Biological colonization and leaf decomposition in a subtropical stream

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ABSTRACT. In small streams, aquatic community obtains energy mainly from leaves of the riparian vegetation. Processing these leaves involves physical, chemical and biological factors that may differ among leaf species. We assessed the effects of litter quality on leaf decomposition and colonization by aquatic invertebrates in a subtropical stream. Leaves from two native, highly represented tree species common in subtropical riparian areas, *Ficus luschnathiana* and *Casearia sylvestris,* were incubated in coarse mesh bags in a stream. Bags were sampled weekly during an incubation of 28 days. The decomposition rates were higher in leaves of *F. luschnathiana* than in those of *C. sylvestris*. In the first seven days of the experiment, leaves of *F. luschnathiana* lost ~60% of the initial mass, while *Casearia sylvestris* lost around 30%. The leaves of *C. sylvestris* were initially more palatable than the leaves of *F. luschnathiana,* perhaps due to their lower lignin and cellulose content, and lignin to N ratio (Lignin:N). These structural compounds enhance leaf toughness thereby protecting them from herbivores and detritivores. However, leaves of *F. luschnathiana* were comparatively softer, which apparently facilitated fungal colonization and conditioning. Thus, leaves of *F. luschnathiana* attained high shredders density, which was reflected in a higher leaf mass loss. Our results suggest that litter decomposition was sensitive to fungal biomass and leaf toughness. We observed that microbial conditioning, rather than the initial chemical quality of the leaf litter, decreased leaf toughness thereby stimulating further colonization by shredders and enhancing the decomposition process. The fungal community has a key-role in the degradation process within aquatic environments, breaking down even low-quality litter (i.e., *F. luschnathiana*) and promoting changes in the composition and structure of the invertebrate community.

[Keywords: shredders, Chironomidae, leaf-toughness, fungal biomass]

RESUMEN. La colonización y descomposición biológica de la hojarasca en un arroyo subtropical. En arroyos de bajo orden, la comunidad acuática obtiene energía principalmente del aporte de la hojarasca que proviene de la vegetación de la ribera. El procesamiento de las hojas implica medios físicos, químicos y factores biológicos que pueden diferir entre especies vegetales. En esta investigación se evaluó el efecto de la calidad del sustrato sobre el proceso de descomposición y sobre la colonización por invertebrados acuáticos asociados, en un arroyo subtropical. Las hojas de dos especies de árboles nativos, *Ficus luschnathiana* y *Casearia sylvestris,* se incubaron en bolsas de malla gruesa en un arroyo durante 28 días, colectándose muestras semanalmente. Las tasas de descomposición fueron mayores en *F. luschnathiana* que en *C. sylvestris*. En los primeros siete días del experimento, *F. luschnathiana* perdió ~60% de la masa inicial, mientras que *C. sylvestris* perdió ~30%. Las hojas de *C. sylvestris* fueron inicialmente más palatables que las de *F. luschnathiana*, tal vez debido a su menor contenido de lignina y celulosa, y a su más baja relación Lignina:N. Estos compuestos estructurales le confieren rigidez a las hojas, las protegen de los herbívoros y las hacen más resistentes a los detritívoros. Sin embargo, la menor dureza de la hoja de *F. luschnathiana* facilitó la colonización fúngica, lo cual se reflejó, a su vez, en una mayor colonización por invertebrados trituradores y, en general, una mayor pérdida de masa. Los resultados sugieren que la descomposición de la hojarasca fue sensible a la biomasa fúngica y a la dureza de las hojas. Se observó que la colonización microbiana y la disminución de la dureza de las hojas tienen un efecto más fuerte sobre la colonización por invertebrados trituradores y sobre el proceso de descomposición que la calidad química inicial de la hojarasca. La comunidad fúngica es clave en los procesos de degradación en el medio acuático, ya que actúa en la descomposición de la hojarasca, aun en aquella de baja calidad (i.e., *F. luschnathiana*), promoviendo cambios también en la composición y en la estructura de la comunidad de invertebrados colonizadores.

[Palabras clave: trituración, Chironomidae, dureza foliar, biomasa fúngica]

Introduction

In low-order streams in which light limitation restricts primary production, the allochthonous material largely support biotic communities and ecological processes (Vanotte et al. 1980; Webster and Benfield 1986). Once the plant debris enter into streams, organic matter and nutrients leach out while leaves travel downstream or remain on the bank, providing resources for detritivores and decomposers (Pettit et al. 2012). These processes overall reduce the size of the particulate organic matter, releasing nutrients that support aquatic communities (Tanaka et al. 2006). The decomposition dynamic may be affected by abiotic and biotic factors such as climate (Bruder et al. 2013), water flow (Fonseca et al. 2013), quantity, quality and diversity of leaf litter (Ferreira et al. 2012; Pettit et al. 2012; Biasi et al. 2013; König et al. 2014), the abundance of shredders (Patrick 2013), among other factors operating on spatial and temporal scales (Pérez-Harguindeguy et al. 2000; Bastian et al. 2007).

The physical and chemical attributes of leaves vary substantially among species, affecting its utilization by microorganisms and shredders (Wright and Covich 2005; Bastian et al. 2007). Further, certain litter types attract different invertebrate assemblages (Schädler and Brandl 2005). Leaf litter with high nitrogen content, low secondary compounds (i.e., polyphenols and tannins), low levels of structural compounds (i.e., lignin and cellulose) and softer is considered of high quality (Graça et al. 2001). Such features determine also that the leaf litter be more attractive to the organisms, thus enhancing its decomposition rate. Low-quality leaf litter is therefore comparatively more stable and long-lasting, being consumed afterwards (Haapala et al. 2001; Graça and Canhoto 2006). In addition, understanding the effects of leaf chemical composition on the decomposition process is important due to the fact that it may reflect on nutrient cycling (Leroy and Marks 2006).

Leaves colonized by microorganisms become more palatable to leaf-shredding macroinvertebrates, which are within the main functional feeding group in low-order streams (Vannote et al. 1980). Leaf conditioning during breakdown reduce leaf toughness (Graça and Zimmer 2005), resulting in maximum leachate of nitrogen (N), protein and phosphorus (P) (Bärlocher 1985; Suberkropp 1992; Graça et al. 1993) and enhancing palatability to shredders.

Feeding and abundance of shredders have been correlated with increased leaf litter conditioning and with fungal biomass on leaf litter (Chergui and Pattee 1993; Graça et al. 1993; Robinson et al. 1998), raising the question of what the proximate cues are for shredder's food preference (Aßmann et al. 2011). Shredders participate actively in nutrient cycling in freshwater; they convert coarse particulate organic matter into to fine particles (CPOM and FPOM, respectively) absorbing partly carbon and other nutrients in the process (Boyero et al. 2014). In the subtropics the community of shredders is composed by a few taxa (e.g., Cogo and Santos 2013; König et al. 2014).

In this investigation we evaluated the effect of litter quality in terms of lignin cellulose and nitrogen contents on the decomposition and invertebrate colonization of two leaf litter types, *Ficus luschnathiana* and *Casearia sylvestris*, in a subtropical stream. We expect leaves bearing higher concentrations of lignin and cellulose that provide rigidity will protect and/or reduce the action of detritivores. On the contrary, high nitrogen concentration in leaf litter that stimulates the activity of fungi will turn the leaf litter more attractive to invertebrates increasing its decomposition rate. Through experimental incubations of leaf litter two hypothesis were tested: i) leaf decay rates are determined by the chemical composition of the detritus and thus, soft and highly nutritious leaf litter will decompose more rapidly than hard leaves, and ii) shredders will select for more nutritious leaf litter.

Materials and Methods

Study area

The decomposition experiment was conducted in a first-order stream located at 29°39′04″ S - 53°31′51″ W in the Planalto and Central Depression areas in a subtropical forest region of southern Brazil (Castillero 1984). The climate of the region is subtropical with annual mean temperature around 19 °C and mean annual rainfall ~1500 mm. Tree diversity in this subtropical forest is relatively high (Cogo and Santos 2013). The riparian vegetation is composed by several tree species, mainly *Cabralea canjerana* (Vell.) Mart.(deciduous),*Campomanesia xanthocarpa* O. Berg. (semi-deciduous), *Casearia sylvestris* Sw. (semi-deciduous), *Cupania vernalis* Cambess. (semi-deciduous)*, Enterolobium*

contortisiliquum (Vell.) Morong (deciduous), *Erythrina falcata* Benth. (deciduous), *Ficus luschnathiana* (Miq.) Miq. (deciduous), *Inga alata* Benoist (evergreen), *Ocotea puberula* (Rich.) Nees (evergreen), *Parapiptadenia rigida* (Benth.) Brenan (evergreen), *Phytolacca dioica* L. (deciduous), *Rollinia emarginata* Schltdl. (deciduous) and *Trema micrantha* (L.) Blume (evergreen).

The stream generally flows year-round, but may eventually dry during extremely hot and dry summers (especially in January and February). It has well preserved riparian vegetation on both sides and low human intervention. During the study period mean temperature was moderate 19.8±0.4 °C, the water showed mean oxygen concentration of 7.9±0.8 mg/L, a mean conductivity of 22.37±5.96 mS/cm and pH averaging 6.1±0.1. The average width of the stream was 73±20 cm, the mean depth was 43±8 cm and the current velocity averaged 0.3±0.0 m/s.

Leaf sampling and chemical analyses

The species used in the experiment were *Ficus luschnathiana* and *Casearia sylvestris.* These species were chosen because they are locally abundant and all of them are native to the biome known as "Mata Atlantica". Senescent leaves of both species were collected along the stream banks in October 2011 (spring) and dried in an oven at 30 °C until constant weight.

The initial chemical quality of the litter was characterized before starting the experiment through measurement of leaf toughness, amount of nitrogen, lignin and cellulose levels. A penetrometer (Nolen and Pearson 1993) was used to determine leaf toughness (expressed as kgf/cm², kilogram-force per square centimeter) by puncturing five points of ten leaves from each species. The total nitrogen content was determined applying the Kjeldahl method (Flindt and Lillebo 2005). Lignin and cellulose percentages were determined through the fiber acid-detergent method following Van Soest (1993).

Leaf decomposition

A total of 32 mesh bags (15x20 cm, 10 mm pore size) containing 4.0±0.1 g of leaf litter which were prepared to perform the experimental incubations in the stream. The experiment was set up with two treatments, one consisting on leaves of *C. sylvestris* and the

second one with leaves of *F. luschnathiana*. The litter bags were placed in the stream at sites with moderate water flow.

Four litter bags from each treatment were randomly removed from the stream during the incubation period at day 7, 14, 21, and 28. Replicates were packed in plastic bags and transported refrigerated to the laboratory. The samples were rinsed in a 0.25 mm mesh sieve to remove sediment and the associated invertebrates. The invertebrates were identified to the lowest possible taxonomic level, using identification guides (Fernández and Domínguez 2001; Merritt et al. 2008; Mugnai et al. 2010). In order to identify the Chironomidae, semi-permanent slides were prepared using Hoyer's solution and the larvae were identified to genus level using the identification guides by Trivinho-Strixino (2011) and Epler (2001). The taxa were classified in trophic groups according to Cummins et al. (2005), Wantzen and Wagner (2006) and Merritt et al. (2008).

Fungal biomass

Fungal biomass was estimated indirectly through the concentration of ergosterol, a natural constituent of most fungal cells that has been used previously as a proxy of the aquatic fungal biomass (Gessner 2005). For this purpose, 10 leaf discs (12 mm) were obtained from each replicate which were maintained at -20 °C until the extraction protocol was applied. The ergosterol was extracted applying the Microwave Assisted Extraction method (Young 1995) and was quantified by high-performance liquid chromatography (HPLC) by injecting 10 µL during 8 minutes. The mobile phase was 100% methanol and the detection wavelength was 282 nm (Gessner 2005). The HPLC system was equipped with a LiChroCART 250-4 LiChrospher 100 RP-18 (5 mm) column (Merck, Darmstadt, Germany) Results were expressed as µg fungal biomass/g dry mass. The leaves were dried in an air-circulation oven at 60 °C during 72 h to determine the remaining dried mass.

Data analysis

The resulting values of the remaining dry mass (W_t) were fitted to a negative exponential model following Webster and Benfield (1986):

$$W_t = W_0 . e^{-kt} \quad (1)$$

where W_0=initial dry mass, k=breakdown constant and t=incubation time.

For each experimental treatment, the total density of organisms and the density of shredders were calculated after Gotelli and Colwell (2001). The results obtained of remaining dry mass, leaf toughness and macroinvertebrate colonization were compared by means of a two-way analysis of variance (Two-way ANOVA), using leaf litter type (2 levels) and incubation time (3 levels) as main factors. The lignin, cellulose and nitrogen content of the leaf litter types were compared by means of t-tests. Invertebrate density values were log-transformed (log[x+1]). All statistical analyses were conducted using soft-ware R (R Development Core Team 2012).

Results

Chemical Quality and Leaf Breakdown

The leaves of *C. sylvestris* and *F. luschnathiana* differed in their lignin, cellulose and N contents, and also showed contrasting Lignin: N ratios and leaf toughness (Table 1). The *C. sylvestris* showed lower lignin and cellulose content, lower Lignin:N ratio and higher N level as compared to *F. luschnathiana*. The latter showed lower initial leaf toughness (Table 1). The leaf toughness decreased with the incubation time and resulted higher in *C. sylvestris* throughout the experiment (Species: GL=1, *F*=3.94, *P*=0.0001; Time: GL=4, *F*=8.10, *P*=0.0001; Species×Time: GL=4, *F*=1.11, *P*=0.0986) (Figure 1). The remaining dry mass differed between the species and among incubation times (*P*=0.0005; Litter type: GL=1, *F*=28.52, *P*=0.0001; Time: GL=4, *F*=113.41, *P*=0.0001; Litter type×Time: GL=4, *F*=4.09).

In the first seven days of the experiment, *F. luschnathiana* lost approximately 60% of the initial mass, while *C. sylvestris* lost around

Table 1. Percentage of lignin, cellulose, nitrogen, lignin to nitrogen ratio and leaf toughness in leaves of *Ficus luschnathiana* and *Casearia sylvestris*.

Tabla 1. Porcentaje de la lignina, celulosa, nitrógeno y dureza de las hojas de *Ficus luschnathiana* y *Casearia sylvestris*.

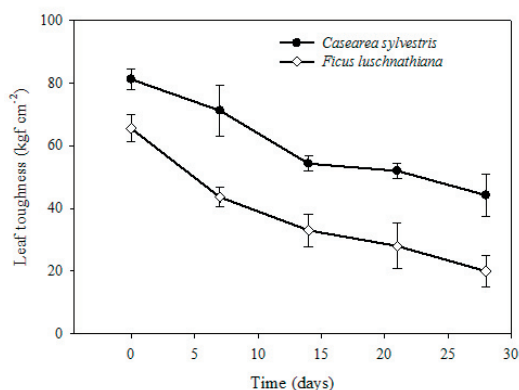|	*Ficus luschnathiana*	*Casearia sylvestris*	*P*
Lignin (%)	20.38±2.81	10.11±0.84	<0.001
Cellulose (%)	14.27±1.38	8.63±0.87	<0.001
Nitrogen (%)	2.12±0.32	3.28±0.39	0.010
Lignin:N	9.61±1.98	3.08±0.75	0.002
Leaf toughness (kgf/cm²)	65.6±4.3	81.2±3.2	<0.001

Figure 1. Changes in the toughness (kgf/cm²) of *Casearea sylvestris* and *Ficus luschnathiana* leaf litter during the decomposition experiment, at day 7, 14, 21 and 28 of incubation in a subtropical stream.

Figura 1. Cambios en la dureza de la hojarasca (kgf/cm²) de *Casearea sylvestris* y *Ficus luschnathiana* durante el experimento de descomposición, a los 7, 14, 21 y 28 días de incubación en un arroyo subtropical.

Figure 2. Percentage of remaining leaf litter mass of *Casearea sylvestris* and *Ficus luschnathiana* during the decomposition experiment, at day 7, 14, 21 and 28 of the incubation time in a subtropical stream.

Figura 2. Porcentaje de peso remanente de hojarasca de *Casearea sylvestris* y *Ficus luschnathiana* durante el experimento de descomposición, a los 7, 14, 21 y 28 días de incubación en un arroyo subtropical.
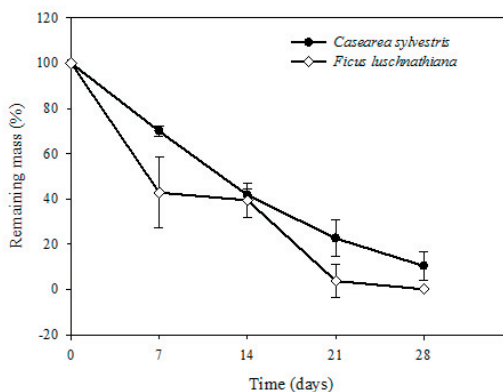
30% (Figure 2). *F. luschnathiana* showed higher breakdown rates than *C. sylvestris* (k=0.238±0.022 1/d and k=0.090±0.91 1/d, respectively).

Biological colonization

The total density of macroinvertebrates varied significantly between leaf litter types (Table 2) and it was dependent on the incubation time. The highest macroinvertebrate density was found in *F. luschnathiana* at day 14 of the experimental incubation. The density of shredders differed between leaf species and also among incubation times (Table 2).

Gathering-collectors were the most numerous group (44%), followed by filtering-collectors
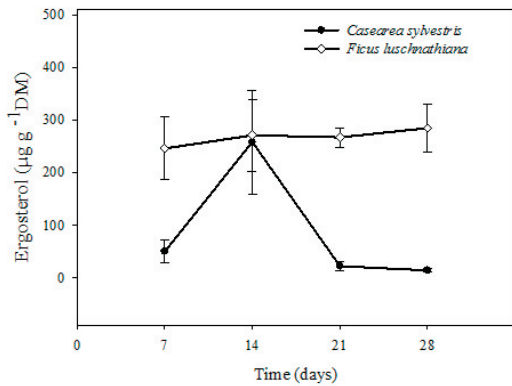
Figure 3. Changes in the concentration of ergosterol (µg/g DM) in leaf litter of *Casearea sylvestris* and *Ficus luschnathiana* in the decomposition experiment, at day 7, 14, 21 and 28 of the incubation time in a subtropical stream.

Figura 3. Concentración de ergosterol (µg/g DM) en la hojarasca de *Casearea sylvestris* y *Ficus luschnathiana* durante el experimento de descomposición, a los 7, 14, 21 y 28 días de incubación en un arroyo subtropical.

Table 2. Results of the Two way ANOVA applied to study the effect of litter type, incubation time and the interaction between these variables on invertebrate and shredders densities (individuals/g dry mass) in experiment of decomposition. d.f.=degrees of freedom.

Tabla 2. Resultados del ANOVA de dos vías aplicado para estudiar el effecto de la especie de hojarasca, el tempo de incubación y su interacción sobre la abundancia de invertebrados y trituradores (individuos/g de materia seca) en el experimento de descomposición. d.f.=grados de libertad.

ANOVA two-way ($P<0.05$)			
	F	P	d.f.
Invertebrate density			
Leaf litter type	7.248	<0.001	1
Time	1.864	0.048	3
Species×Time	1.090	0.022	2
Shredders density			
Leaf litter type	4.083	0.001	1
Time	3.711	0.033	3
Species×Time	1.456	0.075	2

(25%), scrappers (12.4%), shredders (9.6%) and predators (9%). The shredders group were represented by Grypopterigidae, Tipulidae, Calamoceratidae (*Phylloicus*), Hyallelidae (*Hyallela*) and the chironomids *Endotribelos, Stenochironomus, Cricotopus* and *Rheocricotopus*. The inclusion chironomids increased ~ 7.5% the abundance of shredders. *F. luschnathiana* had the highest abundance of shredders (73%), especially after 14 days of incubation (Table 3). The leaves of *F. luschnathiana* showed higher concentrations of ergosterol during the experiment as compared to *C. sylvestris* (Figure 3) and both leaf types showed maximum concentration of this compound at day 14 of the incubation.

Table 3. Total abundance of the macroinvertebrate taxa colonizing experimental enclosures with leaf litter from *Ficus luschnathiana* and *Casearea sylvestris* at different incubation times (7, 14, 21 and 28 days) in subtropical stream.

Tabla 3. Abundancia total de los taxones de macroinvertebrados que colonizan las clausuras con hojas de *Ficus luschnathiana* y *Casearea sylvestris* en las incubaciones experimentales (días 7, 14, 21 y 28) en un arroyo subtropical.

	Ficus luschnathiana				*Casearea sylvestris*			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Decapoda								
Aeglidae								
Aegla	0	3	8	7	3	0	0	7
Amphipoda								
Hyalella	0	9	13	25	2	1	1	9
Colembolla								
Isotomidae	0	0	0	0	1	0	0	0
Coleoptera								
Elmidae	20	27	32	3	9	16	9	12
Psephenidae	0	5	0	0	0	0	4	3
Diptera								
Chironomidae								
Ablabesmyia	2	0	0	0	0	0	0	0
Chironomus	2	0	1	1	0	0	0	0
Corynoneura	469	243	31	131	55	145	41	200
Cricotopus	12	20	15	9	15	13	8	5
Endotribelos	16	181	16	15	5	8	1	16
Paratendipes	3	0	0	0	0	0	0	0
Parametriocnemus	104	35	28	32	55	61	60	90
Pentaneura	21	2	22	12	5	7	8	11
Polypedilum	19	67	16	14	7	40	27	9

Table 3: Continuation
Tabla 3: Continuación

	Ficus luschnathiana				*Casearea sylvestris*			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
Rheocricotopus	5	0	1	4	0	0	1	0
Rheotanytarsus	8	9	3	0	1	5	12	3
Stenochironomus	8	10	5	3	0	2	0	0
Tanytarsus	2	0	0	0	1	4	0	0
Thienemanniella	284	52	199	105	62	65	44	65
Dixidae	20	15	28	1	3	3	6	3
Dytisidae	0	0	0	1	0	0	0	0
Empididae	11	4	14	0	3	5	5	2
Psychodidae	2	3	5	0	2	2	5	1
Simulidae	200	219	108	37	69	77	49	47
Tipulidae	0	0	5	0	1	1	1	0
Ephemeroptera								
Baetidae	13	32	26	83	4	11	11	40
Caenidae	30	33	59	50	6	25	11	48
Lepthobhleliidae	0	0	0	1	0	1	0	0
Hemiptera								
Belostomatidae	0	0	0	1	0	0	0	0
Odonata								
Calopterigidae	0	0	0	1	0	1	1	1
Ceratopogonidae	0	3	0	0	0	0	1	0
Libelulidae	0	0	0	1	0	0	0	0
Plecoptera								
Grypopterigidae	10	12	10	16	4	2	2	5
Trichoptera								
Calamoceratidae								
Phylloicus	5	7	14	1	0	5	1	5
Glossosomatidae	0	0	0	0	0	1	0	0
Helicopsychidae	0	0	0	0	0	0	0	1
Hydrobiosidae	10	12	4	1	1	4	0	5
Hydrophilidae	0	1	9	1	3	4	11	2
Hydropsychidae	69	239	295	25	87	109	22	41
Leptoceridae	9	4	20	6	4	11	5	16
Mollusca								
Gastropoda	158	87	160	84	32	37	82	53

DISCUSSION

Chemical Quality and Leaf Breakdown

C. sylvestris and *F. luschnathiana* present different values as food resource in terms of their chemical quality. Based on Lignin: N ratios, lignin and cellulose concentrations, *C. sylvestris* leaf litter can be considered as a higher quality food resource compared to the leaf litter of *F. luschnathiana,* although the leaves of the latter are softer.

The breakdown rates of *F. luschnathiana* and *C. sylvestris* were classified as rapid based on Petersen and Cumming (1974), who proposed that decomposition rates can be rapid (k>0.01), intermediate (0.005< k<0.001) or slow (k<0.005), based on the remaining dry mass percentage. Decomposition processes in streams may relate also with temperature and prevailing hydrological conditions, such as the water velocity and the slopes of the banks (Hepp et al. 2008; König et al. 2014). The decomposition rates recorded in the present analysis are higher than those reported in other studies performed in subtropical streams (Biasi et al. 2013; Cogo and Santos 2013; König et al. 2014). Compared to these studies in which temperature was moderate (17.6 °C; 13.5 °C; 14.2 °C, respectively), the temperature during our experimental incubations was on average 4.7 °C higher (19.8 °C). Temperature seasonality can be an important factor determining breakdown

rates. The higher temperatures characteristic of the warmer season probably enhanced the activity of the microbial community associated to the leaves thus promoting faster breakdown rates (e.g., Pascoal and Cássio 2004; Ferreira et al. 2006).

F. luschnathiana leaf litter showed the largest mass loss. The leaching recorded in the first days of incubation was apparently the main factor explaining the rapid decomposition. During this process, many hydrosoluble compounds, such as proteins, amino acids, carbohydrates, and lipids are leached, accelerating the loss of leaf mass. The water velocity in the stream (~0.3 m/s) may have contributed to the leaching observed in two species. According to Fonseca et al. (2013), a faster current increases the mass loss during leaching, favoring the transport of fine particulate and dissolved organic matter, and even enhancing the loss of refractory material.

The decomposition rates were not related to the initial quality of the litter. We did not observe an increase in the decomposition rate in litter of *C. sylvestris* that showed a high nutritional quality. However, we observed a high mass loss at low leaf toughness. Recent studies in tropical streams have shown that leaf toughness rather than N and P concentrations controls litter breakdown rates (Ardón et al. 2009; Li et al. 2009). This leaf trait is related with rigid leaves protected from herbivory, and consequently also from detritivores. The leaf toughness can be related negatively with fragmentation and leaf decomposition (Foucreau et al. 2013). Some studies have also reported no clear relationship between litter initial chemical quality and decomposition (Moore and Fairweather 2006; Swan and Palmer 2004; Lecerf et al. 2007; Abelho 2009; Schindler and Gessner 2009). Different species may have particular chemical composition; the leaves may be changed by the immersion in the stream and the leaching, which overall alter their chemical quality (Pettit et al. 2012).

Biological colonization

We observed a high density of shredders colonizing *F. luschnathiana* leaf litter. This species, considered "a priori" as a low quality litter, showed higher mass loss and higher fungal biomass than *C. sylvestris*. Although of nutritionally low quality in terms of nitrogen, *F. luschnathiana* showed the highest fungal biomass during all the experiments, which

likely produced labile detritus enhancing its palatability and favoring consumption by detritivores (Graça and Cressa 2010). The breakdown of herbivore-inhibiting compounds by aquatic fungi may favor assimilation of elements by invertebrates (Graça et al. 2001). Although leaf litter of *F. luschnathiana* can be considered of lower quality compared to *C. sylvestris*, the former has lower leaf toughness. This leaf trait has been considered as an important factor affecting leaf breakdown, since it is related with the resistance to physical abrasion and fragmentation (Ratnarajah and Bermuta 2009; Fonseca et al. 2013). A decrease in leaf toughness has been associated with higher densities of shredders (Foucreau et al. 2013). As the fungal community establishes the resistance of leaves decreases, thereby reducing leaf toughness (Aßmann et al. 2011). Thus, litter conditioning and leaf toughness reduction may be recall to explain the higher rates of decomposition found in leaf litter of *F. luschnathiana*.

Chironomidae was the most numerous group during the entire decomposition process, comprising alone 7.5% of the shredders. The genus *Endotribelos* increased shredders density, likely contributing to enhance leaf decomposition, as has been shown in other related studies (Janke and Trivinho-Strixino 2007; Chará-Serna et al. 2012; Leite-Rossi and Trivinho-Strixino 2012). Similarly, chironomids have been recorded as important components of the invertebrate communities attaining high abundance and richness. These insects play an important role in nutrient recycling (Oertli 1993; Grubbs et al. 1995; Callisto et al. 2007; Hepp et al. 2008) since many of them are generalists and colonize different types of detritus, regardless of its quality and intervening in its breakdown (Moretti et al. 2007; Landeiro et al. 2008; Li et al. 2009; Biasi et al. 2013). In certain cases, chironomids may participate actively in shredding and eroding the leaves, utilizing this detritus as a food resource (Rosemond et al. 1998). However, since chironomids have a wide range of food habits at the genus and species level, their role in leaf fragmentation may be underestimated. The results presented here highlight the importance of chironomids in the fragmentation of detritus. As chironomid shredders are present at high densities in association with detritus, they should be considered co-participants of the decomposition process in tropical aquatic systems.

The dominance of collector-gatherers and collector-filterers may relate to litter conditioning by fungi and shredders that release fine particulate matter and dissolved organic matter, facilitating colonization by these groups. Collector-gatherers and collector-filterers, are not able to directly participate in the rapid breakdown of leaves. They use leaf litter as substratum and leaf fragments as a food resource. Dudgeon and Wu (1999) suggested that collector-filtering organisms, mainly Simuliidae, use leaves as a substratum, which may explain their occurrence in large numbers in our experimental litter bags.

The shredders, represented mainly by *Hyalella*, Grypopterigidae, *Phylloicus*, *Endotribelos*, *Polypedilum* and *Cricotopus* were more numerous in enclosures with leaf litter of *F. luschnathiana*. The density of shredders was determined by the litter species and degradation time. Several studies have recorded a low occurrence of shredders in tropical streams (Wantzen and Wagner 2006; Gonçalves et al. 2007; Moretti et al. 2007), pointing to the microbial community as the primary contributor to the decomposition of leaves. Although chironomids can produce much of the fragmentation as observed here, most studies have underestimated their potential role in the decomposition process. Furthermore, *Aegla*, a macroconsumer that can attain high densities, could also contribute to the fragmentation of coarse organic-matter (Cogo and Santos 2013).

The incubation time of detritus is important for invertebrate colonization as has been also observed by Abelho (2001), Leroy and Marks (2006) and Ligeiro et al. (2010). Many studies evaluating invertebrate colonization of decomposing debris have observed a steady increase in density from the beginning towards the end of the experiment (Dudgeon 1982; Webster and Befield 1986; Benstead 1996; Gonçalves et al. 2012). Apparently, the leaf fragmentation process and the release of nutrients create an appropriate substratum for the development of the insects that use detritus in their diet and over time increase its quality, through microbial conditioning. The changes in the community during incubation time may relate to chemical and microbial conditioning, which together induce changes in the detritus making it more attractive to macroinvertebrates (Abelho 2001; Graça et al. 2001). This result suggests that shredders are able to discriminate advanced stages of leaf decomposition, which is consistent with the observation of Ligeiro et al. (2010) on leaf colonization by shredders. It may be possible that the ergosterol content of tough leaves increases while toughness decreases during the conditioning process. Our results also suggest that *F. luschnathiana* is a resource that becomes palatable depending on its residence time in the stream, during which leaf toughness is reduced by chemical and biological conditioning.

Our results suggest that leaf litter decomposition was driven by fungal biomass and leaf toughness. Contrary to the initial hypothesis, we observed that the microbial colonization and the decrease of leaf toughness rather than the initial chemical quality, determine the colonization by shredders and the decomposition process. Conditioning by the fungal community is particularly relevant to the overall decomposition process, since it breaks down low-quality litter, as in the case of *F. luschnathiana* leaf litter. On the whole, this study indicates that leaf physical traits such as toughness may be more important than leaf chemical traits, thereby driving decomposition rates.

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