Seasonal leaf mass loss estimated by litter bag technique in two contrasting stretches of a tropical headstream

Perda sazonal de massa foliar estimada por sacos com litter em dois trechos contrastantes de um riacho tropical

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Abstract: Aim: The aim of this work was to assess seasonal leaf mass loss of two leaf species, one herbaceous (*Pycreus decumbens*) and other arboreal (*Cabralea canjerana*), in a forested and a deforested area of a tropical stream, during the dry and wet seasons (June/04 and November/04); **Methods:** Leaf bags containing 5.00 ± 0.05 g of fresh and pre-conditioned leaves were installed in the stream bottom and removed after 3 and 13 days of incubation; **Results:** Our results showed differences in the leaf mass loss related to seasonal variation and to leaf quality. The dry mass remaining was small for the conditioned leaves than for unconditioned ones (32 and 69% respectively) and for the wet than for the dry season (42 and 59% respectively). These differences could be caused by leaf quality, related to the initial chemical composition and texture of the leaves, and by differences on physical environmental characteristics, like the current velocity acting directly upon the physical abrasion of the leaves; **Conclusion:** In general, the leaf mass loss was fast when compared with data of a wide variety of plant species found in the literature, mainly in temperate streams. However, these works were studied in streams and with plants of intrinsic characteristics, demanding caution in possible generalizations concerning the leaf mass loss.

Keywords: decomposition, leaf breakdown, litter, tropical stream.

Resumo: Objetivos: Analisar a perda de massa foliar em duas espécies de folhas, uma herbácea (*Pycreus decumbens*) e outra arbórea (*Cabralea canjerana*), em uma área com mata de galeria e outra sem mata de um riacho tropical, durante as estações seca e chuvosa (Junho/04 e Novembro/04); **Métodos:** Pacotes contendo 5,00 ± 0,05 g de folhas frescas e pré-condicionadas foram instaladas no leito do riacho em cada área e estação e removidas após 3 e 13 dias; **Resultados:** Nossos resultados mostraram diferenças na perda de massa foliar quando analisadas a estação e tipo de folha. O material seco remanescente foi menor para as folhas pré-condicionadas do que não condicionadas (32 e 69% respectivamente) e para a estação chuvosa do que para a estação seca (42 e 59% respectivamente). Estas diferenças podem ter sido causadas pela qualidade da folha, relacionada com sua composição química inicial e textura, e por diferenças nas características físicas do ambiente, como a velocidade da correnteza atuando diretamente sobre a abrasão física das folhas; **Conclusões:** No geral, a perda de massa foliar foi rápida quando comparado com dados de uma grande variedade de espécies de plantas encontrados na literatura, principalmente em riachos temperados. Porém, estes trabalhos foram estudados em riachos e plantas com características intrínsecas, necessitando cautela com possíveis generalizações sobre perda de massa foliar.

Palavras-chave: decomposição, perda de massa foliar, folhiço, riacho tropical.

1. Introduction

Headwater streams are particularly influenced by the riparian vegetation which reduces autotrophic production by shading and supplies energy in the form of vegetal matter of allochthonous origin (Abelho, 2001; Graça, 2001; Graça and Canhoto, 2006). Leaf litter is the main component of the allochthonous matter in streams, and its decomposition has received considerable attention (Gessner et al., 1999; Abelho, 2001; Graça, 2001). The leaf litter breakdown is traditionally analyzed as a continuous process with three overlap stages: leaching, conditioning and fragmentation (Boulton and Boon, 1991; Gessner et al., 1999; Abelho, 2001; Graça, 2001).

The time of breakdown is attributed to several factors such as high activity of shredders (Kobayashi and Kagaya, 2005; Ferreira et al., 2006; Tanaka et al., 2006) or microbes (Gessner, 2001; Mathuriau and Chauvet, 2002;

Dobson et al., 2004; Abelho et al., 2005; Gonçalves et al., 2006b; Moretti et al., 2007), and variation in flow regime of streams (Díez et al., 2002; Abelho and Graça, 2006; Gonçalves et al., 2006a). But, as pointed by other authors (Boulton and Boon, 1991; Gessner et al., 1999; Gonçalves et al., 2007), there are a number of other interdependent factors, such as leaf characteristics, climate, latitude, and, consequently, distinct physical and biological characteristics, which can determine different breakdown rates that can interfere in the time of breakdown.

The leaching of soluble compounds is generally fast and may account for a substantial decrease in initial litter mass (Gessner et al., 1999; Abelho, 2001; Graça, 2001). When leaves enter the streams, they are rapidly colonized by microorganisms (Graça, 2001) thus initiating the leaf conditioning process. In this process the microbial assemblages are important not only by enhance breakdown directly by macerating leaves, metabolizing leaf tissue, and incorporating into secondary production, but also indirectly increasing the palatability of detritus to invertebrate shredders (Abelho, 2001; Gonçalves et al., 2006a). Shredders have been shown to feed preferentially on conditioned leaves (Graça, 2001). By feeding on leaves, shredders incorporate some nutrients in secondary production, accelerate leaf fragmentation, and produce abundant FPOM, which are ecologically important for populations of collectors inhabiting lower reaches of streams (Graça, 2001; Graça and Canhoto, 2006).

The abundant literature on leaf litter breakdown in temperate streams contrasts with the scarce information available for tropical streams (Abelho, 2001; Mathuriau and Chauvet, 2002; Graça and Canhoto, 2006). Recent literature based on studies in streams of the Southeastern Brazil found distinct leaf breakdown rates when compared with studies developed in temperate climate (Gonçalves et al., 2006a, 2006b, 2007; Callisto et al., 2007; Moretti et al., 2007). But, the authors were consistent about the low proportion of invertebrates capable of using the leaf litter as energy source and, however, reinforcing the importance of the action of microbes on leaf decomposition.

In a stream located in the southeast of Brazil we intended to evaluate the leaf mass loss through a manipulative experiment in which the variables analyzed were: i) leaf characteristics, by using green leaves of two common riparian species, an invader herbaceous plant (*Pycreus decumbens*) and a native arboreal plant (*Cabralea canjerana*), ii) the previous conditioning, by using leaves previously incubated and fresh ones, iii) intrinsic spatial characteristics, by conducting the experiment in a forested and a deforested stretch of the same stream, and iv) seasonal differences, by investigating the leaf mass loss in a wet and a dry period. We hypothesized that possible differences in the leaf mass loss could be related to intrinsic leaf characteristics and to environmental and climate factors (spatial and seasonal differences) that could accelerate the process of leaf decomposition.

2. Material and Methods

2.1. Study site

The study was carried out in the Ribeirão da Quinta stream (23° 06' 47" S and 48° 29' 46" W), located in the municipality of Itatinga, State of São Paulo, southeastern Brazil, at an elevation of 743 m a.s.l. This is a third order stream (sensu Strahler classification, Stanford, 1996), located on a cattle ranch, with the riparian vegetation partially preserved.

The study was conducted in two consecutive stretches of this stream: 1) the "forested area" shaded by a well-preserved gallery forest, which extended 30 m from each margin, and 2) the "deforested area" located about 300 m downstream and with marginal vegetation composed only by herbaceous vegetation. In spite of this short distance between areas, the intrinsic characteristics observed for each area, as temperature, conductivity, current velocity, light incidence (Table 1), and also the higher amount of aquatic macrophytes in the open area, probably were enough to determine different habitat conditions for leaf decomposition and macroinvertebrates colonization.

The marginal vegetation found in the forested area was composed mainly by *Nectandra* sp. (Lauraceae), *Cabralea canjerana* (Vell.) Mart. (Meliaceae), *Acacia* sp., *Inga* sp., *Piptadenia gonoacantha* (Mart.) J.F. Macbr. (Mimosaceae), *Mollinedia* sp. (Monimiaceae), *Coutarea hexandra* (Jacq.) K. Schum. (Rubiaceae), *Esenbeckia febrifuga* (A. St.-Hil.)

Table 1. Abiotic parameters determined during the experimental study conducted in two areas of the Ribeirão da Quinta stream during the dry (June-July/04) and wet (November-December/04) seasons (mean of 4 measures ± standard deviation).

Parameters	Dry season		Wet season	
	Deforested	Forested	Deforested	Forested
Temperature (°C)	17.5 ± 1.2	16.9 ± 1.5	22.0 ± 0.3	21.7 ± 0.3
pH	8.0 ± 0.07	7.9 ± 0.17	8.0 ± 0.09	7.9 ± 0.07
Conductivity (µS.cm^{-1})	96 ± 11	96 ± 11	80 ± 10	80 ± 10
Current velocity (m.s^{-1})	0.29 ± 0.01	0.12 ± 0.002	0.29 ± 0.02	0.12 ± 0.02
Discharge (m^3.s^{-1})	0.17 ± 0.02	0.18 ± 0.01	0.19 ± 0.02	0.20 ± 0.03
Light incidence (Lux)	32244 ± 27669	685 ± 198	66000 ± 7211	2213 ± 1377

A. Juss. Ex Mart. (Rutaceae), *Smilax* sp. (Liliaceae) and *Urera baccifera* (L.) Gaudich. Ex Wedd. (Urticaceae). The marginal vegetation found in the deforested area was composed mainly by *Pycreus decumbens* T. Koyama (Cyperaceae), *Hedychium coronarium* J. Konig (Zingiberaceae) and *Panicum* sp. (Gramineae).

2.2. Experimental setup

Two types of freshly leaves were used in the experiment, *Cabralea canjerana*, a common tree in the gallery forest area, and *Pycreus decumbens*, an herbaceous plant common in the deforested area. The amount of 5.00 ± 0.05 g of freshly collected leaves was enclosed into a litter bag (20 × 15 cm, with 10 mm mesh opening). Forty (twenty of each plant) of these bags were individually enclosed in another nylon bag (25 × 20 cm, with 0.25 mm mesh opening) to prevent invertebrates colonization and placed in the central area of the stream bottom for 14 days of conditioning (colonization by microorganisms). After this conditioning period, the external nylon bag (0.25 mm mesh openings) was carefully removed from those litter bags, without taken them out of the stream. At the same time a same amount of new litter bags filled with freshly leaves (twenty unconditioned of each plant) was installed. This was considered the day 0 of the experiment, conducted with forty conditioned samples and forty unconditioned samples.

Twenty replicates of each plant species, ten conditioned and ten unconditioned, were randomly removed after 3 and 13 days of experiment. Each sample was placed into a plastic bag and transported to the laboratory in an icebox. The bags were then rinsed with distilled water and each leaf was carefully washed to remove the invertebrates. The leaf material was dried at 70 °C for 48h and then weighed.

The manipulative experiment was performed during the dry (June-July/04) and wet (November-December/04) seasons. The same experimental design was repeated in both areas and seasons. The water characteristics measured during the experimental period were: conductivity (OAKTON-TDSTestr3™), pH and temperature (Horiba model D-14), current velocity and discharge (Leopoldo and Souza, 1979). The light incidence was measured close to the surface of the water and near the experimental area (PANLUX Electronic - GOSSEN).

The chemical composition of the leaves was analyzed at the Department of Animal Nutrition of the State University of São Paulo - UNESP (Faculdade de Ciências Agronômicas, Botucatu, Brazil).

2.3. Leaf mass loss and data analysis

The initial leaf dry mass was obtained by linear regression of data from forty leaf samples of each plant species, during the dry season for *C. canjerana* (R^2 = 0.9939) and *P. decumbens* (R^2 = 0.9943) and during the wet season for *C. canjerana* (R^2 = 0.9934) and *P. decumbens* (R^2 = 0.9909). The percentage of leaf dry mass remaining was computed by the difference between initial and final dry mass, multiplied by 100.

The differences between slopes (comparison of leaf mass loss over time) were tested with an analysis of covariance (ANCOVA), followed by Tukey test (Zar, 1999). The percentage of leaf dry mass remaining and the environmental parameters were analyzed by the Principal Component Analysis (PCA) to determine the relative importance of the environmental parameters on leaf decomposition (Statsoft, 1996).

3. Results

Seasonal differences were observed for temperature, discharge and light incidence data, with high values measured during the wet season; spatial differences were more characteristic for current velocity and light incidence (Table 1). In the deforested area the current velocity was twice higher than in the forested area. The light incidence was higher in the deforested than in the forested area, forty-eight times during the dry season and thirty times during the wet season.

The analysis of leaf mass loss (Figure 1) showed a pronounced difference between unconditioned and conditioned leaves for both plant species, with more dry mass loss for the conditioned leaves (32% for unconditioned leaves and 69% for conditioned leaves). The analysis of the differences between slopes confirmed this difference between treatments for both seasons and the Tuckey test showed a significant difference for conditioned leaves of *P. decumbens* incubated in the forested area at both seasons when compared to the other treatments (Table 2). Besides, the dry mass remaining on the last experimental day (Figure 1) was smaller in the wet than in the dry season (42 and 59% respectively).

The results of the chemical analysis of fresh and conditioned leaves showed higher concentration of acid detergent fibers and cellulose in *C. canjerana* and higher concentration of those two components plus lignin in the conditioned leaves of both plant species (Table 3).

The two axes of the Principal Component analysis, used to verify the importance of the environmental parameters in the leaf decomposition, explained a high percentage of data variance (Table 4). The variables that showed significant positive contribution to the total variance of the first axis were water current, light incidence and experimental area. These variables determined the position of *P. decumbens* in the axes, explained by a high dry mass remaining in the deforested area with high current and light incidence (Figure 2). For the second axis, temperature and season showed positive significant contribution to the total variance (Table 4), determining the position of *C. canjerana*, with a high dry mass remaining related to the dry season with low temperature (Figure 2).

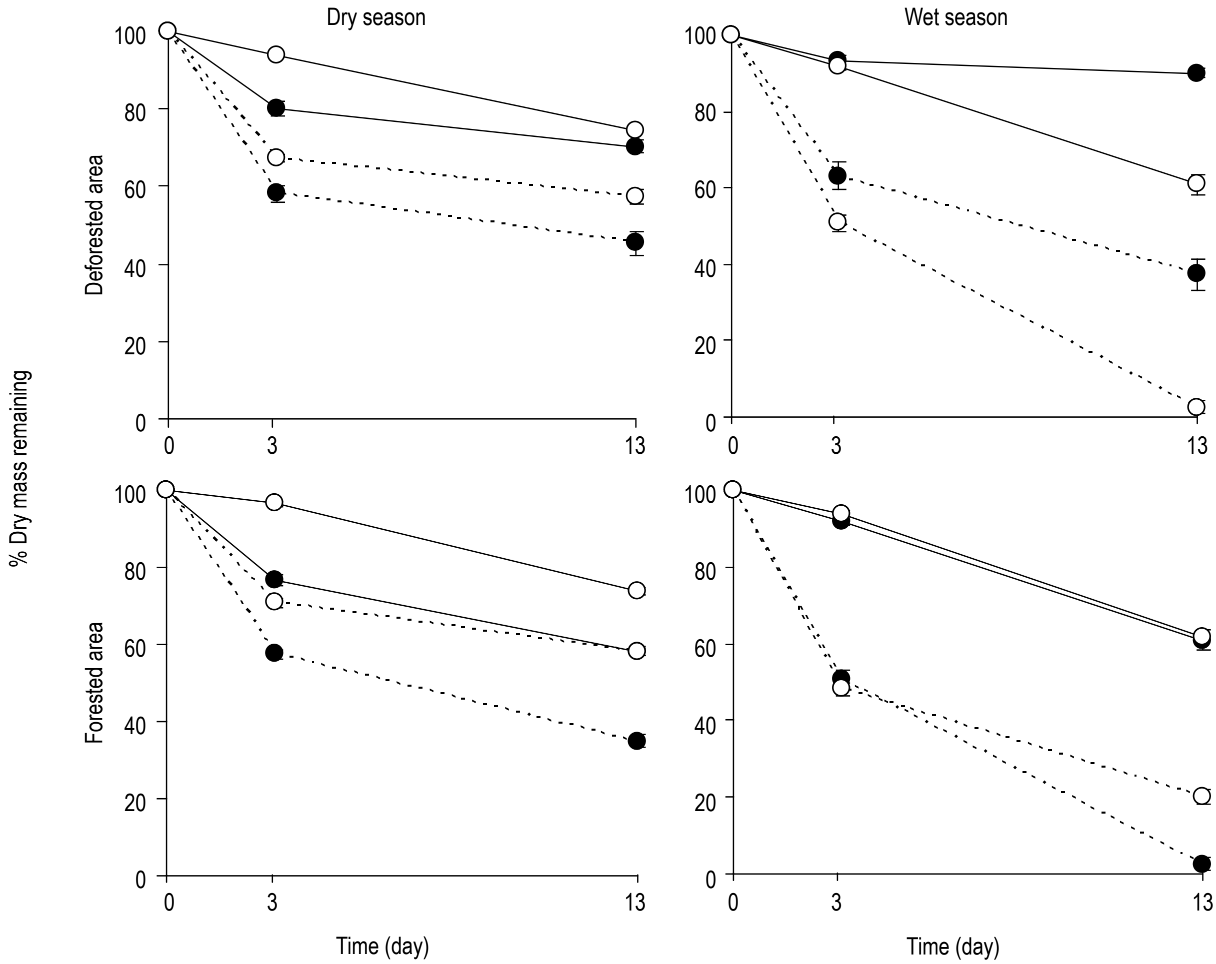

Figure 1. Breakdown of (○) *C. canjerana* and (●) *P. decumbens* leaves, (–) fresh and (…) conditioned, installed in deforested and forested areas during the dry and wet seasons.

Table 2. Results of the multiple comparison between treatments (ANCOVA) applied to leaf dry mass remaining data at each season and results of Tukey HSD test (different letters indicate significant differences) used to verify differences between treatments: two plant species, incubated as fresh and pre-conditioned leaves, at two areas (deforested and forested) and at two seasons (dry and wet).

Results of ANCOVA	Dry season	Wet season
F	7.04	61.65
p	< 0.01	< 0.01
Treatments	Results of Tukey test	
Pycreus decumbens		
Fresh leaves - Deforested area	A	AB
Fresh leaves - Forested area	A	BC
Conditioned leaves - Deforested area	A	ABC
Conditioned leaves - Forested area	B	D
Cabralea canjerana		
Fresh leaves - Deforested area	A	AB
Fresh leaves - Forested area	A	ABC
Conditioned leaves - Deforested area	A	BC
Conditioned leaves - Forested area	A	C

Table 3. Concentration of Acid detergent fibers (ADF), Lignin and Cellulose found in fresh and conditioned leaves of two plant species.

Plant species/treatment	ADF (%)	Lignin (%)	Cellulose (%)
Pycreus decumbens			
Fresh leaves	37.5	15.5	19.2
Conditioned leaves	47.9	16.3	19.5
Cabralea canjerana			
Fresh leaves	48.2	15.3	32.5
Conditioned leaves	55.5	17.6	33.6

4. Discussion

The leaf mass loss of *Pycreus decumbens* and *Cabralea canjerana* was fast when compared to a wide variety of plant species studied by Petersen and Cummins (1974) in a temperate stream. The two studied plants reached about 50% of mass loss between 13-17 days of incubation, a shorter period when compared to 40 days observed by Petersen and Cummins (1974). Other works developed in tropical streams also showed fast leaf mass loss (Mathuriau

Table 4. Results of the Principal Component analysis (PCA), with the variance values extracted for the two principal axes of PCA, used to determine the relative importance of environmental parameters in the leaf decomposition (leaf dry mass remaining data) of *P. decumbens* and *C. canjerana*. The correlation values in bold type were considered of significant contribution to the total variance. Categorical variables: Season = 1-Dry, 2-Wet; Area = 1-Forested, 2-Deforested; Treatment = 1-Fresh, 2-Conditioned.

Variables	PCA 1	PCA 2
Pycreus decumbens	0.785	–0.436
Cabralea canjerana	0.116	–0.774
Temperature	0.116	0.810
Current	0.879	0.260
Luminosity	0.843	0.480
Season	0.036	0.787
Area	0.879	0.260
Treatment	0.426	–0.530
% of variance	41.953	33.906
% of cumulative variance	41.953	75.859

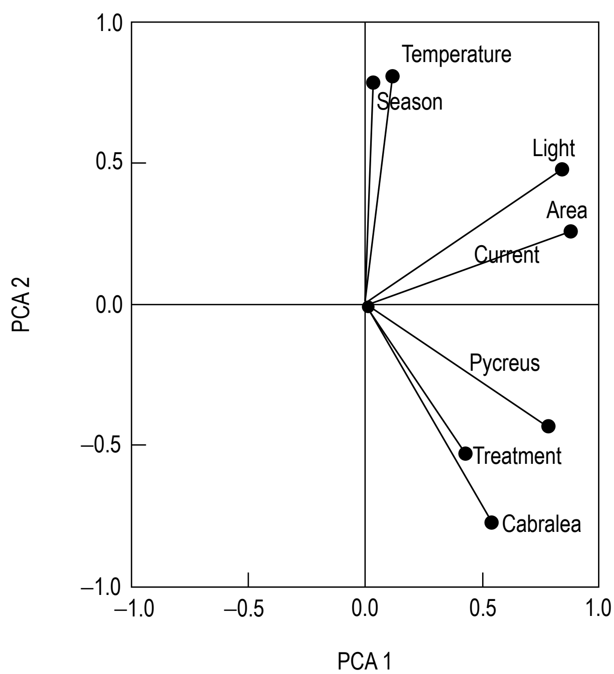

Figure 2. Diagram of PCA ordination axes 1 and 2, constructed with the values of dry mass remaining (%) of *Cabralea canjerana* (CABRALEA) and *Pycreus decumbens* (PYCREUS) leaves, conditioned and unconditioned (TREATMENT), incubated in deforested and forested areas (AREA), during wet and dry seasons (SEASON). The environmental variables also used in the analysis were temperature of water, light incidence and current velocity, measured during the experiment.

and Chauvet, 2002; Dobson et al., 2004; Abelho et al., 2005; Gonçalves et al., 2006a, 2006b, 2007; Moretti et al., 2007) which are comparable with the highest breakdown rates found in the literature (Abelho, 2001). However, these works were conducted in streams and using leaves with intrinsic characteristics, demanding caution in possible generalizations concerning the leaf mass loss.

Our results showed an interaction of factors determining the leaf mass loss, like leaf characteristics, season, water temperature, and current. Similarly to Mathuriau and Chauvet (2002) we used two contrasting fresh-leaf species, one herbaceous with thin soft leaves (*P. decumbens*) and one arboreal with thick tough leaves (*C. canjerana*). The chemical properties of *C. canjerana* could have been the main factor that determined the low mass loss during the initial process of leaf decomposition. Its high concentration of fiber and cellulose could be responsible for a large resistance to the lixiviation process and probably to the action of microorganisms. Lignin and cellulose compounds confer toughness to plant tissues and, consequently, cause slow litter decomposition (Gessner, 2005; Gonçalves et al., 2007). However, *C. canjerana* contain a maximum of 17.6% of lignin, which is among the lowest values presented in the literature (Gessner, 2005; Gonçalves et al., 2006a, 2007) and can also explain the fast leaf decomposition of the present study.

The conditioned leaves lost about 20-40% more mass than unconditioned leaves, although the conditioned leaves concentrated up to 11% of fibers, 1% of lignin and 14% of cellulose more than the fresh leaves. It is certain that this high percentage of mass loss is partially related to the previous incubation of 14 days for conditioning in the stream, the leaching of soluble compounds and microbial degradation possibly acting simultaneously during this initial leaf decomposition. Gonçalves et al. (2006a, 2006b, 2007) and Abelho et al. (2005), working in tropical streams, considered the microbial community responsible for the fast leaf litter decomposition.

After two weeks of incubation the leaf mass loss was higher in the wet than in the dry season, suggesting an important physical environmental effect on leaf decomposition. It may be argued that current may be more important in the late stages of decomposition when leaves are more fragile due to digestion of tissues by microbial action (Ferreira et al., 2006). As the effects of physical abrasion can change temporally according to discharge fluctuations (Kobayashi and Kagaya, 2005), the increase of water discharge during the wet season probably increased the physical abrasion and, consequently, played a strong effect on leaf structure disintegration. According to Ferreira et al. (2006), the presence of fine sediments in water had the potential to amplify the effect of current velocity. However, during both seasons a clear difference was observed for the current velocity among the forested and deforested areas, but with a significant higher leaf mass loss in the area with the slower value of current (forested area).

Thus, if current is a potential source of variability, then many results of decomposition studies could be influenced by the place where leaves are located during the experiments

(Ferreira et al., 2006). However, it is necessary to have caution in evaluating the relative importance of current velocity in the decomposition of leaves, because many other factors can be working synergistically. The positive correlation of current and luminosity upon *P decumbens* decomposition and the negative correlation of temperature upon *C. canjerana* decomposition reinforced not only the effect of different physical parameters but also of leaf characteristics. It is possible that the increase on temperature during the wet season optimized the decomposition process of the arboreal *C. canjerana* plant species. Ferreira et al. (2006) also considered the higher water temperature as a parameter that explained the higher breakdown, regardless current velocity.

The work of Kochi et al. (2009) also emphasized differences on the distribution, retention pattern and decomposition of green and senescent leaves in a step-pool streambed. Senescent leaves may serve as case-building material and green leaves as food, considering that differences in the duration and retention of green leaves of different tree species provide varied food resources for macroinvertebrates under various physical conditions, as season, depth, current (Kochi et al., 2009).

The differences in the decomposition rates between tropical and temperate streams may be related to the velocity at which microbes colonize and decompose leaf material (Abelho et al., 2005). Although the degradation by microbes and fragmentation by invertebrates are considered the main mechanisms determining the mass loss (Gessner et al., 1999), in some streams invertebrates can be considered as unimportant in energy transference in detritus based systems, while in other cases they may be the key elements (Graça and Canhoto, 2006; Callisto et al., 2007). Several recent papers have showed the occurrence of shredders in tropical streams, but presenting low density (Gonçalves et al. 2006a, 2006b; Moretti et al., 2007). Moreover, shredding invertebrates may be less important in tropical streams because there are alternative decomposition pathways for leaves, such as faster microbial processing due to higher temperatures (Boulton and Boon, 1991; Abelho, 2001; Mathuriau and Chauvet, 2002). We did not analyze the degradation by microbes, but data of another study (Carvalho and Uieda, unpublished data) corroborate the low presence of shredders in the studied stream and reinforces the possible small influence of these organisms on leaf fragmentation.

We concluded that the fast leaf breakdown found in this study may have been caused by differences in leaf quality, related to the initial chemical composition and texture of the leaves, and by differences on physical environmental characteristics, like the current velocity acting directly through the physical abrasion of the leaves. Future researches should aim to elucidate the dynamics of litter in natural conditions, as well as the period of input, storage and transport. Besides, studies as composition and diet of invertebrates on litter are necessary to elucidate their importance on the decomposition process in streams.

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