**ECOLOGY**

# Effects of microbial conditioning and temperature on the leaf-litter shredding activity of *Phylloicus* sp.

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**ABSTRACT.** Few studies try to explain the effects in tropical lotic ecosystems of an increase in water temperature on the shredding activity of invertebrate shredders, particularly in association with the quality of the leaf litter and the degree of litter conditioning. Therefore, the aims of this study were as follows: i) to better understand how this key invertebrate shredder group affects the decomposition of different species of leaf litter under gradual increases in temperature and microbial conditioning; and ii) to verify the possible consequences on leaf mass loss (LML). Three species of leaf litter were used in two experiments. In experiment I, the litters of three species (*Protium spruceanum*, *Richeria grandis* and *Inga laurina*) at three conditioning levels (1, 7, 14 days) were tested under five different temperatures (20, 22, 24, 26 and 28°C). In experiment II, the leaf litters of three species were used, without conditioning, under four temperatures (20, 22, 26 and 27°C). The shredding performed by *Phylloicus* sp. was largely dependent on the lignin and cellulose concentrations in each leaf species, independent of conditioning. The presence or absence of conditioning may cause the shredders to use different energy compensation strategies in response to the temperature increases.

**Keywords:** global warming; shredding behavior; conditioning; stream.

## Introduction

In lotic ecosystems, the source region displays heterotrophic metabolism due to both the presence of well-developed marginal vegetation and the low light penetration (Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Therefore, the input of organic matter from allochthonous origins constitutes the primary source of energy (Cummins, Wilzbach, Gates, Perry, & Taliaferro, 1989; Graça, 1993). In this context, the decomposition of leaf matter becomes fundamental for the functioning of these ecosystems. This process is the result of physical and chemical activity (leaching, abrasion, and shredding) as well as biological activity (microbial colonization and the activity of invertebrate shredders) (Gessner, Chauvet, & Dobson, 1999; Rincón & Martínez, 2006).

The invertebrate shredders present in these lotic environments possess jaws capable of transforming coarse particulate organic matter (CPOM) in fine particulate organic matter (FPOM) (Merrit & Cummins, 1996). This shredding ability allows for leaf matter to be used as food and case-building to be constructed dorsoventrally, contributing to the leaf shredding process within the aquatic trophic chain (Wantzen & Wagner, 2006; Wiggins, 1996).

The feeding preference of shredders is intimately associated with the quality of the leaves, as measured by physical characteristics (such as hardness) and chemical characteristics (such as nutritional content and the soluble and insoluble organic compounds in the litter) (Gessner & Chauvet, 1994; Hladyz, Gessner, Giller, Pozo, & Woodward, 2009). Furthermore, invertebrates tend to consume leaf litter that is labile and has reached a certain level of conditioning by microorganisms, as opposed to non-conditioned litter (Cummins et al., 1989; Graça, 1993; 2001). Secondary compounds, such as polyphenols, are lost during the conditioning/leaching process (Bärlocher & Graça, 2005), whereas the protein, nitrogen, and phosphorus levels increase (Bärlocher, 1985; Suberkropp, 1992; Graça, Maltby, & Calow, 1993) with decreasing leaf hardness (Graça & Zimmer, 2005). These effects of the conditioning process tend to make the leaf litter more palatable to invertebrate shredders. The microbial community associated with litter is primarily

composed of fungi, which are considered the main decomposing microorganisms and represent up to 16% of the total litter mass (Abelho, 2001; Gessner, Gulis, Kuehn, Chauvet, & Suberkropp, 2007).

The level of conditioning can be measured by the exposure time of the leaves in streams, the nitrogen content and microbial biomass of the litter, and the fungi species present (Suberkropp, 1992; Graça, 1993; Rong, Sridhar, & Bärlocher, 1995). The selection of leaf litter by fragmenting is linked to a higher degree of conditioning of food resources in order to ensure greater survival rate, growth and increased reproductive efficiency (Bueler, 1984; Canhoto & Graça, 1995; Bärlocher, 1985; Jacobsen & Jensen, 1994; Suberkropp, 1992; Graça et al., 1993). Furthermore, the shredding effect of invertebrates can be affected by extrinsic factors, such as thermic alterations in streams which can affect the assimilation of N, P, and C in detritivorous species (Villanueva, Albariño, & Canhoto, 2011).

There are many studies predict a worldwide temperature increase of 1.4 – 5.8°C in the next 100 years, which would also lead to an increase in the temperatures of rivers (Houghton et al., 2001). This temperature increase can lead to serious consequences, such as physiological alterations in various organisms, species extinctions, alterations in competitive interactions and ecological processes, and exponential increases in metabolic rates (Mooney et al., 2009; Brown, Gillooly, Allen, Savage, & West, 2004). In the aquatic communities that guide the process of leaf decomposition, these consequences of warming may affect the functioning of lotic ecosystems at the individual, population or community levels (Villanueva et al., 2011). This elevation is able to stimulate the respiratory activity and fungal production, which contributes to enhance the decomposition process mediated by microorganisms (Ferreira & Chauvet, 2012; Geraldes, Pascoal, & Cássio, 2012).

Few studies have attempted to explain the effects of a water temperature increase on the decomposer biota, particularly on the invertebrate shredders present in tropical lotic ecosystems. Consequently, little research has been performed discussing the effects of higher river temperatures on lotic environments. Boyero et al. (2011) observed a decrease in the participation of detritivores and an increase in microbial activity with an increase in water temperature. Additionally, other studies reported a higher sensitivity to temperature increases in invertebrate shredders with low quality diets (Villanueva et al., 2011).

This study was performed with a new species of invertebrate shredder, belonging to the genus *Phylloicus* Muller (1880) that is yet to be described. The genus is part of the family Calamoceratidae and contains 61 known species that are distributed from South America to Central America. It is a very diverse genus in Brazil, Peru, and Venezuela (Huamantinco, Dumas, & Nessimian, 2005; Prather, 2003). The aims of this study were as follows: i) to better understand how this key invertebrate shredder group affects the decomposition of different species of leaf litter under gradual increases in temperature and microbial conditioning, and ii) to verify the possible consequences on leaf mass loss. Based on the premise that the fragmentation activity of *Phylloicus* sp. is influenced by the quality of the leaf litter influenced by temperature, two hypotheses were assessed. (1) The leaf mass loss of better-quality litter is high, independent of the temperature or conditioning; an increase in temperature leads an increase in individual metabolisms, causing a higher consumption of these more palatable resources (Experiments I and II). (2) The increase in temperature affects the leaf mass loss of only the most highly conditioned leaf litter, and a higher consumption of this resource will be observed during an increase in temperature (Experiment I).

## Material and methods

### Experiments I and II

Individuals of *Phylloicus* sp. (Trichoptera) were collected by "active search" between March and April in the Capetinga stream of the Água Limpa farm, which belongs to the *Universidade de Brasilia* (UnB) and comprises 4,500 hectares. The invertebrates were transported to the laboratory in isothermal boxes. In the laboratory, the invertebrates in leaf cases were acclimatized for 24 hours in 60-L aquariums containing stream water and a layer of fine gravel. The aquariums were continuously aerated during the acclimatization period.

The leaf litter used originated from three plant species: *Richeria grandis*, *Protium spruceanum*, and *Inga laurina*. According to a study by Navarro, Rezende, and Gonçalves Jr. (2013), *Richeria grandis* contains 7% g$^{-1}$ dry mass of polyphenols, 400 g of hardness, 29% g$^{-1}$ of lignin, and 21% g$^{-1}$ of cellulose. *Inga laurina* displays 5% g$^{-1}$ dry mass of polyphenols, 364 g of hardness, 44% g$^{-1}$ of lignin, and 29% g$^{-1}$ of cellulose. *P. spruceanum* was characterized by 5% g$^{-1}$ dry mass of polyphenols, 351 g of hardness, 34% g$^{-1}$ of lignin, and 18% g$^{-1}$ of cellulose. *Inga laurina* was considered lower quality than *P. spruceanum* and *R. grandis* because of its higher concentrations of lignin and cellulose.

For experiment I, 15 g of each litter species was placed in litter bags (0.5 mm mesh). The litter was then submerged in the Capetinga stream for microbial conditioning, remaining there for periods of 1, 7 or 14 days. This experiment consisted of five treatments differentiated by the water temperature: Treatment 1 occurred at 20°C, Treatment 2 at 22°C, Treatment 3 at 24°C, Treatment 4 at 26°C, and Treatment 5 at 28°C. The lowest level in this temperature gradient was defined based on the mean temperature of Capetinga. Two invertebrates without leaf cases, as well as 24 disks conditioned leaf disks (8 disks of each level of conditioning, either 1, 7, or 14 days) of each type of litter, were placed in each 60-L aquarium. The disks of each litter species were arranged in groups, and the groups were placed in distinct positions inside the aquarium.

Experiment II consisted of four treatments differentiated only by the water temperature: Treatment 1 at 20°C, Treatment 2 at 22°C, Treatment 3 at 26°C, and Treatment 4 at 27°C. Two invertebrates without leaf cases and 24 disks non-conditioned leaf disks (8 disks of each level of conditioning, either 1, 7, or 14 days) of each litter species were placed in each 60-L aquarium. The disks of each litter species were arranged in one group, and the groups were placed in distinct positions inside the aquarium.

For both experiments, the aquariums had automatic thermostats to maintain their temperature, aerators to maintain oxygenation, and submersion pumps to simulate the lotic flow rate. Additionally, water quality parameters (such as dissolved oxygen and pH) were measured daily.

### Percentage of leaf mass loss

The leaf disks of the plant species studied were cut using a cork drill (1.8-cm diameter), weighed with a precision scale (0.00001 g) to quantify the wet weight, and distributed among the treatments of both experiments. These disks were attached to the substrate of the aquarium with pins and colored rubber fragments to facilitate the identification of the disks. Leaf disks homologous to those used in the experiments were also cut from each species and used to correct the initial moisture percentage (initial dry weight) by being dried in an oven dryer at 62°C for 48 hours.

For both experiments, the incubation period of the leaf disks for each treatment in the aquariums was 12 days. After this period, the disks were dried in an oven dryer at 62°C for 48 hours and then weighed in a precision scale (0.00001 g) to measure their final dry weights. The leaf mass loss (LML) among the different litter species was calculated by subtracting the final dry weight from the initial dry weight. The experiments were performed in the Experimental Laboratory of the AquaRiparia Program of the Department of Ecology/IB/UnB.

### Statistical analysis

To test hypothesis 1, the percentage of leaf mass loss in experiment I (dependent variable) due to the invertebrates of the genus *Phylloicus* was tested using a split-plot analysis of variance (SPANOVA) for the three leaf litter species—*Richeria grandis*, *Protium spruceanum*, and *Inga laurina* (categorical variable)—at the different temperatures (categorical variable) (Crawley, 2007). The level of conditioning (categorical variable of lower hierarchy) was used as an error variance for the quality of each leaf litter type. In experiment II, the leaf loss mass (dependent variable) was tested using a two-way analysis of variance (ANOVA) for the different qualities of the leaf litter (categorical variable) at the different temperatures (categorical variable). To distinguish the categorical variables, a contrast analysis was performed with a significance level of $p < 0.05$ (Crawley, 2007).

To test hypothesis 2, the percentage of leaf loss mass for each conditioning stage of each leaf litter species (dependent variable) at the different temperatures (categorical variable) was tested using a one-way ANOVA. To distinguish the categorical variables, Tukey's test was used with a significance of $p < 0.05$ (Zar, 1996). The normality of the data was evaluated by the Kolmogorov-Smirnov test and, when necessary, the data were logarithm-transformed (Massey Jr., 1951).

## Results

### The influence of the temperature gradient on the leaf mass loss is dependent on the quality of the leaf litter

In the experiment I (SPANOVA, $F_{118,2} = 18.244$, $p < 0.001$) and II (two-way ANOVA, $F_{66,2} = 10.589$, $p < 0.001$), we found that the leaf mass loss was significantly influenced by the different leaf litter species

studies. The interaction between the leaf litter species and the temperature also was significantly (SPANOVA, $F_{118,8}$ = 4.187, p < 0.001) in the experiment I (Table 1). On the other hand, in the experiment II we did not find significance in the interaction between the temperature and the leaf litter species (two-way ANOVA, $F_{66,2}$ = 0.694, p = 0.503) (Table 1).

In the experiment I, the leaf mass loss of *I. laurina* was significantly lower than those of *R. grandis* or *P. spruceanum*, whereas *R. grandis* was significantly higher than *P. spruceanum* (contrast analysis, p < 0.05) (Table 1). The leaf mass loss was affected by temperature gradient in different litter types (SPANOVA, $F_{118,4}$ = 2.454, p = 0.0495) (Table 1). The 22, 26, and 28°C temperatures have had similar and lower the leaf mass loss, than those at 20 and 24°C (contrast analysis, p < 0.05). Furthermore, the 20°C experiment had leaf mass loss higher than 24°C (contrast analysis, p < 0.05) (Table 1).

In experiment II, the leaf mass loss of *I. laurina* was significantly lower than *R. grandis* or *P. spruceanum* (contrast analysis, p < 0.05), but we did not differ between them (contrast analysis, p > 0.05) (Table 1). However, temperature did not significantly influence in the leaf mass loss of the different leaf litter species (two-way ANOVA, $F_{66,1}$ = 2.853, p = 0.096) (Table 1).

**Table 1.** The mean values and standard error of the leaf mass loss (LML %), as well as the statistical results of the two-way ANOVA for non-conditioned leaf litter species and the results of the split-plot analysis of variance (SPANOVA) for conditioned lead litter species, at the different temperatures.

| (°C) | Leaf Litter Species | (LML %) | (°C) | Leaf Litter Species | (LML %) |
|---|---|---|---|---|---|
| | | (Conditioned) | | | (Non-Conditioned) |
| | *R. grandis* | | | *R. grandis* | |
| 20°C | | 23.46 ± 7.85 | 20°C | | 37.99 ± 10.16 |
| 22°C | | 36.21 ± 12.94 | 22°C | | 14.59 ± 8.23 |
| 24°C | | 62.53 ± 10.91 | 26°C | | 4.11 ± 11.15 |
| 26°C | | 1.82 ± 7.30 | 27°C | | 23.9 ± 6.63 |
| 28°C | | 13.85 ± 8.34 | | | |
| | *P. spruceanum* | | | *P. spruceanum* | |
| 20°C | | 43.78 ± 11.69 | 20°C | | 32.49 ± 6.27 |
| 22°C | | -2.84 ± 1.14 | 22°C | | 28.53 ± 7.48 |
| 24°C | | -1.62 ± 1.29 | 26°C | | 14.07 ± 2.27 |
| 26°C | | 6.23 ± 11.95 | 27°C | | 32.14 ± 3.23 |
| 28°C | | -8.35 ± 15.14 | | | |
| | *I. laurina* | | | *I. laurina* | |
| 20°C | | -11.68 ± 7.79 | 20°C | | 5.08 ± 6.67 |
| 22°C | | -9.18 ± 5.22 | 22°C | | 2.25 ± 1.96 |
| 24°C | | -11.99 ± 4.53 | 26°C | | 1.43 ± 9.39 |
| 26°C | | -11.79 ± 3.51 | 27°C | | 3.05 ± 4.19 |
| 28°C | | -1.04 ± 3.60 | | | |
| | Statistics | | | | |
| | $P_{\text{leaf litter species}}$ | <0.001 | | | <0.001 |
| | $P_{\text{temperature}}$ | 0.049 | | | 0.096 |
| | $P_{\text{temperature X leaf litter species}}$ | <0.001 | | | 0.503 |

## The influence of the temperature gradient on leaf mass loss is dependent on the level of conditioning of each leaf litter species

The leaf of *R. grandis* that was subjected to the longest conditioning period (14 days) and showed higher leaf loss mass with increasing temperatures (one-way ANOVA, $F_{4,14}$ = 4.9, p = 0.018). In the treatment at 24°C was significantly higher than those at 26 or 28°C (Tukey's test, p < 0.05) (Figure 1A). This trend was also observed in leaf litter of the same species subjected to only 1 day of conditioning (one-way ANOVA, $F_{4,14}$ = 4.89, p = 0.019; Tukey's test, p < 0.05) (Figure 1A). However, no difference was found along the temperature gradient for leaf litter of *R. grandis* at the intermediate conditioning stage (7 days) (one-way ANOVA, $F_{4,14}$ = 0.92, p = 0.492; Tukey's test, p > 0.05; Figure 1A).

The leaf of *P. spruceanum* showed a significant difference in the leaf loss mass only leaves subjected to the shortest conditioning period (1day). We observed a significant reduction between the litter at 20°C and those at 24 and 26°C (one-way ANOVA, $F_{4,14}$ = 6.95, p = 0006; Tukey's test, p < 005, Figure 1B).

The leaf of *I. laurina* was not significantly dependent on the temperature or on the conditioning stage (one-way ANOVA, $F_{4,14}$ = 0.117, p = 0.934; Tukey's test, p > 0.05; Figure 1C).
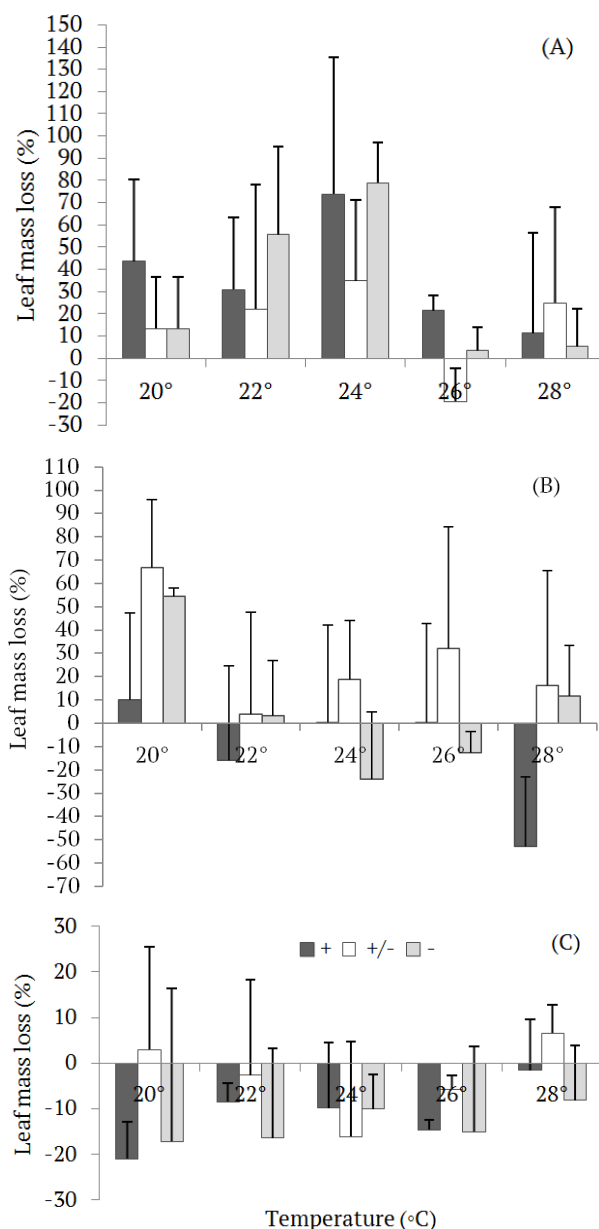
**Figure 1.** The means and standard deviations of the leaf loss mass percentages of *Richeria grandis* (A), *Protium spruceanum* (B), and *Inga laurina* (C) by invertebrates of the genus *Phylloicus*; tests took place under different conditioning stages (1 day = -, 7 days = +/-, and 14 days = +) and at different temperatures.

# Discussion

### The Influence of the temperature gradient on leaf mass loss is dependent on the quality of the leaf litter

In both experiments, the use of leaf resources was higher when the leaf litter was of higher quality (*R. grandis* and *P. spruceanum*) than of lower quality (*I. laurina*). Because the hardness and the polyphenol concentration did not differ among the three species, the low quality of species *I. laurina* must be related to its higher lignin and cellulose contents. This poor quality likely reduced the litter's palatability and digestibility to shredders, hindering the degradation of this leaf litter species. These results corroborate other studies that have shown a higher feeding preference of *Phylloicus* sp. for leaf litter with higher nutrient contents and lower lignin and cellulose concentrations (Ríncon & Martínez, 2006; Navarro et al., 2013).

The increase in the leaf mass loss percentage of conditioned leaf litter at 20°C can be justified by *Phylloicus* sp. has increased consumption at temperatures closer to that of its natural environment. We observed its increase at 24°C is due to an increase in its metabolic rate. The optimal temperature condition (20°C) of Neotropical consumers is associated with a higher flexibility in life history and in mobility, which could lead to an ability to flexibly use feeding resources (Covich, 1988). This flexibility may have generally

contributed to the increased leaf consumption found in this study. However, higher temperatures (24°C), which likely are still within a possible optimal gradient, may have contributed to an increase in the energy demand of the invertebrates. In its turn, this higher demand may have caused an increase in feeding consumption, particularly of leaf litter with a lower quantity of refractory compounds that would rapidly yield energy with high nutritional value. These results corroborate other studies that also show an increase in the amount of matter cycling in aquatic ecosystems when the water temperature increases due to the metabolic acceleration of the biota (for example, increased ingestion) (Brown et al., 2004; Villanueva et al., 2011; Ward & Stanford, 1982). The increase in temperature might also lead to increased metabolism due to the higher respiration and excretion rates in some invertebrate shredders (*Sericostoma vittatum*, Villanueva et al., 2011), the fact that the leaf litter (*Eucalyptus globulus*) remained in the intestines for a longer period of time could increase nutrient acquisition without requiring increased consumption (Villanueva et al., 2011).

Furthermore, this reduction in litter leaf mass loss at higher temperatures (26 and 28°C) could be a result of reduced body metabolism and shredding activity, a strategy the shredder would use to reduce energy expenditure and guarantee survival. Studies show that in gradients above or below the optimal temperature, most of the performance values tend to decline (Scriber & Slansky, 1981; Villanueva et al., 2011). Therefore, it is possible that temperatures above 26°C are overly high, leading to alterations in the behavioral patterns and metabolic performance of *Phylloicus* sp.

### Increase in temperature X Leaf mass loss dependent of the level of conditioning of each leaf litter species

Despite the decreased use of better quality resources at 26°C in comparison to 20°C, the latter being considered closer to that of the shredders' natural environment. The preference of *Phylloicus* sp. for resources that guarantee a more effective and efficient nutritional gain was verified in the preferential use of highly conditioned (14 days) *R. grandis*. The advanced stage of conditioning likely allowed for a higher microbial diversity and proliferation in the leaf litter, making it more palatable than leaf litter subjected to only 7 days of conditioning (Gonçalves Jr., Rezende, Martins, & Gregório, 2012). After 7 days, there was a total leaching of soluble compounds from this leaf species (Gomes, Medeiros, & Gonçalves Jr., 2016). However, according to Abelho (2001), a significant proportion of the microbial colonization is usually complete within the first two weeks, which can increase the nutritional value of the organic matter (Gonçalves Jr., França, Medeiros, Rosa, & Callisto, 2006). In addition to the characteristics of the biomass, the diversity of the fungal species in the leaf litter can also determine the feeding pattern preference of invertebrates (Suberkropp, 1992; Graça, 1993; Rong et al., 1995). Therefore, a longer conditioning period may guarantee an increase in the nitrogen content due to the fungal biomass as well as a higher level of leaf degradation, favoring the action of microbial enzymes on lignin/cellulose compounds and enabling a higher energy input for *Phylloicus* sp. (Graça et al., 1993). Invertebrates are capable of consuming both the leaf fragments and the fungal biomass present in leaf litter (Graça et al., 1993; Graça et al., 2001).

At the highest temperature (28°C), no increase in the consumption of better-quality leaf litter was found. Possibly, the conditioning that indirectly increases the nutritional value and the palatability of the leaf litter (Barlöcher & Graça, 2005) also ensures a better assimilation of nutrients than in the non-conditioned leaf litter at 27°C. The invertebrates increased their consumption to try to elevate their nutritional assimilation efficiency. This result was also verified in studies by Villanueva et al. (2011), who found that an increase in temperature with better-quality leaf litter led to a higher assimilation of minerals, such as phosphorus, compared to resources with lower nutritional value.

Conditioning the leaf litter seems to increase the use of only the higher quality resources. This, it has contributed to a more specialized behavior of *Phylloicus* sp. at intermediate temperatures. The higher-grade plant matter, when enriched with a primarily fungal microbial community (conditioned), can be more palatable (Martin, 1979; Dangles & Chauvet, 2003, Colpo, Ribeiro, Wesz, & Ribeiro, 2012). Furthermore, conditioned plant matter possesses a higher nutritional value because of the protein and lipid contents in the fungal tissues, which may comprise 50% of the leaf litter mass (Martin, 1979; Colpo et al., 2012). This leaf litter was capable of meeting the likely increased energy demands of *Phylloicus* sp. caused by the temperature increases. However, the non-conditioned litter (which did not suffer leaching) most likely hindered microbial colonization due to the effect of secondary compounds (Barlöcher & Graça, 2005). Reduced colonization makes these resources less palatable and less nutritive for the shredders (Abelho, 2001; Allan & Castillo, 2007) and leads to a more generalist behavior of the shredders at all temperatures.

## Conclusion

The shredding behavior of *Phylloicus* sp. depends primarily on the lignin and cellulose concentrations of the plant species used as feeding resources, regardless the conditioning. The presence or absence of conditioning may cause the shredders to adopt different strategies for energy compensation in response to the increase in temperature, for the temperature affected the leaf mass loss of the conditioned litter only. The invertebrate shredders with conditioned resources seemed to increase the selectivity of this decomposer biota, particularly at intermediate temperatures. Furthermore, the higher palatability and nutritional value caused by the conditioning process tends to reduce the consumption of higher-quality litter at extreme temperatures. These behavioral variations in different water temperatures, which are associated with the presence or absence of conditioned resources, could lead to changes in the quality (in minerals such as carbon, nitrogen, and phosphorus) or quantity of the fine particulate organic matter. Such changes lead to alterations in the energy flow and matter cycling of lotic ecosystems.

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