Exploring the impacts of non-native leaf litter on invertebrate community and leaf decomposition in a Atlantic Forest stream

Explorando o impacto da presença de detritos exóticos na comunidade de invertebrados aquáticos e no processo de decomposição foliar em um riacho na Mata Atlântica

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**Abstract:** **Aim:** In this study, we examined the effects of non-native leaf litter on the functioning of an Atlantic Forest stream ecosystem. **Methods:** Were tested two predictions: (i) Leaf litter from the native trees with high nutritional quality will have higher decomposer’s activity and faster litter decomposition; (ii) Given the presence of anti-grazing defenses, we also hypothesized that non-native leaf litter would be colonized by fewer invertebrates and that native species would be more species-rich. For this, in a forest stream (Florianópolis, SC, Brazil) we conduct the experiment to understand the decomposition and biological colonization of leaf litter among two non-native (*Eucalyptus* sp. and *Pinus radiata* D. Don) and two native trees (*Ficus eximia* Schott and *Alchornea triplinervia* (Spreng) Mull. Arg.). **Results:** Our predictions were partially corroborated. The percentage of dry mass remaining was lower for the native leaf litter. The invertebrate abundance and richness, and functional feeding groups vary between native and non-native leaf litter. Invertebrate abundance was higher in non-native *Eucalyptus* detritus, largely due to the high larval abundance of Chironomidae (Diptera). **Conclusions:** Our results indicate that the presence of non-native riparian species can modify leaf decomposition and aquatic invertebrate communities in subtropical streams, with potential consequences for ecosystem functioning.

**Keywords:** aquatic assemblages; detritus; exotic species; leaf breakdown; lotic ecosystem.
Mull. Arg). **Resultados:** Especificamente, as nossas hipóteses foram parcialmente corroboradas. A porcentagem de massa seca remanescente foi menor para a serrapilheira nativa. A abundância e riqueza de invertebrados e grupos funcionais de alimentação variam entre serrapilheira nativa e não nativa. A abundância de invertebrados foi maior em detritos de *Eucalyptus* sp, em grande parte devido à grande abundância de Chironomidae (Dípteros). **Conclusões:** Os nossos resultados indicam que, a presença de espécies vegetais não nativas na zona ripária podem modificar a decomposição foliar e as comunidades de invertebrados aquáticos em riachos subtropicais, com potenciais consequências para o funcionamento do ecossistema.

**Palavras-chave:** assembleias aquáticas; detritos foliares; espécies exóticas; decomposição foliar; ecossistemas lótico.

### 1. Introduction

Riparian vegetation is an important part of riverine ecosystems and are essential for the structure and functioning of these ecosystems (Dufour et al., 2019; Zelnik et al., 2020). However, despite the variety of ecosystem services provided by riparian vegetation, many riparian ecosystems have experienced widespread degradation, and the presence of non-native tree species is becoming a common worldwide concern (Dudgeon et al., 2006; Richardson et al., 2007; Castro-Díez & Alonso, 2017). The distribution of non-native tree species are expected to continue increasing in extent, with their spread facilitated by agriculture, urbanization and climatic changes, including increasing temperatures and altered precipitation patterns (Castro-Díez et al., 2019). The presence of non-native tree species can cause severe ecological, social and economic losses, and they are considered as one of the leading direct causes of biodiversity loss (Ferreira et al., 2016; Cordero-Rivera et al., 2017). On islands where space is a limiting factor, the presence of non-native trees may reduce the diversity of natural ecosystems by displacing species and displaying a high local dominance (Tershy et al., 2015). In the simplified ecosystems of islands, the native species are more vulnerable than the continental species, because they have smaller population sizes and less genetic diversity (Tershy et al., 2015).

Allochthonous litterfall supplied by riparian vegetation, which mainly comprises leaves, represents an important energy source for aquatic communities in shaded headwater streams (Webster & Benfield, 1986; Abelho, 2001). Allochthonous litterfall can be retained on the streambed and used as food and habitat resource by aquatic microorganisms and invertebrates (Mathurin et al., 2008). Litterfall are decomposed through a combination of chemical, physical and biological in-stream processes that result in its fragmentation, transformation and incorporation into stream food webs in which both microorganisms (especially aquatic hyphomycetes) and detritivorous shredders have key roles (Hieber & Gessner, 2002; Graça et al., 2015). Compared to native leaf litter, the non-native leaf litter, in some case, can show lower nitrogen and phosphorous concentrations and higher quantities of lignin, oils, tannins and other recalcitrant compounds, thus affecting the availability of palatable organic matter for in-stream decomposers (Baker et al., 2007; Cordero-Rivera et al., 2017; Pereira & Ferreira, 2021). Aquatic invertebrates generally colonize soft leaf litter with high nutrient concentration and low structural and secondary compounds and decompose it faster than more recalcitrant litter (Canhoto & Graça, 1995; Balibrea et al., 2017). Aquatic invertebrates are sensitive to leaf litter quality, and they tends to be more diverse when riparian vegetation and litter inputs are high (Ferreira et al., 2015).

*Eucalyptus* (Myrtaceae, a tree species originating especially from Australia) and *Pinus* (Pinaceae, a conifer species originating especially from Central America and North America) species have been introduced into several regions of the world (Valduga et al., 2016), mainly for use in the paper industry (Canhoto et al., 2004). Specific traits, such as having harder leaves, being a pyrophytic species and having a high capacity to adapt to different environmental conditions are responsible for their high invasiveness. In a meta-analysis, Ferreira et al. (2016) suggested that *Eucalyptus* plantations can have stronger negative effects on leaf litter decomposition rates in streams naturally receiving high-quality litter inputs and where detritivore abundances are high. In the present study, we conducted an *in situ* experiment to (1) quantify differences in decomposition of leaf litter of two non-native and two native tree species, (2) determine how aquatic invertebrate communities respond to the presence of non-native leaf litter in a third-order subtropical island stream. We compared leaf-associated invertebrate colonization and
leaf decomposition rates using leaf litter from two native tree species (*Ficus eximia* Schott and *Alchornea triplinervia* Spreng Mull. Arg) and two non-native tree species (*Eucalyptus* sp. and *Pinus radiata* D. Don) placed in a third-order subtropical island stream. Based on the previous research and differences in the leaf chemical among the native and non-native species we hypothesized that (i) leaf litter from the native species, probably with a high nutritional quality (e.g., soft and with high initial nitrogen concentration), will have higher decomposer activity and faster litter decomposition and; (ii) due the presence of anti-grazing defenses (Graça et al., 2002), the non-native leaf litter would be colonized by fewer invertebrates and that native species would be more species-rich compared to the colonization of leaf litters from the non-native species.

### 2. Material and Methods

#### 2.1. Study area

The study was conducted in a stretch of a third-order small stream located within a legally protected area with predominant dense Atlantic Rainforest in Florianópolis, Santa Catarina State, Southern Brazil (27° 44' S and 48° 31' W). We conducted the experiment in a forested stream to simulate possible ecological implications for the input of non-native riparian plants on associated invertebrate fauna and leaf litter decomposition. This nearly pristine stream drains an area of 1.66 km² with primary forest in the higher regions close to the sources of the stream, and it is covered by dense mixed Atlantic Rainforest in an advanced stage of regeneration. The dominant plant families are Myrtaceae, Fabaceae, Rutaceae, Lauraceae, Meliaceae, Apocynaceae and Palmae (Lisboa et al., 2015). The stream flows year-round, but the discharge can greatly increase in summer (December to March) due to a higher volume of aurstral rainfall. The climate in the area is typically subtropical, with rainfall well distributed throughout the year; although it is more intense in the spring and summer months (October–March) (Hennemann & Petrucio, 2011; Lemes-Silva et al., 2016).

#### 2.2. Environmental parameters

At the occasion of installation (incubation), the electric conductivity, water temperature (°C), pH and dissolved oxygen concentration were measured *in situ* with portable optical probes WTW 350ii. The water velocity was estimated by measuring the time that a plastic float needed to travel 1 m of the stream (Couceiro et al., 2007). The stream had a water temperature of 23.0 ± 2.3°C (mean ± standard deviation) and, and the water velocity of 0.15 ± 0.08 m s⁻¹. Stream waters had a pH neutral (pH = 7.0 ± 0.3), with higher levels of dissolved oxygen (8.0 ± 2.3 mg L⁻¹) and electrical conductivity (55.0 ± 2.3 mS cm⁻¹). The mean rainfall during the studied period (see below the description of the study period) was 177.6 ± 81.0 mm, with the highest precipitation in March (292.2 mm) and the lowest precipitation in April (116.2 mm).

### 2.3. Data collection

The leaf decomposition experiment was conducted from February to May 2016. We evaluated the leaf litter of two native (*A. triplinervia* and *F. eximia*) and two non-native trees (*Eucalyptus* sp. and *P. radiata*). The non-native trees species were selected among the dominant riparian species to represent differences in terms of leaf litter quality and because they are found year-round in the watershed studied. *Alchornea triplinervia* and *F. eximia* are native species and occur frequently in the riparian zone of the studied stream (Lisboa et al., 2015). The senescent leaf litter from *A. triplinervia* and *F. eximia* were collected from the floor immediately after natural abscission in the forest. *Eucalyptus* sp. leaves were directly collected from trees of similar age and vigour, and the leaf litter from *P. radiata* was collected from the floor during the season of peak litter fall. After the collection, the litter of each species was taken to the laboratory, oven-dried at 60 °C for 72 h and weighed to obtain the wet weight (g WW). We weighed 3.0 ± 0.1 g WW of each species and enclosed individually in single-species litterbags of 15 × 20 cm (10 mm mesh size). The litterbags were incubated in five stretches of the stream, in low flow current areas in five similar reaches of the stream. At each reach, five iron bars with 16 litterbags (4 replicates by species) were anchored to the streambed ~ 0.3 m in depth on the stream. Four replicate samples of each leaf species were randomly withdrawn after 1, 7, 30 and 45 and 60 days. We prepared 80 litterbags (5 chain/segment × 4 bags × 4 leaf litter species, Figure 1).

The retrieved litterbags were enclosed in individual plastic bags and taken to the laboratory. The litter remaining in bags was gently rinsed with distilled water into a 125 µm mesh sieve to remove small litter fragments and to retain the associated invertebrate fauna (see below). The remaining litter was placed in aluminium trays, oven-dried at 70°C for 72 hours and the leaf material was...
weighed to obtain dry biomass (DM). From dry biomass, we calculated the percentage of dry mass remaining (% DMR) for each replicate. Following that, we used %DMR to calculate the decay constant ($k$) by first taking the natural log of %DMR for each collection day using the negative exponential model, where the remaining mass was expressed in grams, and time was expressed in days considering the following equation: $k = -(\ln \frac{\text{final mass}}{\text{initial mass}})/\text{time}$. This model (Webster & Benfield, 1986) assumes that there is a constant and fractionated loss of material at any given moment.

### 2.4. Associated invertebrates

After rinsing the leaf material over 125 µm sieves the invertebrate fauna retained on the sieves were preserved in 70% ethanol until they were later counted and identified to family level using identification keys (Cummins et al., 2005; Mugnai et al., 2010) and organisms were assigned to functional feeding groups as either as gathering-collector (CG), collector-filterer (CF), shredder (SD), scraper (SC) and predator (PD) (Cummins et al., 2005). Chironomidae were classified as generalists in the functional feeding group classification because of the large number of generalist-feeding chironomid (Lemes-Silva et al., 2014). We measured invertebrate diversity within our colonized litterbags using species richness as the number of taxa present in each litterbag.

### 2.5. Data analysis

We analyzed our response variable % DMR and invertebrates’ colonization as a function of type of litter, if native or not. To do so, we ran a generalized linear mixed models (GLMM) with a Poisson (to aquatic invertebrates) and Gamma (% DMR) distributions. Gamma distribution is often used for nonnegative continuous variables and the Poisson distribution is often used to describe the distribution of a count variable. In our analysis the stream stretch was used as a random effect to account for the spatial autocorrelation of sampling points within streams and litter types (native and non-native detritus) as a fixed effect. To control temporal autocorrelation due to the repeated measures design, the period of exposure (1, 7, 30, 45 and 60) was included as a random effect (intercept and slope), and the residual diagnostics plotted from the ‘simulateResiduals’ function in the DHARMa package (Hartig, 2020) were used to examine the assumptions of each GLMM model. We used the glmmTMB function in R to conduct Tukey’s post hoc tests on our models, elucidating

Figure 1. Scheme illustrating of litter decomposition experiment with leaf litter from two native and two non-native trees realized in third-order small stream, Florianópolis, Santa Catarina, Brazil. This illustration was created in BioRender.com.
pairwise differences in the aquatic invertebrates, functional feeding groups and % DMR between native and non-native species. All generalized models were built using the packages in R.

Indicator value analyses (IndVal) were performed to identify which taxa of invertebrates were most important to the invertebrate community structure during the process of the colonisation of the leaf litter for each leaf litter species (Dufrêne & Legendre, 1997). The frequency and relative abundance of each group in the different leaf litter species were used in the analysis. The IndVal values were tested for significant differences among the native and non-native invasive plants using a Monte Carlo procedure (10,000 permutations). We graphically verified the normality and homogeneity of variance assumptions. When data transformation was necessary to approach a normal distribution and homogeneity of variance, the nature of the transformation is specifically indicated. All statistical analyses were performed using the open-source software R 4.0.3 (R Development Core Team, 2020).

3. Results

3.1. Decomposition

We found a significant effect of detritus type (if native or not) and time of incubation on %DMR (z value = –5.274; p = 0.001, Figure 2). However, no difference was observed when the time and type of detritus was considered together (p = 0.244). A fast mass loss was observed for *A. triplinervia* (native species, k = 0.0973 ± 0.1215 d⁻¹) followed by *F. eximia* (native species, k = 0.0884 ± 0.0130 d⁻¹), *P. radiata* (non-native, k = 0.0633 ± 0.0579 d⁻¹) and *Eucaliptus* sp. (non-native, k=0.0616 ± 0.0591d⁻¹) during the days of incubation (~70% mass loss). However, there were no differences observed between two native species and two non-native during the incubation (GLMM p > 0.05). At day 1, 7, 30 and 45, *A. triplinervia* and *F. eximia* detritus had lost 20 to 50% more biomass than *Eucaliptus* sp. and *P. radiata* and significant differences was observed on the %DMR between the leaf litter (GLMM p < 0.05). By day 60, no significant difference was observed on the %DMR among the species. Unfortunately, there were no data for *P. radiata* at 60 days because the detritus were lost during the experiment.

3.2. Invertebrates associated

During the experiment, we recorded a total of 1, 561 invertebrates belonging to 18 families, with 600 invertebrates found in the native leaf litter (234 in *F. eximia* and 366 in *A. triplinervia*), and 961 invertebrates in the non-native leaf litter (721 in *Eucaliptus* sp. and 240 in *P. radiata*). Chironomidae (Diptera) and Leptophlebiidae (Ephemeroptera) were the predominant aquatic invertebrate taxa found on the leaf litter. The results of the indicator species analysis indicated Chironomidae (Indval = 50.38, p = 0.044) as the taxon associated with *A. triplinervia* and Leptophlebiidae (Ephemeroptera) were the predominant aquatic invertebrate taxa found on the leaf litter. The results of the indicator species analysis indicated Chironomidae (Indval = 50.38, p = 0.044) as the taxon associated with *A. triplinervia* and Leptophlebiidae (Indval = 45.07, p = 0.001) as the taxon exclusively associated with *F. eximia*. No indicator species were found on the *Eucaliptus* sp. and *P. radiata* leaf litters.

![Figure 2](image-url)
The invertebrates abundance was different between the leaf litter from the native and non-native trees (F = 43.6, p = 0.0197, Figure 3A) and incubation time (p < 0.001). Total abundance was greater in Eucalyptus sp. on the day 7 and was significantly different between incubation periods, whereas the abundance on A. triplinervia, F. eximia and P. radiata were similar to each other. Taxon richness values were statistically different among litter types (GLMM, z value = 2.955; p = 0.003) and between incubation periods (Figure 3B, p < 0.05). The leaf litter of A. triplinervia, F. eximia and P. radiata had a higher taxonomic richness of aquatic invertebrates than those of the Eucalyptus sp.

### 3.3. Functional feeding groups

The invertebrate community structure, in terms of the functional trophic groups, differed significantly among the types of leaf litter (if native or not) and sampling period (time). No significant interaction between these two factors were found. Shredders, the functional feeding group' directly involved in litter decomposition, showed higher abundance in the native species (A. triplinervia = 15.0 ind.g⁻¹ AFDM and F. eximia = 10.2 ind.g⁻¹ AFDM). No shredder invertebrates were found in the Eucalyptus sp. The highest values were observed after 7 days, peaking on A. triplinervia on the 60 day of incubation. The most abundant functional feeding groups were generalists (here represented by the Chironomidae family), with the highest values observed on Eucalyptus sp. (Figure 4). The highest values were observed on the 7 days and the remaining sampling times were similar to each other. Abundances of gathering-collectors also differed, peaking on F. eximia. This functional feeding group showed the lowest densities after 45 and 60 days and the highest at 30 days. Predators varied in abundance and were most numerous on P. radiata and A. triplinervia. Predators reached their highest abundance after 45 days. Scrapers also differed in abundance with the lowest levels observed on the non-native detritus and the highest F. eximia. The lowest abundances were observed on the 7 and 45 days. Finally, the abundance of collector-filterer was significantly different between the detritus types (high abundance on the non-native leaf litter),

![Figure 3](image-url)
although no difference was observed between the incubation periods.

4. Discussion

The introduction of non-native invasive riparian species is deemed one of the major agents of global environmental changes (Pereira et al., 2012; Valduga et al., 2016), but information on the effects of invasive tree species on stream ecosystem functioning in an island ecosystem is still limited (Chauvet et al., 2016; Ferreira et al., 2016; Raposeiro et al., 2014). As predicted, *A. triplinervia*...
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and F. eximia decomposed much more rapidly than Eucalyptus sp and P. radiata, the two non-native tree species. Similarly slow decomposition of Eucalyptus (Graça et al., 2002; Cizungu et al., 2014) and P. radiata (Robson et al., 2009) has been observed in other forested streams (Graça et al., 2002). However, other cases have shown a faster decomposition rate (Bachega et al., 2016) or absence of differences (Guo & Sims, 1999) in various parts of Brazil. For the genus Alchornea, similar rapid decomposition has been reported in the savannah streams, with up to 80% biomass loss (~ 50 day of incubation) upon coarse mesh bags (Tenkiana & Chauver, 2018).

In our study, as well as observed in previous studies in Spain and Portugal on the effects of Eucalyptus plantations in streams ecosystems (Abelho & Graça, 1996; Larrañaga et al., 2006), we found changes in invertebrate communities (mainly shredders invertebrates) with consequences for the breakdown process. We believed that the difference observed in the leaf decomposition process can be explained by the low resource quality (e.g. lignin, cellulose and/or tannin content) of Eucalyptus sp. and P. radiata (Martínez et al., 2013). Studies have demonstrated that aquatic invertebrates, mainly shredders, can detect leaves of higher nutritional quality and reject leaves of lower nutritional quality (Danger et al., 2012). In fact, invertebrates prefer nitrogen-rich leaves with low amounts of secondary compounds (Cassoti et al., 2015). When exposed to these leaves, invertebrate shredders demonstrate high rates of consumption, and the high-quality leaves are processed more rapidly (Canhoto & Graça, 1995). On the other hand, the presence of non-native leaf litter in the streambed can influence the feeding behaviour of the shredders, which can exhibit low consumption rates and high mortality (Abelho & Graça, 1996; Cassoti et al., 2015; Kiffer Junior et al., 2018). Here, we observed differences in the abundances of certain taxonomic groups, among which generalists (here as Chironomidae) and sensitive taxa such as the orders Ephemeroptera, Plecoptera and Trichoptera, including the shredders on leaves of non-native species.

The structural and biological composition of the forested riparian areas are important not only as determinants of the quality, quantity and seasonality of food sources to the heterotrophic aquatic food chains (Kominoski et al., 2013) but also as stabilisers of the stream banks. Therefore, the quality of leaf litter entering freshwater ecosystems can alter aquatic communities (invertebrates and microbes), with potential breakdown rate effects (Barlocher & Graça, 2002; Wright & Covich, 2005). Litter decomposition is strongly related to the chemistry of leaf litter, and the contents of nitrogen and lignin are often used as proxies for litter quality (Schindler & Gessner, 2009; Marcarelli et al., 2011). Phosphorous, carbon and leaf toughness have also been documented as good predictors of litter breakdown rates in streams (García et al., 2014; Frainer et al., 2015). As predicted, F. eximia and A. triplinervia leaves, the fastest-decomposing leaves, were associated with the highest taxonomic richness of invertebrate taxa throughout the entire experiment. This suggests that invertebrate communities, mainly shredders, aggregate on the litter with the highest quality, which in turn increases the rate of litter breakdown. Inversely, we observed that the leaf litter of non-native invasive species had lower taxa richness, which in turn decreased the rate of litter breakdown.

One aspect evaluated in the present study, the colonisation by invertebrates, supported the hypothesis that the invertebrates prefer eating the leaf litter from native species to non-native species, corroborating other studies (König et al., 2014). This preference was reflected mainly in the abundance of the shredders and collector-gatherers. The abundance of shredders was greater for A. triplinervia throughout the period in which the leaves of this species were incubated in the stream. This preference can be related to different leaf properties that determine leaf palatability (Graça, 2001). Lower levels of tannins and higher nitrogen contents in the A. triplinervia leaves may have favoured invertebrate colonisation, as tannins are one of the main secondary metabolites produced by plants and are generally negatively correlated with the feeding preferences of invertebrates (König et al., 2014).

The take-home message is clear: The replacement of native riparian vegetation, a major energy source for aquatic decomposers, by non-native species can alter the breakdown of leaf litter, which is an important ecosystem function. In conclusion, we believe that the evaluation of ecosystem-level processes in streams that have changing environmental conditions can be an alternative for a better understanding of the effects that different disturbances (here replacement of native vegetation by non-native species) have on aquatic invertebrates and stream ecosystem functioning. Therefore, understanding the relationship between riparian vegetation cover and stream ecosystem processes has become increasingly relevant to understanding the
changes that have occurred in recent years in aquatic ecosystems. This knowledge will help to support current and future research in stream ecosystems, as the process of litter breakdown is considered a proxy for stream health and ecological integrity (Gessner & Chauvet, 2002; Clarke et al., 2008; Kominoski et al., 2013).

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**References**


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