Shredders prefer soft and fungal-conditioned leaves, regardless of their initial chemical traits

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ABSTRACT. Through field and laboratory experiments we investigated the effects of leaf traits of two tree species and microbial conditioning on the abundance, biomass, and feeding preference of a typical macroinvertebrate shredder. In the field, we compared the association of Phylloicus (Calamoceratidae, Trichoptera) with two tree species commonly found in riparian zones, which are representative of high and low nutritional quality, respectively: Nectandra megapotamica and Chusquea tenella. In the laboratory, we investigated the feeding preference of Phylloicus using unconditioned leaves and leaves conditioned by aquatic fungi. The same tree species used in the field experiment were used in the laboratory. Initially, C. tenella leaves were proved to be more nutritious and softer, while N. megapotamica leaves were harder and more lignified. The shredders preferred conditioned leaf detritus of reduced toughness (field: C. tenella; laboratory: N. megapotamica, both conditioned for 14 days). These leaf traits seem to be crucial for the choice process of Phylloicus. After 14 days, N. megapotamica leaves showed a decreased toughness associated with the microbial conditioning, which explained its consumption rate by Phylloicus. In both field and laboratory experiments, we found evidence that Phylloicus is a selective feeding shredder, and that the leaf traits, especially leaf structure (e.g., leaf toughness and lignin content), determine its association and preferences.

KEYWORDS. Phylloicus, C:N ratio, feeding preference, leaf toughness, hyphomycetes.

The input of allochthonous material to forested streams is the primary source of nutrients, energy, and shelter for invertebrates, especially shredders, which consume a significant part of decomposing leaves in aquatic environments (Wallace et al., 1997; Pettit et al., 2012). These organisms are responsible for turning coarse particulate organic matter into fine particulate and dissolved organic matter, making it available to other aquatic invertebrates (Wallace & Webster, 1996).

The shredder activity is a significant process in the decomposition of organic matter, representing 50-74% of the decomposition rates in temperate regions, depending on the plant species (Cuffney et al., 1990). In tropical regions, shredders have a low participation in leaf decomposition (Bovero et al., 2015). Often, tropical streams have ~2.5 times lower shredders density than temperate regions (Bovero et al., 2011). However, there are reports of tropical streams with shredders densities similar to temperate streams (Cheshire et al., 2005; Yule et al., 2009). In subtropical streams, studies about the importance of shredders to the ecosystem are scarce. Some data suggest that the low density of this trophic group in subtropical streams can be compensated by the large size and biomass of typical shredders (Tonin et al., 2014) when compared with temperate streams. Also, leaf features, microbial activity, and environmental variables acting on streams should be further investigated to determine...
Shredders prefer soft and fungal-conditioned leaves, regardless of their effects on the shredder community in both tropical and subtropical regions (Rezende et al., 2015; Biasi et al., 2016).

The genus *Phylloicus* (Müller, 1880) (Calamoceratidae, Trichoptera) is the main shredder of aquatic communities in the subtropical region (Tonin et al., 2014; Tonello et al., 2016). Their larval stages are found under leaves on the banks of streams headwaters (Wantzen & Wagner, 2006), feeding and case-building activities of these organisms lead to the typical leaf fragmentation observed in those environments (Vannote et al., 1980). Larvae feed on leaves with a higher nutritional quality (Rincón & Martínez, 2006), while harder and more lignified leaves with higher polyphenols content are used for case-building (Moretti et al., 2009; Cerézier et al., 2016).

Intrinsic leaf traits, such as toughness and nutrients and secondary metabolites content, are limiting factors explaining shredders preferences among different leaf species (Graça, 2001; Ratnarajah & Barmuta, 2009; König et al., 2014). Harder leaves (i.e., resistant to physical abrasion) and those with a higher concentration of secondary compounds (e.g., polyphenols) may be difficult to consume and restrict shredders activity (Rincón & Martínez, 2006). Besides, more nutritious leaves (i.e., higher concentrations of nitrogen and lower Carbon:Nitrogen ratio — C:N ratio) (König et al., 2014) are more palatable and appealing to shredders (Mathurau & Chauvet, 2002).

The process of microbial conditioning of leaves is known to influence shredders choice (Canhoto & Graça, 2008). This process is the transformation of the leaf litter by aquatic hyphomycetes into a more palatable resource for detritivore animals. The colonization by hyphomycetes starts with the conidia fixation on the substrate surface. Afterward, these microorganisms macerate the litter through the activity of their external enzymes, mineralize the organic carbon, convert it into mycelium and reproductive structures, and release fine particulate organic matter (Hieber & Gessner, 2002; Cornut et al., 2010). This process is affected by physical (e.g., toughness, shape, and roughness) and chemical (e.g., amount of nitrogen) traits of the litter (Gulis et al., 2004; Biasi et al., 2017). The importance of leaf conditioning on shredder preference has been the focus of classic studies which point to a positive effect of microbial colonization on invertebrate feeding (Kaushik & Hynes, 1971; Barlocher & Kendrick, 1975). However, some shredders species have shown different patterns. *Lepidostoma complicatum* (Kobayashi, 1968), Trichoptera and *Sternmoera rhaca* (Kuribayashi, Mawatari & Ishimaru, 1996), Amphipoda, consume unconditioned freshly abscised leaves or even green leaves that retain compounds that would normally prevent herbivory (Kochi & Yanai, 2006). Understanding the relationship between *Phylloicus* and food resources is crucial due to its large size compared with other shredders and because it is the main responsible for leaves fragmentation in subtropical streams (Tonin et al., 2014; Tonello et al., 2016).

In this study, we conducted field and laboratory experiments to investigate the effects of leaf traits and microbial conditioning of two tree species on abundance, biomass, and feeding preference of a typical macroinvertebrate shredder. In the field, we compared the association between *Phylloicus* and two contrasting tree species concerning their leaf traits (*Nectandra megapotamica* and *Chusquea tenella*).

In the laboratory, we investigated the feeding preference of *Phylloicus* amongst the same tree species used in the field experiment; leaves conditioned by aquatic fungi and unconditioned leaves were used. The both tree species are found of riparian zones and they are representative of good (*N. megapotamica*) and poor quality resources (*C. tenella*) for shredders, so they are good models for testing our hypotheses. We expect that larvae prefer and consume a higher quantity of softer, nutritious, and conditioned leaves. Thus, regardless of the environment field or laboratory, they will prefer items with reduced leaf toughness, lower C:N ratio, and conditioned by the microbial community.

**MATERIAL AND METHODS**

**Leaf sampling.** We collected senescent leaves of *N. megapotamica* (Spreng.) Mez. (Lauraceae) and *C. tenella* Nees (Poaceae) from riparian zones of stream from South Brazil during the 2013 autumn. Both species are native to South America and the choice was based on their wide occurrence and abundance in stream riparian zones. The leaves were air-dried at room temperature (~20°C) until the beginning of the experiments. Approximately 5 g of leaves of each species were used to perform the leaf traits characterization.

**Leaf traits.** The leaf traits characterization of both tree species was performed prior to the beginning of the experiments. The leaves (1 mm) were ground to powder in an analytical mill to estimate the concentration of nitrogen, polyphenol, lignin, cellulose, carbon, and metals. The nitrogen concentration was estimated according to the Kjeldahl method (Flindt & Lillebo, 2005). For polyphenol extraction, samples were exposed to the Folin-Denis reagent and sodium carbonate. The polyphenols concentration was estimated through OD (optical density); results were read in a 725-nm wavelength spectrophotometer (Schwarze, 1958). Approximately 3 g of leaves of each species was incinerated in a muffle furnace (at 550°C, for 4 hours) for the determination of the ash-free dry mass (AFDM). The resulting inorganic material was diluted in HNO₃ (1 mol L⁻¹) and analyzed using an atomic absorption spectrophotometer with metals determination (calcium, magnesium, and potassium).

The carbon concentration in leaves was estimated through OD (optical density); results were read in a 725-nm wavelength spectrophotometer (Schwarze, 1958). Approximately 3 g of leaves of each species was incinerated in a muffle furnace (at 550°C, for 4 hours) for the determination of the ash-free dry mass (AFDM). The resulting inorganic material was diluted in HNO₃ (1 mol L⁻¹) and analyzed using an atomic absorption spectrophotometer with metals determination (calcium, magnesium, and potassium). The carbon concentration in leaves was estimated through OD (optical density); results were read in a 725-nm wavelength spectrophotometer (Schwarze, 1958). Approximately 3 g of leaves of each species was incinerated in a muffle furnace (at 550°C, for 4 hours) for the determination of the ash-free dry mass (AFDM). The resulting inorganic material was diluted in HNO₃ (1 mol L⁻¹) and analyzed using an atomic absorption spectrophotometer with metals determination (calcium, magnesium, and potassium).
Shredders prefer soft and fungal-conditioned leaves, regardless of conditioning time, and display strong preferences that are unaffected by leaf toughness or environmental variables. The larvae were deposited in the scientific collection of the Carcinology Laboratory of the Universidade Federal de Santa Maria, RS.

Data analyses. The chemical variables of both plant species were tested by a t-test. The abundance and biomass of Phylloicus and leaf toughness (field experiment) of each plant species were compared by a two-way ANOVA, using the streams as factors. The consumption data of both plant species from the laboratory experiment were compared by a two-way ANOVA, using the conditioning time as a
Shredders prefer soft and fungal-conditioned leaves, regardless of species. Leaf toughness from the laboratory experiment was compared by a t-test. The abundance and biomass data were log-transformed (x+1). All analyses were performed on R software (R Development Core Team, 2012).

**RESULTS**

**Leaf traits.** There were differences between the initial physical and chemical traits of both plant species, except for polyphenols and cellulose (Tab. II). *Chusquea tenella* leaves had higher concentrations of nitrogen, magnesium, and potassium, while *N. megapotamica* leaves were harder and had higher lignin, carbon, C:N ratio, and calcium content (Tab. II). In the field experiment, leaf toughness of *C. tenella* was lower ($F_{1,24} = 122.6, p < 0.001$; Fig. 1A). In the laboratory experiment, *N. megapotamica* had lower leaf toughness after 14 days ($t = 1.2, df = 9, p = 0.004$; Fig. 1B). The streams had no effect on the leaf toughness lost ($F_{2,24} = 0.1, p = 0.46$).

<table>
<thead>
<tr>
<th></th>
<th>C. tenella</th>
<th>N. megapotamica</th>
<th>t (df=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf toughness (kgf cm$^{-2}$)</td>
<td>37.2 ± 2.1</td>
<td>49.2 ± 2.8</td>
<td>-19.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Nitrogen</td>
<td>2.2 ± 0.0</td>
<td>1.5 ± 0.0</td>
<td>17.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Carbon</td>
<td>46.5 ± 0.5</td>
<td>57.2 ± 0.2</td>
<td>-9.1</td>
<td>0.006</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>20.4 ± 0.3</td>
<td>38.1 ± 0.5</td>
<td>19.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Lignin</td>
<td>6.9 ± 1.0</td>
<td>29.5 ± 1.9</td>
<td>7.4</td>
<td>0.008</td>
</tr>
<tr>
<td>% Cellulose</td>
<td>7.8 ± 0.8</td>
<td>6.4 ± 0.0</td>
<td>1.7</td>
<td>0.117</td>
</tr>
<tr>
<td>Phenolic (DO g$^{-1}$ DM)</td>
<td>170.3 ± 5.9</td>
<td>167.2 ± 3.7</td>
<td>0.6</td>
<td>0.307</td>
</tr>
<tr>
<td>Calcium (mg g$^{-1}$ DM)</td>
<td>1.1 ± 0.0</td>
<td>3.8 ± 0.1</td>
<td>26.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Magnesium (mg g$^{-1}$ DM)</td>
<td>2.2 ± 0.0</td>
<td>0.2 ± 0.0</td>
<td>243.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Potassium (mg g$^{-1}$ DM)</td>
<td>6.8 ± 0.0</td>
<td>3.3 ± 0.0</td>
<td>382.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Field approach – Phylloicus abundance in streams.** There were variations in the abundance of *Phylloicus* depending on which plant species it was associated ($F_{1,8} = 6.95, p = 0.014$) (Fig. 2A). *Phylloicus* individuals were more abundant when associated with *C. tenella* (70%). In the Horto stream, the total abundance of *Phylloicus* was 58% ($F_{1,8} = 16.0, p = 0.001$). There was no relationship between leaf species and neither one of the streams ($F_{1,8} = 0.5, p = 0.58$). Shredders biomass varied according with the plant species ($F_{1,8} = 5.0, p = 0.02$), but no difference was observed between streams ($F_{1,8} = 3.3, p = 0.09$) (Fig. 2B).

**Laboratory approach – Feeding preference of Phylloicus.** *Phylloicus* had a feeding preference for 14-day conditioned *N. megapotamica* ($F_{1,264} = 67.1, p = 0.001$; Fig. 3). The unconditioned disc leaves of *C. tenella* and *N. megapotamica* were also consumed by shredders.

![Fig. 1. Leaf toughness values (mean ± SE) for Nectandra megapotamica and Chusquea tenella and in (A) during the period of experiment in the field and (B) during the period of experiment in the laboratory (H, Horto stream; D, Dourado stream).](image-url)
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DISCUSSION

Both evaluated plant species differed in their nutritional traits. While C. tenella leaves are more palatable and nutritious, N. megapotamica leaves are harder, more lignified, and have a more resistant cuticle (Zanon et al., 2009). These leaf traits are essential to the fragmentation process in streams; C. tenella will have a faster decomposition rate than N. megapotamica since it is more easily consumed by shredders; N. megapotamica will remain longer in the streams, so it will be later consumed by shredders. As expected, the leaf toughness of both plant species, in the field and in the laboratory, decreased with time. This decreased toughness promotes a consequent reduction in the friction resistance of leaves (Ratnarajah & Bermuta, 2009). Thus, detritus with increased immersion time in the stream become softer and more palatable for shredders (Ligeiro et al., 2010; Biasi et al., 2013). However, N. megapotamica leaves showed a marked decrease in toughness after 14 days of laboratory experiment, which did not happen in the field. This result was unexpected and suggests intense microbial conditioning of N. megapotamica leaves.

Phylloicus individuals were more abundant and had higher biomass when associated with C. tenella leaves throughout the field experiment. Based on the previously estimated leaf traits, C. tenella leaves are more palatable for these shredders. Detritus of C. tenella had a higher amount of nitrogen and a lower C:N ratio, lignin content, and leaf toughness during the field experiment. It has been demonstrated that there is higher shredder density associated with decaying leaves with higher nutritional quality (Rincón & Martinez, 2006; Graça & Cressa, 2010; Garcia et al., 2012; Bruder et al., 2014; König et al., 2014). There is often a negative correlation between fragmentation rates and leaf toughness (Abelho, 2008; Li et al., 2009) and lignin quantity (Wright & Covich, 2005), which are important physical attributes promoting the resistance of leaves (Ratnarajah & Bermuta, 2009). High consumption rates of fast decomposing leaves are also related to a larger shredder size (González & Graça, 2003). Chusquea tenella leaves have features that allow a fast association of shredders, such as low lignin: N and low toughness, which explain the higher Phylloicus abundance and biomass and probably the faster leaf breakdown. Thus, the higher Phylloicus abundance and biomass when associated with C. tenella is related to the leaf traits.

In the laboratory experiment, shredders had a preference for the less palatable species (N. megapotamica). However, they preferred 14-day conditioned leaves, which had a significant toughness reduction. In this case, the preference of Phylloicus for N. megapotamica is related to leaf traits. The decreased leaf toughness along with the increased conditioning time explained this consumption...
pattern. There are reports that the microbial conditioning, especially hyphomycetes, promotes increased palatability of detritus as it influences the breakdown of structural leaf compounds and reduces its toughness (Pearson & Connolly, 2000; Graça et al., 2001; Assmann et al., 2011).

In both field and laboratory experiments, shredders had a preference for more conditioned detritus with reduced toughness, which seem to be determining factors for the Phylloicus choice. These parameters are interrelated, leaf toughness tends to decrease as microbial colonization is established, which was observed in the leaves of both species (Foucreau et al., 2013). Unconditioned leaves of C. tenella were consumed by Phylloicus; while unconditioned leaves of N. megapotamica were used only to case repairing. This is probably related to the fact that N. megapotamica is a more resistant low-quality resource and it was only consumed by Phylloicus when its toughness is reduced and the conditioning time is increased. As for C. tenella, due to its high initial quality, it was consumed even without being conditioned by the microbial community.

Laboratory studies have shown that shredders prefer consuming conditioned leaves (Chung & Suberkropp, 2009) with higher nitrogen content (Rincón & Martínez, 2006; Casotti et al., 2015) and lower toughness (Li et al., 2009). As for the case-building, they prefer leaves with higher toughness and lignin and polyphenols content (Moretti et al., 2009; Cerezer et al., 2016). This corroborates our results; although we did not quantify it, we observed that some Phylloicus individuals used lower quality leaf discs with higher toughness for case repairing, and used leaves of better quality for feeding.

In both field and laboratory experiments, we found evidence that Phylloicus is selective concerning food choice. These organisms select detritus with reduced toughness and conditioned by the microbial community. Leaf traits characteristics of both tree species explained the association of shredders with the detritus, even experiencing a reversal preference between the field (higher association with C. tenella) and laboratory (N. megapotamica was the most consumed species) experiments. In conclusion, our results indicate that leaf traits determine the association, feeding preference, and biomass of Phylloicus. This study points out to the importance of combining laboratory tests with field observations. Our results also support the importance of conservation of riparian vegetation since it provides a diversity of detritus to aquatic communities. We demonstrated a strong relationship between shredders (Phylloicus) and the plant material entering the streams. This relationship demonstrates that alterations in the allochthonous material can affect the food activity of shredders which can affect the secondary production and the food web in low-order streams. Importantly, ecosystem processes that are performed by shredders, such as leaf decomposition, can be also altered.

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