomass, suggesting a relationship between decomposition stage and fungal colonization. Higher fungal biomass was observed in the green leaves of *Helicostylis tomentosa* and *Dipteryx odorata*, which had less than 30% remaining mass. In contrast, fungal biomass exceeded 300 μg g^{-1} in the senescent leaves of *Dipteryx odorata*, *Croton lanjouwensis*, *Helicostylis tomentosa*, *Eugenia stipitata*, and *Carapa guianensis*, all of which had over 50% remaining mass. This pattern may reflect trade-offs among leaf quality, substrate stability, and heterogeneity (Ligeiro et al. 2010; Tiegs et al. 2019). However, the low predictive power of random forest models suggests that additional environmental factors (e.g., oxygen, temperature, and water nutrients; Martins et al., 2015, 2017b; Pereira et al., 2024) and biological interactions (e.g., bacterial biomass; Hayer et al., 2022; Cao et al., 2024) can affect fungal colonization.

For shredder biomass, only the model incorporating leaf traits, remaining leaf mass, and fungal biomass outperformed the null model, identifying C and S as the key traits. These nutrients can limit microbial conditioning and result in a low shredder biomass (Zubrod et al., 2014). The remaining leaf mass had a minor effect on shredder biomass, suggesting a link between the decomposition stage and shredder colonization; however, this relationship varied among plant species. For example, *Dipteryx odorata* had 28% remaining leaf mass and supported 48 mg of shredder, whereas *Eperua glabriflora* and *Clusia* sp., with 62–73% remaining leaf mass, had similar shredder biomass (46 mg). This variation may reflect differences in how shredders utilize detritus, either as food or habitat, depending on the leaf quality and structural characteristics.

Leaf condition was also included as a predictor in the model, resulting in a higher biomass of shredders in the green leaves. Previous studies have reported higher foliar consumption and colonization of different species of shredder on green leaves than on senescent leaves (Stout et al., 1985; Yeates & Barmuta, 1999). Higher colonization of shredders was also recorded in green leaves owing to their lower toughness (Martins et al., 2015). Although the average tannin value was 1.8 times higher in green leaves, this compound is water-soluble and is rapidly leached after the leaf enters the stream; therefore, it does not inhibit shredder colonization over time (Ostrofsky, 1993; Campbell &

Fuchshuber, 1995). Furthermore, according to Yates and Barmuta (1999), for some species of shredder, the benefits of consuming green leaves with a higher concentration of nutrients outweigh the costs of higher concentrations of secondary compounds in this detritus. Interestingly, in our results, the relationship between shredders and fungal colonization was not as strong as is often assumed. A previous study in the Amazon found that the relationship between fungal biomass and shredder consumption varies among plant species (Martins et al., 2022). Additionally, the fungal biomass peaks may have occurred before our sampling period, with bacteria potentially playing a more dominant role in the later stages of decomposition (Gomes et al., 2017). Moreover, the weak correlation between shredders and both trait intensity and direction change suggests that shredders can use a wide range of leaf types, making leaf traits alone insufficient predictors of biomass. Finally, the limited explanatory power of the random forest models indicates that external ecological factors, such as environmental conditions (Firmino et al., 2023), biological competition (Firmino et al., 2022), and predator–prey dynamics (Ferreira et al., 2023), may play a more significant role in determining shredder biomass.

The decomposition rate ranged from -0.004 d^{-1} for both the green and senescent leaves of *Eperua glabriflora* to -0.045 d^{-1} for the green leaves of *Tapira guianensis*. According to the classification suggested by Gonçalves Jr. et al. (2019) for Brazilian aquatic environments, one green taxon and one senescent taxon were classified as slow ($k > -0.0041$ d^{-1}). Eight taxa of green leaves and 14 taxa of senescent leaves were classified as intermediate ($-0.0041 > k > -0.0173$ d^{-1}), and one taxon of senescent leaves and seven taxa of green leaves were classified as fast ($k < -0.0173$ d^{-1}). In general, slow decomposition rates (k) are rare in tropical regions (Abelho 2001). Previous studies in the Amazon have shown patterns similar to our findings, with most species (both green and senescent leaves) exhibiting intermediate or rapid decomposition rates (Rueda-Delgado et al., 2006; Landeiro et al., 2010; Martins et al., 2015; Gonçalves Jr. et al., 2017; Alves et al., 2021; Firmino et al., 2021). Our study significantly advances the understanding of leaf decomposition in tropical streams by providing comprehensive data on green and senescent leaves of 16 species. This represents an increase over previous research conducted in Amazonian streams, which analyzed a total of 15 plant species, including 4 exotic species. The inclusion of a broader range of native species offers a more robust foundation for understanding the decomposition dynamics in these ecosystems.

Conclusion

Our findings highlight the substantial diversity in leaf traits among the green and senescent leaves of the Amazonian plant species. Understanding the ecological dynamics of green leaf decomposition in Amazonian streams is becoming increasingly important, particularly in the context of climate change and deforestation. Increased wind disturbances and forest loss may enhance green leaf inputs into aquatic ecosystems, potentially altering the decomposition dynamics and energy flow in tropical streams. Moreover, leaf traits were highly important in our study, playing a more significant role than species identity. Therefore, they should be considered in decomposition studies in aquatic environments.

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Data availability Data supporting the findings of this study are available upon request from the corresponding author.

Declarations

Conflict of interest The authors declare they have no financial interests and/or conflict of interest.

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