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RESEARCH ARTICLE

Galled leaves as an improved resource for benthic detritivores

Marina Tagliaferro[1] · **Verónica Díaz Villanueva**[2] · **Laura Wolinski**[1] · **Claudia Clementina Boy**[1]

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Abstract

Galled leaves are very frequent in southern beech (*Nothofagus pumilio*) forests. We compared the consumption of leaves (galled and ungalled) by two detritivores and the effect of this consumption on detritivore nutrient content. The amphipod *Hyalella curvispina* and larvae of the caddisfly *Monocosmoecus hyadesi* were fed with two diet treatments for 30 days: (1) ungalled leaves and (2) galled leaves. Fungal biomass, carbon (C), nitrogen (N), and phosphorus (P) content were measured in leaves and detritivores, and C:N:P molar ratios were calculated. We hypothesized that galls produce changes in litter nutrient content, affecting invertebrate consumption and nutrient content. Galled leaves had higher N and ergosterol content, and lower C:N ratios than ungalled leaves. Galled leaf consumption was higher than the ungalled for both detritivores. The two species differed in the content of N and C (caddisfly > amphipod) and P (caddisfly < amphipod). The N:P ratio of the amphipods was similar to the ungalled leaf ratio and increased when fed on galled leaves. In contrast, the N:P ratio of the caddisflies was considerably higher than the ungalled leaves and decreased when fed on galled leaves. Galls alter nutrient content in leaves and increase fungal biomass. Both detritivore species were highly homeostatic with regard to C:N ratio, but caddisflies could overcome the limited P content by increasing consumption. We highlight the impact of galls on aquatic detritivores, which in turn are relevant in forested streams for recycling organic matter and cascading effects through brown detritus-based aquatic food webs.

Keywords Stoichiometry · Cross-ecosystem resources · Macroinvertebrates · Streams · Patagonian Andes

Introduction

In forested areas, trees input much organic matter into streams (Anderson and Sedell 1979; Hall et al. 2000; Evans-White and Halvorson 2017). Thus, alterations to forests can induce changes in the aquatic environments. One natural alteration is produced by pathogen infections in leaves (e.g., galls formations due to egg-laying by aphids) with effects ranging from individual to ecosystem levels (LeRoy et al. 2020). For instance, the development of galls is characterised by an increase in tannins, lignin, and cellulose in leaves (Findlay et al. 1996; Schweitzer et al. 2005; LeRoy et al. 2020), making galled litter more recalcitrant, which in turn can reduce detritivore consumption capacity and litter decomposition (Schweitzer et al. 2005)**.** On the other hand, gall-inducers can promote early leaf abscission, which prevents trees from reabsorbing nutrients from the leaves (Choudhury 1988; Mazía et al. 2012), leading to nutrient-enriched leaf litter.

Leaves that enter aquatic systems rapidly lose soluble compounds (leaching) and are colonised ("conditioned") by bacteria and fungi. Detritivore invertebrates, mainly shredders, then feed on this conditioned litter (Cummins 1973). This process can be directly influenced by gall presence due to leaf structure and nutrient changes (Schweitzer et al. 2005; LeRoy et al. 2020). An increase in litter phosphorus and nitrogen content can accelerate litter decomposition (Wardle et al. 2002) by promoting conditioning, which in turn makes leaves more palatable for detritivores (Cross et al. 2007). However, nutrient content alone cannot explain differences in decomposition rates among species (Longhi et al. 2008). The quantity of recalcitrant molecules such as tannins and lignin can reduce organic matter decomposability

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Marina Tagliaferro
azulmarinita@gmail.com; marina.tagliaferro@conicet.gov.ar

[1] Centro Austral de Investigaciones Científicas (CADIC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Houssay 200, 9410 Ushuaia, Tierra del Fuego, Argentina

[2] Instituto de Investigaciones en Biodiversidad y Medioambiente (INIBIOMA), Quintral 1250, R8400 San Carlos de Bariloche, Río Negro, Argentina

(Hättenschwiler and Jørgensen 2010). Thus, the effect of galls on litter might increase nutrient content but alter litter structural characteristics (i.e., tannin content) making consumption more difficult than that of ungalled litter.

Leaf litter nutrient content can affect detritivore consumption, because consumers maintain their body elemental composition (body stoichiometry) within certain bounds (Sterner and Elser 2002). Nutrients are less concentrated in plant litter than in detritivores (Elser et al. 2000; Frost et al. 2002; Cross et al. 2003). Thus, litter detritivores should differentially adjust the acquisition, incorporation (allocation and storage), and release of chemical elements to achieve a constant elemental composition (Frost et al. 2005). Alternatively, some organisms may change their internal elemental ratios (carbon:nitrogen:phosphorus; C:N:P) to some degree. Changes in litter nutrient content may alleviate nutrient limitation and potentially lead to long-term alterations of community structure in leaf litter-based ecosystems (Cross et al. 2003, 2007; Frainer et al. 2016).

In southern South America, Southeast Australia, and New Zealand, *Nothofagus* (Nothofagaceae) forms extensive forests (McQuillan 1993; Quintero et al. 2014), increasing the relevance of "brown" detritus-based food webs (Zou et al. 2016). The most common and widespread species in Chile and Argentina is *N. pumilio*, a deciduous species known as southern beech (Hildebrand-Vogel et al. 1990). These deciduous forests dominate the inputs of organic matter to the streams and lakes of the region (Albariño et al. 2009). Due to its biogeographic history, highly endemic flora, and climate, a rich gall community (90 morphospecies) has become closely associated with these forests (Vergara and Jerez 2010; Quintero et al. 2014). The most frequent ones for *N. pumilio* identified are: Pteromalidae (Hymenoptera), Cecidomyiidae (Diptera), and Eriophyoidea (Acari) (Quintero et al. 2014). Although the mean gall frequency was reported to be as high as 19% (Quintero et al. 2014), the association of gall-inducers and trees has been primarily focused on the consequences for terrestrial ecosystems.

Due to the vast range of *N. pumilio* along the Patagonian Andes, the high frequency of galls, and its importance in the contribution to basal resources, we selected this species to evaluate leaf litter quality and the possible impacts on aquatic ecosystems. To achieve this, we conducted a 30-day experiment in which two highly abundant detritivore species with different feeding habits (*Monocosmoecus hyadesi* and *Hyalella curvispina*) were fed on galled and ungalled *N. pumilio* leaves. The caddisfly *M. hyadesi* is a shredder (Moorman et al. 2010; Zagarola et al. 2017), whereas the amphipod *H. curvispina* is a collector-gatherer (Velásquez and Miserendino 2003; Zagarola et al. 2017), that can eventually feed as shredder (Saigo et al. 2009). Our hypotheses were: (1) galls produce changes in litter nutrient content and hence in fungal biomass; (2) invertebrate consumption is affected by the presence of galls in litter, but the effect depends on invertebrate feeding habit (shredder or collector-gatherer); and (3) changes in litter nutrient content affect invertebrate nutrient content. We predicted that: (1) galled leaves would have a higher N and P content because of early leaf abscission (Blundell and Peart 2000), which will lead to higher fungal biomass; (2) the shredder feeding galled leaves would have a lower consumption rate (due to higher nutrient content) than the shredder feeding ungalled leaves, whereas the collector-gatherer would be less affected by leaf type by consuming fine particulate organic matter generated from the leaf tissue; and (3) detritivores would change their body stoichiometry depending on food and functional feeding group; we expected a stronger response from the shredder than from the collector-gatherer.

Materials and methods

Sampling

We collected recently fallen leaves of *N. pumilio* in early autumn 2019 from two different forest areas, one with a high percentage of gall infection and another with a reduced proportion of galls (%g galled/g ungalled leaves ± SE = 2.40 ± 0.69% and 0.47 ± 0.01%, respectively). Both forests are close to Ushuaia, Tierra del Fuego (54° 48′ 58.28″ S; 68° 19′ 33.13″ W), at approx. 80 ± 10 m.a.s.l., and are connected through a 5 km-forest continuum separated by a main road. We air-dried and separated three types of leaves: (1) leaves from the infected forest without galls; (2) leaves from the infected forest with galls; and (3) ungalled leaves from the reference forest.

For the experiment, we collected two detritivore species: early juvenile amphipod *Hyalella curvispina* from Tunel stream (54° 49′ 00.25″ S, 68° 10′ 10.89″ W) and mid-instar larvae of the caddisfly *Monocosmoecus hyadesi* from Piloto stream (54° 50′ 08.32″ S, 68° 28′ 29.69″ W). Both streams are oligotrophic (soluble reactive phosphorous = 0.002 ± 0.001 mg/L) that run through *N. pumilio* forest with low gall prevalence (temperature 4.6 ± 0.8 °C, mean ± SE).

Leaf characteristics

To test the effect of galls on leaf litter nutrient content, the amount of carbon, nitrogen, and phosphorus was measured for the four types of leaf structure using $n = 4$ replicates of each: (1) whole ungalled leaves from the reference forest, (2) ungalled leaves from the infected forest, (3) gall tissue, and (4) leaf tissue from galled leaves. Leaves were dried at 60 °C for 72 h, and a subsample was ground to powder with a mortar and pestle. Then C and N were quantified in a CN analyser (Thermo Finnigan Flash EA 1112). To measure P content, another subsample was weighed and combusted at 500 °C for 4 h, and the ashes were dissolved in MiliQ water to measure soluble reactive phosphorus (SRP) through the ascorbate-reduced molybdenum method (APHA 2005). For each leaf tissue and type of leaf, lignin and cellulose content (%) were measured by INTA-Balcarce (Laboratorio de Nutrición y Evaluación de Calidad de Forrajes) following methods explained in Van Soest (1975) and Ankom (2005).

Also, nutrient loss by leaching was compared between galled and ungalled leaves. Leaf litter (1.00 ± 0.01 g) was dried at room temperature and placed in 100 mL of MilliQ water at 4 °C for 48 h to obtain leachates (Anesio et al. 2000) in four replicates per leaf type. The resulting solution was filtered through a pre-combusted GF/F filter (Osmonics). All glassware was previously acid-washed and combusted at 450 °C for 1 h. Inorganic phosphorus ($P–PO_4$), nitrate ($N–NO_3$), and nitrite ($N–NO_2$) concentrations were measured in a Hach DR/2700 spectrophotometer using Hach reagents (Hach Company, CO, USA) as follows: ascorbic acid method for $P–PO_4$, diazotization method for $N–NO_2$ and cadmium-reduced $N\text{-}NO_3$ (APHA 2005). Ammonia ($N–NH_4$) concentration was determined using the phenol–hypochlorite method (APHA 2005).

Fungal biomass of conditioned galled and ungalled leaves was estimated by the ergosterol content in leaves (Bärlocher 2005; Gessner 2005). We used freeze-dried leaves (c. 45 mg) to perform the ergosterol extraction with hot potassium hydroxide and methanol, purified by solid-phase filtration. We quantified the ergosterol using an HPLC (high-performance liquid chromatography, Waters 600E) with a reverse phase C18 column (LUNA C-18, Phenomenex, Torrance, CA, USA).

Experimental design and determinations

A laboratory experiment was conducted to measure the consumption rates of galled leaves and whole ungalled leaves (from an uninfected forest) by two aquatic detritivores and the effect on their nutrient content. Organisms were acclimated to laboratory conditions (5.0 ± 0.3 °C and 12 h:12 h dark:light) and fed with 1-week conditioned ungalled leaves for 10 days. Leaves were conditioned in fine mesh bags (0.5-mm pore size) in a local stream (4.6 ± 0.3 °C) for 7–10 days to allow for microbial colonization and then kept aerated under laboratory conditions for 5–7 days.

After acclimation, organisms were placed into individual glass jars. We used four replicates per treatment, with seven experimental jars each (28 individuals per treatment) to meet the mass requirement for C:N:P analyses, with 2 cm height of habitat (a mix of coarse sand and gravel) and 250 cm^3 of dechlorinated filtered water with artificial aeration. All leaves for the experiment were pre-conditioned together with leaves offered in the acclimation of the consumers. Water was changed every 2 days and invertebrates were fed ad-libitum (previously weighed). Consumption rates were calculated as the difference in leaf dried weight given to organisms and the remaining leaf tissues every 2 days. Initial dried mass of leaves and detritivores was estimated from a similar amount of leaves and ten detritivores of each species. The experiment lasted 30 days.

To measure elemental composition (C, N, and P content) of detritivores, individuals were dried at 60 °C for 72 h. A subsample was ground to a powder with a mortar and pestle and C, N, and P were measured as explained for leaves.

Data analysis

We analysed the data with INFOSTAT (Di Rienzo et al. 2016). Leaf ergosterol content and leachates characteristics in galled and ungalled leaves were compared using a *t* test. Leaf nutrient, lignin, and cellulose content were analysed using an ANOVA; C:N, and C:P were evaluated by comparing the four leaf structures using a Kruskal–Wallis test followed by paired comparisons. The individual consumption rates, nitrogen N and phosphorus P content, and invertebrate C:N, C:P and N:P were compared between galled and ungalled leaves and species using a two-way ANOVA with two fix effects (factor 1: leaf type; factor 2: species). When significant differences were found, a posteriori Tukey test was used to identify differences.

Results

Leaf characteristics

Ungalled leaves from the two forests (reference “control” forest and infected forest) did not differ in N or C content (Table 1). Gall tissue and leaf tissue from galled leaves showed the highest lignin, cellulose, and N content but no differences in P or C content (Table 1). Leaf C:N ratio was significantly different among the leaf tissues, with higher values in ungalled reference leaves (Table 1). On the contrary, C:P and N:P were higher in galled leaves than ungalled reference leaves or gall tissue (Table 1). Leachates of galled leaves had lower $P–PO_4$ concentration than ungalled leaves (Table 2) and did not differ in dissolved inorganic nitrogen. Galled leaves had nearly twice the ergosterol content than ungalled leaves ($F_{1,7} = 10.31$, $p = 0.0183$; mean ± SE = 262 ± 46, 142 ± 27, respectively).

Leaf consumption

Organism survival was higher than 90%, with only a few deaths during the galled and ungalled treatments (1–2

Table 1 Leaves elemental contents (mg/g of tissue) and molar ratios: N (nitrogen), P (phosphorous), C (carbon)

	Mean ± SE				p value
	Ungalled—reference	Ungalled—infected	Gall	Galled leaf	
Cellulose (%)	18.7 ± 0.3 (a)	16.1 ± 0.2 (b)	32.4 ± 0.1 (d)	25.4 ± 0.4 (c)	$p < 0.0001$
Lignin (%)	12.4 ± 0.1 (a)	14.2 ± 0.2 (b)	35.0 ± 0.3 (d)	28.4 ± 0.8 (c)	$p < 0.0001$
N (mg/g)	6.1 ± 0.3 (a)	5.1 ± 0.4 (a)	9.5 ± 1.2 (b)	13.0 ± 1.4 (b)	$p = 0.0003$
P (mg/g)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	$p = 0.5028$
C (mg/g)	440 ± 6	411 ± 5	443 ± 11	467 ± 17	$p = 0.2548$
Median ± IQ					
C:N	84.8 ± 4.8 (a)	95.1 ± 4.0 (a)	54.8 ± 3.0 (ab)	40.3 ± 2.7 (b)	$p = 0.0004$
C:P	1,621 ± 92 (a)	1,511 ± 43 (a)	1,670 ± 92 (a)	2,178 ± 147 (b)	$p = 0.0018$
N:P	19.1 ± 2.4 (a)	15.7 ± 4.4 (a)	28.8 ± 6.4 (b)	54.0 ± 14.6 (c)	$p = 0.0001$

Values are expressed as mean ± standard error (SE) or median ± interquartile (IQ). Ungalled refers to leaves without galls from the uninfected forest (reference) and gall-infected forest (infected). Different letter after indicates statistically different values

Table 2 Chemical characterization of 48 h leachates

	Mean ± SE		Statistic	p value
	Ungalled	Galled		
N-NH_4	8.0 ± 2.0	7.0 ± 2.1	$W_{4,4} = 16.0$	$p = 0.628$
N-NO_2	0.10 ± 0.04	0.5 ± 0.2	$W_{4,4} = 19.5$	$p = 0.087$
N-NO_3	0.8 ± 0.3	1.3 ± 0.6	$W_{4,4} = 25.0$	$p > 0.999$
P-PO_4	960 ± 120	350 ± 60	$W_{4,4} = 10.0$	$p = 0.029$

Ammonia (N–NH_4), nitrite (N–NO_2), nitrate (N–NO_3), and phosphorous (P–PO_4) measured in leaves leachate are expressed as μg nutrient per g of dried leaf

deaths out of 28 individuals for each species), which kept the experiment balanced. Mean leaf consumption relative to invertebrate dried weight (DW) was significantly higher in galled than in ungalled leaves ($F_{1,12} = 15.52$, $p = 0.0020$), and for the amphipods (0.48 ± 0.13 and 1.30 ± 0.12 mg DW/day, fed on ungalled and galled leaves, respectively) than for the caddisflies (0.11 ± 0.03 and 0.16 ± 0.03 mg DW/day, fed on ungalled and galled leaves, respectively) ($F_{1,12} = 95.29$, $p < 0.0001$; Fig. 1a), without significant interaction ($F_{1,12} = 2.8430$, $p = 0.1176$). However, consumption rates per individual were similar within each leaf treatment between species ($F_{1,12} = 0.7074$, $p = 0.4168$ for species; $F_{1,12} = 7.0408$, $p = 0.0210$ for leaf treatments; Fig. 1b), and no interaction occurred ($F_{1,12} = 0.1569$, $p = 0.6990$). Consumption was not reflected in animal growth, which did not differ from initial dried mass (36 ± 3 mg and 33 ± 4 mg vs. 140 ± 10 mg and 136 ± 8 mg for *H curvispina* and *M. hyadesi* selected for ungalled and galled, respectively), in some cases being negative, and in both species with zero values.

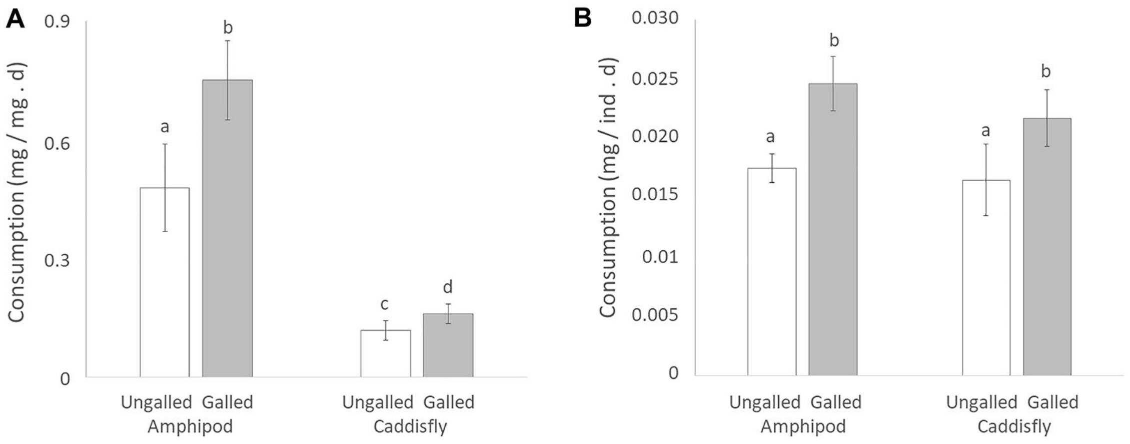

Fig. 1 Consumption related to dry weight (left panel, relative consumption) and to individuals (right panel). Different letters indicate significant differences found by Tukey a posteriori test. Vertical bars represent standard error

Consumer stoichiometry

The two species differed in N and C content (caddisfly > amphipod), and in P content (caddisfly < amphipod), but there were no differences in N and P between feeding treatments (Fig. 2; Table 3). In contrast, C content was higher in animals fed on ungalled than on galled leaves (421 ± 7 and 517 ± 8 mg C/g DW ungalled fed vs. 407 ± 8 and 474 ± 11 mg C/g galled in amphipods and caddisflies, respectively). While C:N ratios did not differ between species nor between treatments (Fig. 3; Table 3), the C:P and N:P ratios differed in organisms according to the diet (Table 3). The highest C:P was found in the caddisflies fed on ungalled leaves and the lowest in the amphipod fed on ungalled leaves (Fig. 3). The N:P was also different between species according to the treatment (Fig. 3; Table 3). The amphipods were balanced with the ungalled leaves (N:P = ~20) and increased in N:P when fed on galled leaves. By contrast, the caddisflies were highly imbalanced when fed on ungalled leaves ($N:P_{consumer} = 104$ vs. $N:P_{leaf} = 17$) and became more balanced when fed on galled leaves (N:P ~ 60) (Fig. 3).

Discussion

We found that (1) galled leaves present a higher N content, lower C:N, and higher C:P, resulting in higher fungal biomass; (2) galled leaves were consumed in greater amounts than ungalled ones by both the shredder and the collector-gatherer species; (3) organisms were more homeostatic for N than for P, and the differences between species in their nutrient requirements led to different responses to the supply of a resource enriched in N.

Galled leaves have higher N content, which is consistent with the early abscission of the leaves (Blundell and Peart 2000). The lack of differences between non-infected leaves from different types of forests showed that differences in elemental composition between galled and ungalled reference leaves might be due to the presence of the galls and not to the forest of origin. Furthermore, whereas galled leaves had the most N content, the gall itself had less than the leaf itself. One possible explanation is that early leaf abscission does not allow the tree to absorb nutrients and larvae in the gall consume part of these nutrients. Consequently, naturally low P content tended to decrease in the galled leaf. However, the gall content was comparable to that of the ungalled leaf, which might be due to absorption of P in the gall tissue. These results are consistent with the concept that galls often act as mobilizing sinks for plant resources (Larson and Whitham 1991; Kmieć et al. 2018) and might result in differential nutrient absorption in *Nothofagus* species (Diehl et al. 2003; Bahamonde et al. 2019). On the other hand, higher P

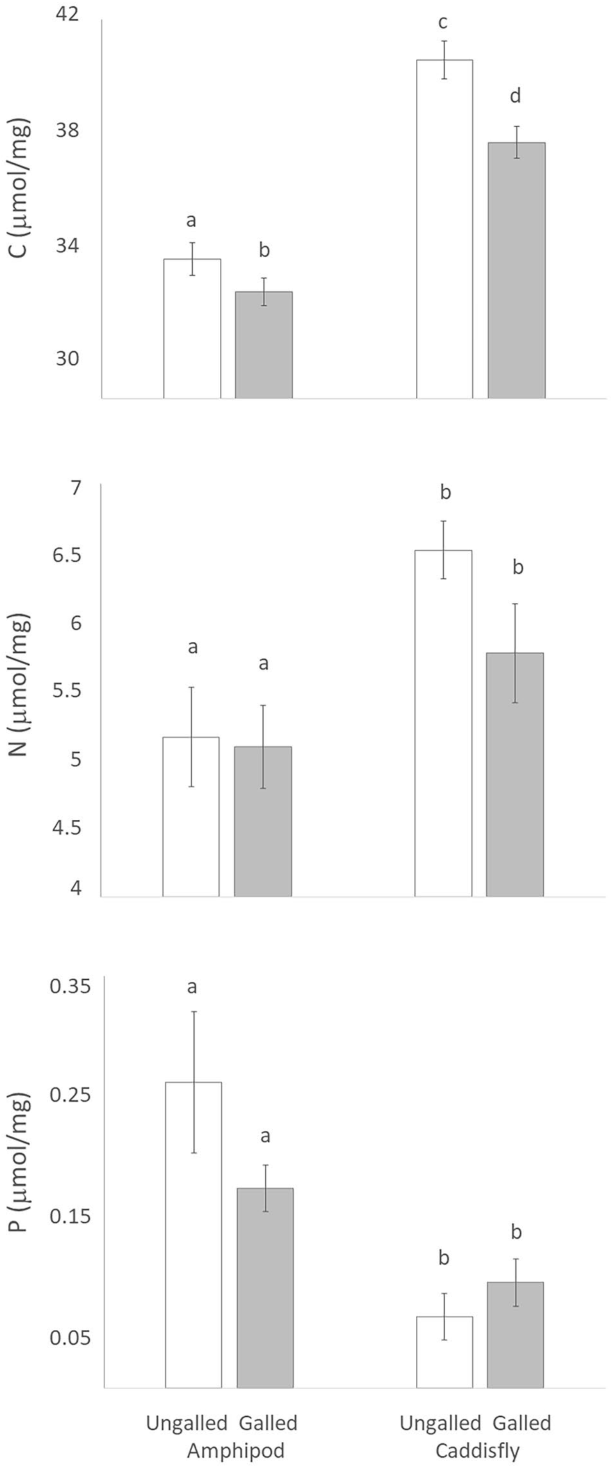

Fig. 2 Nitrogen (N), phosphorus (P), and carbon (C) content for detritivores fed on ungalled and galled leaves. Different letters indicate significant differences found by Tukey a posteriori test. Vertical bars represent standard error

Table 3 Results of the two-way ANOVA for the comparisons of the elemental N, P, and C content and molar ratios for species (*Hyalella curvispina* and *Monocosmoecus hyadesi*) fed on ungalled and galled leaves

	Feeding treatment	Species	Interaction
N	$F_{1,8}=0.89, p=0.3712$	$F_{1,8}=9.06$, $\boldsymbol{p=0.0168}$	$F_{1,8}=0.67, p=0.4389$
P	$F_{1,8}=0.78, p=0.4015$	$F_{1,8}=21.84$, $\boldsymbol{p=0.0016}$	$F_{1,8}=4.04, p=0.0799$
C	$F_{1,8}=9.43$, $\boldsymbol{p=0.0153}$	$F_{1,8}=73.62$, $\boldsymbol{p<0.0001}$	$F_{1,8}=2.30, p=0.1681$
C:N	$F_{1,8}=0.02, p=0.8920$	$F_{1,8}=0.03, p=0.8787$	$F_{1,8}=0.14, p=0.7250$
C:P	$F_{1,8}=139.58$, $\boldsymbol{p<0.0001}$	$F_{1,8}=2{,}416.28$, $\boldsymbol{p<0.0001}$	$F_{1,8}=350.28$, $\boldsymbol{p<0.0001}$
N:P	$F_{1,8}=0.3512, p=0.5698$	$F_{1,8}=446.78$, $\boldsymbol{p<0.0001}$	$F_{1,8}=52.34$, $\boldsymbol{p<0.0001}$

Statistic parameters and p value are separated for treatments and species. Significant effects of individual factors or interactions between species and treatment are indicated in bold letters

released in leachates of ungalled leaves indicates that this element is in a highly soluble form and is rapidly lost when the leaf enters the water. Other studies with *N. pumilio* leaf litter from lower latitudes (North Patagonian Andes, 41° S) found that they are a substantial source of soluble P to the streams (Navarro et al. 2019; Villanueva 2019).

Leaf differences in ergosterol content support our first prediction that the early abscission of galled leaves would indirectly increase fungal biomass. Although we could not identify if the ergosterol was in gall or leaf tissue, we assume that the fungi could be in the leaf tissue due to the higher N content. Fungi play an important role in changing the leaf's palatability (Graça et al. 1993; Baldy et al. 1995), and consequently altering detritivore feeding rates and growth (Albariño et al. 2008; Evans-White and Halvorson 2017). Leaf colonization by fungi can change detritus elemental composition (Danger 2020) and its palatability (Graça et al. 1993; Baldy et al. 1995), which might be the cause of the differences we found in the response of invertebrates (higher consumption).

Galling-induced changes to leaf chemistry also had consequences for detritivore consumption and elemental composition. Differences in consumption rates expressed as body biomass, which were higher for the amphipod, may be attributed to differences in mobility, *H. curvispina* being highly mobile and *M. hyadesi* being less mobile, benthic and case-building. Changes in motility and metabolism have been associated with different factors affecting invertebrates, such as environmental conditions and predatory pressure (Glazier et al. 2011, 2020). In addition, smaller non-metabolous invertebrates may exhibit higher N and P demands compared to larger holometabolous larvae, with greater C demands for lipid storage (Halvorson 2019). Demi et al. (2018) suggested that animal growth and secondary production regulation might be more sensitive to P than N content. In our experiment, the caddisfly larvae responded differently from the amphipod to detritus nutrient changes, with a stronger response to detrital P than to N.

On the other hand, in contrast to our prediction, both detritivores consumed galled leaves more quickly than ungalled leaves. Although organisms are expected to increase consumption rates to compensate for low food quality (e.g., Flores et al. 2014), there is also evidence showing increased consumption of nutrient-rich resources that might usually be limited for organisms (e.g., Danger et al. 2013; Evans-White and Halvorson 2017; Halvorson et al. 2017). In our experiment, the organisms consumed more of the resource with higher N and ergosterol content (galled leaves) despite higher cellulose and lignin content.

The degree of homeostasis of a given species may depend on its feeding habits (Cross et al. 2003; Persson et al. 2010; Kendrick and Benstead 2013). Since caddisflies are shredders feeding on leaf fragments and the amphipods are collector-gatherers, we expected the response to changes in leaf quality to be higher in the caddisflies. Amphipods "scrape" biofilm on leaves and ingest lower C:N and C:P resources compared to the leaf litter, while caddisflies "chew" leaf litter in bulk and thus feed less selectively on leaf litter microbes. The absence of C:N differences and higher C:P ratios in *M. hyadesi* than in *H. curvispina* confirm previous comparisons of shredders and collector-gatherers (Cross et al. 2003).

Our experiment revealed that *H. curvispina* and *M. hyadesi* were not homeostatic in relation to C:P and N:P. Both species were affected by the N:P content in leaves, but they differed in their responses. While *H. curvispina*. was more balanced with the ungalled leaves and less affected by food diet, *M. hyadesi* was highly imbalanced with the ungalled leaves and strongly affected (Fig. 3). The low natural values of N:P in *H. curvispina* in comparison to *M. hyadesi* is consistent with Elser et al. (2000), who proposed that small-fast growing species are expected to have lower N:P ratios than large-slow growing species. Notably, high N:P values of *M. hyadesi* when feeding on ungalled leaves were reduced when feeding on galled leaves, the resource with higher N:P. Although this result is counterintuitive, other studies (Halvorson 2018) found that the response to detrital nutrient improvement was driven by greater consumption rates but lower assimilation efficiencies on higher nutrient detritus. The caddisflies might be experiencing internal regulation and differential elimination of elements (N), which decreased N:P.

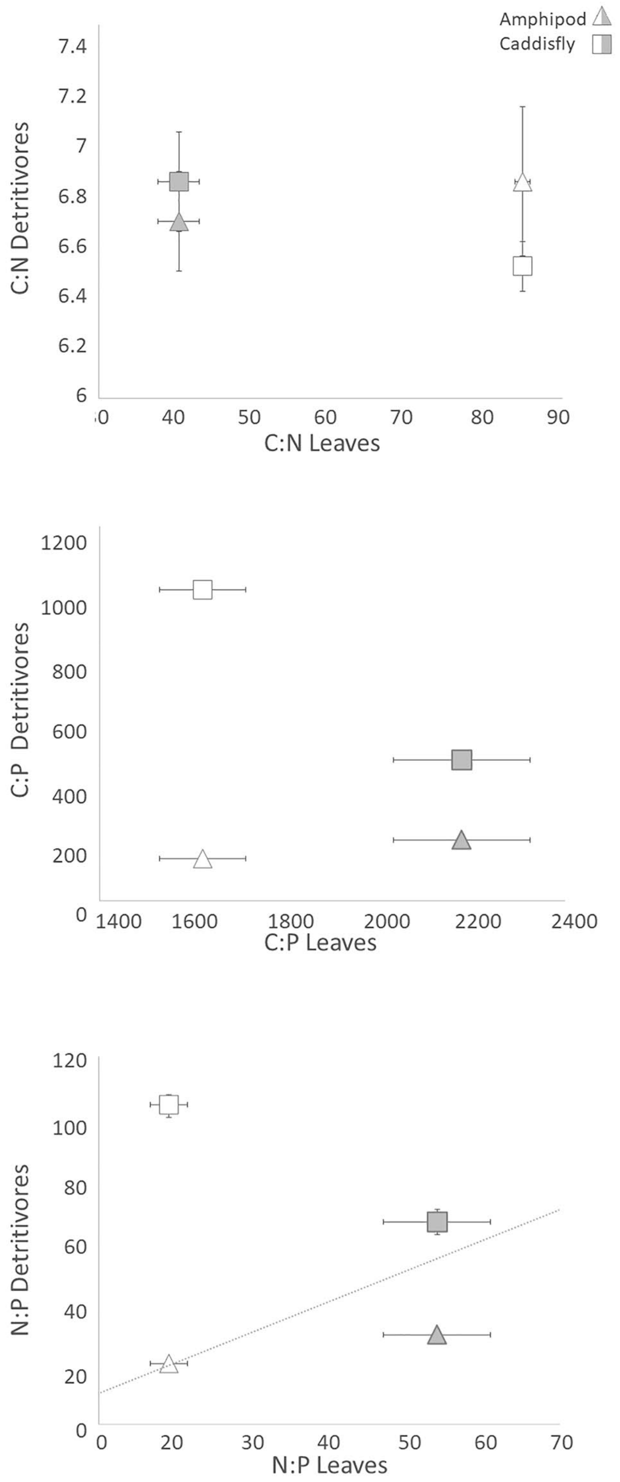

Fig. 3 Detritivore C:N, C:P and N:P ratios as a function of leaf C:N, C:P and N:P ratios. Squares represent caddisflies; triangles, amphipods. Grey colour indicates galled fed and white ungalled fed organisms. Bars represent standard error for both variables

Halvorson et al. (2015) reported that another caddisfly species that fed on oak litter exhibited flexible body C:P during growth and in response to altered C:P diet, low P incorporation efficiencies, and P excretion at peak growth. High N:P ratios are common in slow developing taxa (Beck et al. 2021). In particular, although there are no studies on the life history of *M. hyadesi*, the large body size in comparison with other insect larvae would indicate that this is a slow growing species. This would also explain the lack of measurable growth after 4 weeks of the experiment. Evans-White et al. (2005) and Beck et al. (2021) also reported that amphipods usually have lower N:P values than caddisflies. Beck et al. (2021) also found a relation between N:P of the entire invertebrate community and N:P in stream water. Although we did not measure N:P in the stream community, the high values of N:P in the streams could also explain the high N:P values found in both detritivores. Therefore, caddisflies might be able to reduce their C:P and N:P ratios by consuming higher fungal biomass and nutrients in galled leaves.

In the present study, we evidenced the stoichiometric changes in benthic organisms as a consequence of the variation in the quality of the resource subsidies. These changes in the quality of resources are due to the action of gall forming herbivores on leaves from headwaters stream riparian areas. These resource subsidies from the terrestrial environment to streams greatly contribute to detritivores and aquatic food webs at least during part of their life cycle (Polis et al. 1997; Richardson and Sato 2015). Thus, changes in the quantity or quality of terrestrial inputs can affect the energy, carbon and nutrient budgets (Polis et al. 1997; Collins et al. 2016) with consequences in population size and production and ecosystem functioning (Stoker et al. 2017; Halvorson et al. 2018, 2019). In turn, the close association and reciprocal dependence between streams and the riparian forest will generate a cross-resource subsidy between both systems (Klemmer et al. 2020; Tolkkinen et al. 2020), with allochthonous material due to the presence of galls affecting detritivores' food quality and emerging invertebrates subsiding the terrestrial environment. Although this study focused on the two most abundant detritivores, other detritivores might change their consumption and/or their stoichiometry in a different way. Consequently, changes transferred to terrestrial habitats from benthic caddisflies might reflect only one part of the complete scenario. Thus, although a broader scale experiment is needed, the chemical changes in leaves due to galling might also generate consequences in benthic community structure (i.e., fungi and invertebrates) and ecosystem function (i.e., leaf litter decomposition rates) (LeRoy et al. 2020).

In conclusion, through this study, we highlight the influence of galls on two aquatic detritivores and evidence the importance of cross-ecosystem resource subsidies in headwater streams. Considering that galls´ influence reach up to 44% of leaves depending on trees species (Quintero et al.

2014; Altamirano et al. 2016) and that this proportion may increase in the future (e.g., due to temperature increase or deforestation; Garibaldi et al. 2011; Mazía et al. 2012; Quintero et al. 2014), it is considered relevant to evaluate how this resource might generate cascading effects on "brown" detritus-based aquatic food webs.

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References

Albariño RJ, Villanueva VD, Canhoto C (2008) The effect of sunlight on leaf litter quality reduces growth of the shredder *Klapopteryx kuscheli*. Freshw Biol 53:1881–1889

Albariño RJ, Villanueva VD, Buria L (2009) Leaf litter dynamics in a forested small Andean catchment, Northern Patagonia, Argentina. In: Oyarzún C, Verhoest N, Boeckx P, Godoy R (eds) Ecological advances on Chilean temperate rainforests. Academic Press, Ghent, pp 183–211

Altamirano A, Valladares G, Kuzmanich N, Salvo A (2016) Galling insects in a fragmented forest: incidence of habitat loss, edge effects and plant availability. J Insect Conserv 20:119–127

Anderson NH, Sedell JR (1979) Detritus processing by macroinvertebrates in stream ecosystems. Annu Rev Entomol 24:351–377

Anesio AM, Theil-Nielsen J, Granéli W (2000) Bacterial growth on photochemically transformed leachates from aquatic and terrestrial primary producers. Microb Ecol 40:200–208

Ankom (2005) Method for determining acid detergent lignin in beakers. ANKOM Technology

APHA (2005) Standard methods for the examination of water and wastewater, 21st edn. American Public Health Association

Bahamonde HA, Fernández V, Gyenge J, Mattenet F, Peri PL (2019) Essential nutrient and trace element foliar resorption of two co-existing *Nothofagus* species grown under different environmental conditions in southern Patagonia. Front Plant Sci 10:1–13

Baldy V, Gessner MO, Chauvet E (1995) Bacteria, fungi and the breakdown of leaf-litter in a large river. Oikos 74:93–102

Bärlocher F (2005) Leaf mass loss estimated by litter bag technique. In: Graça MAS, Bärlocher F, Gessner M (eds) Methods to study litter decomposition: a practical guide. Springer, pp 37–42

Beck M, Mondy CP, Danger M, Billoir E, Usseglio-Polatera P (2021) Extending the growth rate hypothesis to species development: can stoichiometric traits help to explain the composition of macroinvertebrate communities? Oikos 130:879–892

Blundell AG, Peart DR (2000) High abscission rates of damaged expanding leaves: field evidence from seedlings of a Bornean rain forest tree. Am J Bot 87:1693–1698

Choudhury D (1988) Herbivore induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. Oikos 51:389–393

Collins SM, Kohler TJ, Thomas SA, Fetzer WW, Flecker AS (2016) The importance of terrestrial subsidies in stream food webs varies along a stream size gradient. Oikos 125:674–685

Cross WF, Benstead P, Rosemond AD, Wallace JB (2003) Consumer-resource stoichiometry in detritus-based streams. Ecol Lett 6:721–732

Cross WF, Wallace JB, Rosemond AD (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. Ecology 88:2563–2575

Cummins KW (1973) Trophic relations of aquatic insects. Annu Rev Entomol 18:183–206

Danger M (2020) Ecological stoichiometry in detritus-based ecosystems with a special focus on forested headwater streams. Limnetica 39:335–352

Danger M, Arce Funck J, Devin S, Heberle J, Felten V (2013) Phosphorus content in detritus controls life-history traits of a detritivore. Funct Ecol 27:807–815

Demi LM, Benstead JP, Rosemond AD, Maerz JC (2018) Litter P content drives consumer production in detritus-based streams spanning an experimental N:P gradient. Ecology 99:347–359

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2016) InfoStat versión 2016. Universidad Nacional de Córdoba, Córdoba

Diehl P, Mazzarino MJ, Funes F, Fontenla S, Gobbi M, Ferrari J (2003) Nutrient conservation strategies in native Andean-Patagonian forests. J Veg Sci 14:63–70

Elser JJ, Sterner RW, Gorokhova E, Fagan WF, Markow TA, Cotner JB, Harrison JF, Hobbie SE, Odell GM, Weider LW (2000) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550

Evans-White MA, Halvorson HM (2017) Comparing the ecological stoichiometry in green and brown food webs—a review and meta-analysis of freshwater food webs. Front Microbiol 8:1–14

Evans-White MA, Stelzer RS, Lamberti GA (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. Freshw Biol 50(11):1786–1799

Findlay S, Carreiro M, Krischik V, Jones C (1996) Effects of damage to living plants on leaf litter quality. Ecol Appl 6:269–275

Flores L, Larrañaga A, Elosegi A (2014) Compensatory feeding of a stream detritivore alleviates the effects of poor food quality when enough food is supplied. Freshw Sci 33:134–141

Frainer A, Jabiol J, Gessner MO, Bruder A, Chauvet E, McKie BG (2016) Stoichiometric imbalances between detritus and detritivores are related to shifts in ecosystem functioning. Oikos 125:861–871

Frost PC, Stelzer RS, Lamberti GA, Elser JJ (2002) Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C:N:P ratios in lentic and lotic habitats. J N Am Benthol Soc 21:515–528

Frost PC, Cross WF, Benstead JP (2005) Ecological stoichiometry in freshwater benthic ecosystems: an introduction. Freshw Biol 50:1781–1785

Garibaldi LA, Kitzberger T, Ruggiero A (2011) Latitudinal decrease in folivory within *Nothofagus pumilio* forests: dual effect of climate on insect density and leaf traits? Glob Ecol Biogeogr 20:609–619

Gessner MO (2005) Ergosterol as a measure of fungal biomass. In: Graça MAS, Bärlocher F, Gessner MO (eds) Methods to study litter decomposition: a practical guide. Springer, Dordrecht, pp 189–195

Glazier D, Butler E, Lombardi S, Deptola T, Reese J, Satterthwaite E (2011) Ecological effects on metabolic scaling: amphipod responses to fish predators in freshwater springs. Ecol Monogr 81:599–618

Glazier DS, Gring JP, Holsopple JR, Gjoni V (2020) Temperature effects on metabolic scaling of a keystone freshwater crustacean depend on fish-predation regime. J Exp Biol 223:1–14

Graça MAS, Maltby L, Calow P (1993) Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*: feeding strategies. Oecologia 93:139–144

Hall RO, Wallace JB, Eggert SL (2000) Organic matter flow in stream food webs with reduced detrital resource base. Ecology 81:3445–3463

Halvorson HM, Sperfeld E, Evans-White MA (2017) Quantity and quality limit detritivore growth: mechanisms revealed by ecological stoichiometry and co-limitation theory. Ecology 98:2995–3002

Halvorson HM, Fuller CL, Entrekin SA, Scott JT, Evans-White MA (2018) Detrital nutrient content and leaf species differentially affect growth and nutritional regulation of detritivores. Oikos 127:1471–1481

Halvorson HM, Fuller CL, Entrekin SA, Scott JT, Evans-White MA (2019) Interspecific homeostatic regulation and growth across aquatic invertebrate detritivores: a test of ecological stoichiometry theory. Oecologia 190:229–242

Hättenschwiler S, Jørgensen HB (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. J Ecol 98:754–763

Hildebrand-Vogel R, Godoy R, Vogel A (1990) Subantarctic-Andean *Nothofagus pumilio* forests. Vegetation 89:55–68

Kendrick MR, Benstead JP (2013) Temperature and nutrient availability interact to mediate growth and body stoichiometry in a detritivorous stream insect. Freshw Biol 53:1820–1830

Klemmer AJ, Galatowitsch ML (1929) McIntosh AR (2020) Cross-ecosystem bottlenecks alter reciprocal subsidies within meta-ecosystems: bottlenecks to reciprocal subsidies. Proc R Soc B Biol Sci 287:20200550

Kmieć K, Złotek U, Jakubczyk A, Karaś M (2018) Biochemical alterations in *Ulmus pumila* L. leaves induced by galling aphid *Tetraneura ulmi* L. Acta Scientiarum Polonorum Hortorum Cultus 17:175–183

Larson KC, Whitham TG (1991) Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. Oecologia 88:15–21

LeRoy CJ, Fischer D, Schweitzer JA, Bailey JK (2020) Aphid gall interactions with forest tree genotypes influence leaf litter decomposition in streams. Forests 11:1–14

Longhi D, Bartoli M, Viaroli P (2008) Decomposition of four macrophytes in wetland sediments: organic matter and nutrient decay and associated benthic processes. Aquat Bot 89:303–310

Mazía N, Chaneton EJ, Dellacanonica C, Dipaolo L, Kitzberger T (2012) Seasonal patterns of herbivory, leaf traits and productivity consumption in dry and wet Patagonian forests. Ecol Entomol 37:193–203

McQuillan PB (1993) Nothofagus (Fagaceae) and its invertebrate fauna—an overview and preliminary synthesis. Biol J Lin Soc 49:317–354

Moorman MC, Anderson CB, Gutiérrez AG, Charlin R, Rozzi R (2010) Aquatic invertebrate diversity and watershed conservation. Alberto D´Agostini National Park

Navarro MB, Villanueva VD, Modenutti BE (2019) High phosphorus content in leachates of the austral beech *Nothofagus pumilio* stimulates bacterioplankton C-consumption. Freshw Sci 38:435–447

Persson J, Fink P, Goto A, Hood JM, Jonas J, Kato S (2010) To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119:741–751

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28:289–316

Quintero C, Garibaldi LA, Grez A, Polidori C, Nieves-Aldrey JL (2014) Galls of temperate forest of Southern South America: Argentina and Chile. In: Fernandes GW, Santos JC (eds) Neotropical insect galls. Springer, Dordrecht, pp 429–462

Richardson JS, Sato T (2015) Resource subsidy flows across freshwater-terrestrial boundaries and influence on processes linking adjacent ecosystems. Ecohydrology 8:406–415

Saigo M, Marchese M, Montalto L (2009) Hábitos Alimentarios de *Hyalella Curvispina* Shoemaker, 1942 (Amphipoda: Gammaridea) en Ambientes Leníticos de la Llanura Aluvial del Río Paraná Medio. Natura Neotropicalis 1:43–59

Schweitzer JA, Bailey JK, Hart SC, Wimp GM, Chapman SK, Whitham TG (2005) The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. Oikos 110:133–145

Van Soest PJ (1975) Physio-chemical aspects of fiber digestion. In: McDonald IW, Warner ACI (eds) Digestion and metabolism in the ruminant. The University of New England Publication Unit, Armidale

Sterner RW, Elser JJ (2002) Ecological stoichiometry: biology of elements from molecules to the biosphere. Princeton University Press, Princeton

Stoker D, Falkner AJ, Murray KM, Lang AK, Barnum TR, Hepinstall-Cymerman J, Conroy MJ, Cooper RJ, Pringle CM (2017) Decomposition of terrestrial resource subsidies in headwater streams: does consumer diversity matter? Ecosphere 8:e01868

Tolkkinen MJ, Heino J, Ahonen SHK, Lehosmaa K, Mykrä H (2020) Streams and riparian forests depend on each other: a review with a special focus on microbes. For Ecol Manag 462:117962

Velásquez SM, Miserendino ML (2003) Analisis de la materia organica alocctona y organizacion funcional de macroinvertebrados en relacion con el tipo de habitat en rios de montaña de Patagonia. Ecologia Austral 13:67–82

Vergara O, Jerez V (2010) Insectos e infestaciones asociadas al follaje de *Nothofagus antarctica* (Forst) Oerst (Nothofagaceae) en la cuenca del río Baker, Región de aysén, Chile. Gayana 74:83–93

Villanueva VD (2019) Dissolved organic matter as p source for biofilms in two contrasting low-order streams. Fundam Appl Limnol 193:131–142

Wardle DA, Bonner KI, Barker GM (2002) Linkages between plant litter decomposition, litter quality, and vegetation responses to herbivores. Funct Ecol 16:585–595

Zagarola JPA, Martínez Pastur G, López ME, Anderson CB (2017) Assessing the effects of urbanization on streams in Tierra del Fuego. Ecol Austral 27:45–54

Zou K, Thébault E, Lacroix G, Barot S (2016) Interactions between the green and brown food web determine ecosystem functioning. Funct Ecol 30:1454–1465