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The leaf breakdown of *Picramnia sellowii* (Picramniales: Picramniaceae) as index of anthropic disturbances in tropical streams

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Abstract

There are few studies in tropical regions exploring the use of leaf breakdown process as a functional tool to assess anthropic disturbance on aquatic ecosystems. We assessed the effects of water pollution due to human activities on the leaf breakdown rate of *Picramnia sellowii* in streams of the southeastern Brazil. The experiment was carried out for 60 days in three reference streams and three streams impaired by organic pollution and absence of riparian vegetation. Three litter bags were incubated in each stream containing 3 ± 0.05 g of *P. sellowii* leaves. The reference streams presented higher values of dissolved oxygen and lower values of nutrients, turbidity, electrical conductivity, total impermeable area and water temperature. The leaf breakdown rate (k) differed significantly between the reference ($k = 0.014 \pm 0.003$ d^{-1}) and impaired streams ($k = 0.005 \pm 0.001$ d^{-1}). The leaves incubated in the reference streams contained greater fungal biomass (measured as ergosterol concentration) and abundance of invertebrates, as well as greater presence of shredders, with k values being related to the biomass of these organisms. Overall, there were clear differences between the leaf mass loss in the reference and impaired streams. These results reinforce the negative effect of urbanization on leaf breakdown and fungal and shredder biomass.

Keywords: fungal biomass, leaf decomposition, functional tool, shredding invertebrates, urbanization.

Decomposição de folhas de *Picramnia sellowii* (Picramniales: Picramniaceae) como índice de distúrbio antrópicos em riachos tropicais

Resumo

Na região tropical são escassos os estudos que exploram o uso do processo de decomposição foliar como ferramenta funcional para avaliar os efeitos das perturbações antrópicas sobre os ecossistemas aquáticos. Nosso objetivo foi avaliar os efeitos dos impactos antrópicos sobre a taxa de decomposição de *Picramnia sellowii* em riachos no Sudeste do Brasil. O experimento foi realizado durante 60 dias em três riachos referências e três impactados por poluição orgânica e sem cobertura vegetal. Em cada riacho foram incubados três sacos contendo 3 ± 0,05 g de folhas secas de *P. sellowii*. Os riachos referência apresentaram maiores concentrações de oxigênio dissolvido e menores valores de nutrientes, turbidez, condutividade elétrica, TIA (área total impermeável) e temperatura. O coeficiente de decomposição (k) diferiu entre os riachos referência ($k = 0{,}014 \pm 0{,}003$ d^{-1}) e os impactados ($k = 0{,}005 \pm 0{,}001$ d^{-1}). Nas folhas incubadas nos riachos referência foi observada maior biomassa de fungos e abundância de invertebrados, assim como a presença de fragmentadores, estando o k relacionado com a biomassa destes organismos. A decomposição se mostrou sensível para avaliar impactos antrópicos nos ecossistemas aquáticos uma vez que foram observadas nítidas diferenças entre a perda de massa foliar nos riachos referências e impactados. Estes resultados reforçam o efeito negativo da urbanização sobre a decomposição e biomassa de fungos e fragmentadores.

Palavras-chave: biomassa de fungos, decomposição de folhas, ferramenta funcional, invertebrados fragmentadores, urbanização.

1. Introduction

The decomposition of the material from riparian vegetation is an important source of energy for low-order streams (Kominoski and Rosemond, 2011). This process is influenced by the interaction of physical, chemical and biological factors (Brum et al., 1999). Among the abiotic factors, water temperature, water velocity, dissolved oxygen, pH, electrical conductivity and nutrient levels can be considered variables that influence the leaf breakdown process (Pascoal and Cassio, 2004; Ferreira and Chauvet, 2011).

In temperate aquatic environments, invertebrate shredders exercise an important role in leaf breakdown transforming coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) (Wallace et al., 1999). On the other hand, in tropical regions, shredders generally present low abundance and diversity in comparison with temperate regions. This is probably due to the low leaf quality, characterized high concentrations of lignin and polyphenols (Ligeiro et al., 2010; Boyero et al., 2011). In tropical region, hyphomycete fungi are mainly responsible for accelerating the detritus degradation, either directly releasing enzymes able to degrade lignin (Fenoglio et al., 2006) or indirectly converting leaf carbon into microbial biomass (Gessner et al., 1999).

The increased concentration of nutrients in polluted streams increases the microorganisms biomass and activity (Suberkropp et al., 2010; Krauss et al., 2011), accelerating the leaf breakdown. However, a high nutrient composition can also be associated with reduced oxygen availability, consequently diminishing the microorganisms activity (Pascoal and Cassio, 2004; Lecerf and Chauvet, 2008; Medeiros et al., 2009) and producing a negative effect on the breakdown rates. Additionally, the increased water temperature caused by removal of riparian vegetation can reduce or even eliminate the presence of shredding organisms (Couceiro et al., 2007), slowing the leaf breakdown (Chadwick et al., 2012).

Recently, studies have focused on the use of leaf breakdown as a tool to assess various anthropic impacts on aquatic environments, such as deforestation (Mckie and Malmqvist, 2009), nutrient enrichment (Abelho et al., 2010), organic pollution (Moulton and Magalhães, 2003; Lecerf and Chauvet, 2008; Colas et al., 2013) and urbanization (Moulton et al., 2009; Imberger et al., 2010). Different impacts can be detected by the reduction on leaf breakdown rate caused by the reduced microorganisms and invertebrate shredders biomass (Gessner and Chauvet, 2002; Baldy et al., 2007). However, few such studies have been conducted in tropical areas.

Therefore, our aim was to assess the impacts of human activities (removal of riparian vegetation and discharge of domestic sewage) on the leaf breakdown of *Picramnia sellowii* Planch (Picramniaceae) in streams of the southeastern Brazil. We hypothesized that a disturbed ecological condition (characterized by the absence of riparian vegetation, lower dissolved oxygen content and higher dissolved nutrients, turbidity, electrical conductivity and water temperature) negatively affect the fungal and shredders biomass, and consequently reduce the leaf breakdown rate in relation to reference streams.

2. Material and Methods

2.1. Study area

The study was carried out in six low-order streams located in the Ribeirão Marmelos Basin, in Juiz de Fora, Minas Gerais, southeastern Brazil (Figure 1). Three of these streams were classified as references (presence of riparian vegetation and absence of any visual human impact) and the other three as impaired (absence of riparian vegetation and presence of organic pollution).

2.2. Field experiment

Green leaves of *Picramnia sellowii* were collected directly from the trees and dried at air temperature. This arboreal species is common along the reference streams. We used green leaves because they were recorded in higher quantity than senescent leaves in litter banks on the streambed. The experiment was conducted in July and August 2012 (dry season) and involved the use of 18 litter bags (3 in each stream) measuring 20 × 20 cm and 1 cm mesh, containing 3 ± 0.05 g of dried leaves. In each stream, one litter bags weres immersed near the bottom of each of three pools (distant approximately 40 meters apart). All the litter bags were removed after 60 days of incubation, stored in plastic sacks with water from the same stream and then placed in ice chests.

The removal date was chosen to be near the point where 50% of the initial mass had decomposed, because complete leaf breakdown reduces the ability to detect site-specific differences (Young et al., 2008; Riipinen et al., 2009). The estimation of the interval necessary for 50% of leaf breakdown was determined in advance by removal of 27 additional litter bags after 7, 15 and 30 days (3 removal dates x 3 litter bags x 3 reference streams). These additional litter bags were placed one week before experiment start in each reference streams selected for our study.

2.3. Environmental variables

At the time of removing the litter bags, the following parameters were measured in each stream using a multi-sensor (Horiba model U-10): pH, electrical conductivity, dissolved oxygen and water temperature. The turbidity was measured with a portable turbidity meter (Lutron model TU-2016). The water velocity was estimated by the time that a plastic float needs to roam one meter of stream (Couceiro et al., 2007). Water samples were collected in plastic bottles (500 ml) to determine the concentration of nitrite (NO_2), nitrate (NO_3) and ammonia (NH_4) according to the methods described by Wetzel and Likens (2000). The total impermeable area (TIA) in each stream catchment was calculated by Landsat images taken in 2010. Around the collection site in each stream, circles were laid out with radius of 500 m, and the size of the area covered by primary and/or secondary forest and the

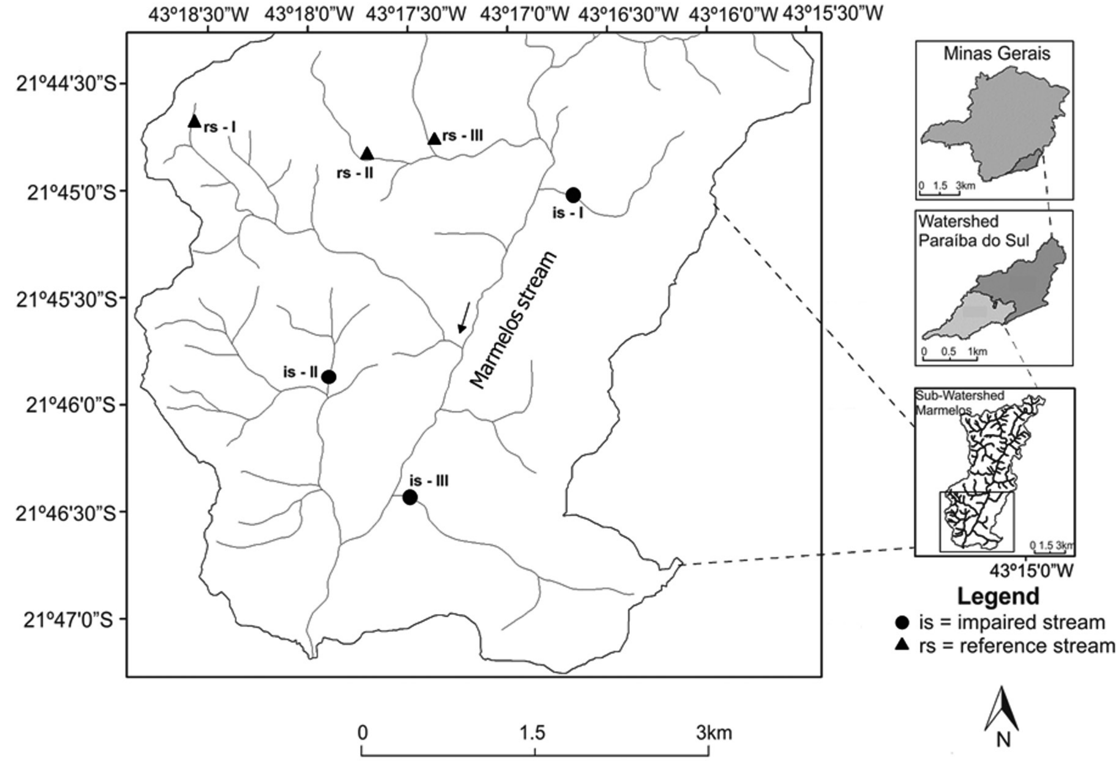

Figure 1. Map of the selected low-order streams from Marmelos Stream Basin, southeastern Brazil. rs (Triangles) = reference streams; is (circules) = impaired streams.

area without plant cover were measured. To determine the impermeable surface, the fractions of urban coverage and exposed ground were grouped (Chadwick et al., 2012).

2.4. Aquatic invertebrates

The remaining material in each litter bag was washed under running water through a sieve with 0.21 mm mesh and observed under a stereomicroscope. The invertebrates sorted out were preserved in alcohol 70 °GL and then identified to the family level according to Brinkhurst (1989) and Cummins et al. (2005). Only *Triplectides* spp. (Trichoptera: Leptoceridae) and *Phylloicus* spp. (Trichoptera: Calamoceratidae) were identified to the genus level. Because of the shortage of information on the functional trophic groups in the Neotropical region, we took the conservative approach by Landeiro et al. (2010) and only classified these two genera as shredders. The biomass of shredders invertebrates was obtained by drying them in a drying chamber at 60 °C for 48 h and then weighing them on a precision balance (accuracy = 0.001 g).

2.5. Fungal biomass

Five leaves were selected from each litter bag and 10 disks were removed (60 mm in diameter), five were used to determine the ash-free dry mass (AFDM) and the others to determine the concentration of ergosterol (as indicative of the hyphomycete fungi biomass; Gessner et al., 1991). The discs for fungal biomass measurements were frozen at –20 °C until ergosterol extraction, by means of methanol and potassium hydroxide. The extract obtained was purified by passage through solid phase extraction (SPE) cartridges. The ergosterol retained in the column was eluted with isopropyl alcohol for reading by high-performance liquid chromatography (HPLC) (Gessner et al., 1991).

2.6. Remaining dry mass and ash-free dry mass

After removing the sets of disks, the remaining leaves were dried in a drying chamber at 60 °C for 72 h and weighed on a precision balance (0.001 g) to determine the remaining dry mass. To estimate the AFDM to correct for the inorganic matter in the disks, they were dried and weighed according to the same process. After that the dried disks were placed in porcelain crucibles, which had been previously weighed on the same precision balance, and then the dried material was incinerated in a muffle furnace at 550 °C for 4 h and weighed again (Pozo and Elosegi, 2005). The leaf breakdown rate (k) was calculated according to the negative exponential model (e.g.: Petersen and Cummins, 1974).

2.7. Data analysis

Principal component analysis (PCA) was used to verify the ranking of the selected streams, employing the following environmental variables: dissolved oxygen, water temperature, pH, electrical conductivity, NH_3, NH_4, NO_2, turbidity, water velocity and TIA. The number of principal components analyzed was determined by considering the largest eigenvalues, generated by the broken stick method. For this analysis, the environmental variables were standardized ([observed-mean]/standard deviation).

Simple linear regression was used to verify the influence of fungal and shredders biomass on leaf breakdown rate. This same analysis was applied to check the influence of the urbanization (PCA-Axis I) on the fungal biomass, shredders biomass and leaf breakdown rate. To perform simple linear regression we considered the stream as replicates.

The *t*-test was used to check for significant differences of the environmental variables, fungal biomass and remaining dry mass in the litter bags between the reference and impaired streams. Prior to perform *t* test we evaluated data normality and homoscedasticity using Shapiro-Wilk test and Levene test, respectively. Moreover, to perform *t*-test we considered the stream as replicates. Since there were no recorded shredders in the impaired streams, it was not possible to use their abundance and biomass to test the difference between the two ecological conditions. All the analyses were carried out in the R program (R Core Team, 2012).

3. Results

3.1. Environmental variables and mass loss

The values of electrical conductivity, water temperature, turbidity and NO_2 were greater in the impaired streams ($p < 0.05$), while the dissolved oxygen values were higher in the reference streams ($p < 0.05$; Table 1).

The first PCA axis presented an eigenvalue higher than those generated by the broken-stick method, explained 71.24% of the total variance of the data and showed the separation between the stream categories (reference and impaired). The reference streams were positively related to axis 1 and were associated with dissolved oxygen and pH, while the impaired streams were negatively related to this axis and directly related to NH_4, NO_2, NO_3, temperature, turbidity, water velocity, electrical conductivity and TIA (Figure 2).

The leaf breakdown rate of *P. sellowii* varied from 0.004 d^{-1} to 0.018 d^{-1}. This rate was higher in the reference streams, which presented *k* values of 0.014 ± 0.003 d^{-1} (mean ± SD), than in the impaired streams, which presented *k* values were 0.005 ± 0.001 d^{-1}. In Figure 2 the size of the symbols is related to the remaining dry mass. Therefore, the smaller size of the symbols in the reference streams represents a lower percentage of remaining dry mass of leaves, 45.19 ± 5.19% (mean ± SD), and while in the impaired streams the remaining dry mass was 74.27 ± 1.17%. It was possible to observe a significant difference in the remaining dry mass between the preserved and impaired streams ($T = -9.46$, $df = 4$, $p = 0.001$). We recorded a negative relationship between urbanization (PCA-axis 1) and leaf breakdown rate ($R^2 = 0.87$, $F_{(1,4)} = 35.36$, $p = 0.004$).

3.2. Fungal colonization

The fungal biomass (i.e., ergosterol concentration) was significantly higher in the leaves incubated in the reference streams (881.13 ± 137.67 µg/g AFDM), than in the impaired streams (523.64 ± 113.01 µg/g AFDM;

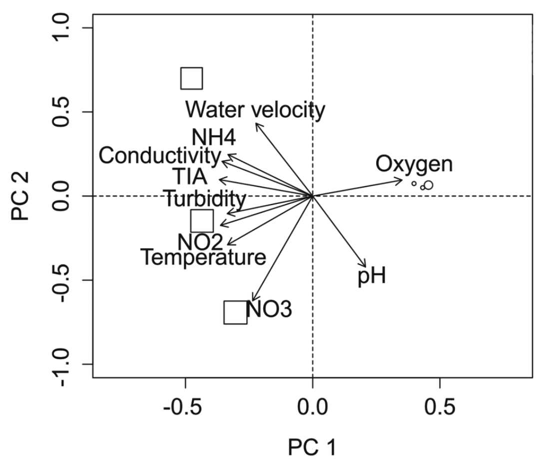

Figure 2. Principal component analysis (PCA) of the environmental date from six selected low-order streams from Marmelos Stream Basin, southeastern Brazil. The circles represent the reference streams and the squares the impaired streams. The sizes of these symbols are proportional to the remaining dry mass of the *Picramnia sellowii* leaves.

Table 1. Environmental variables (mean ± standard deviation) of the reference and impaired selected streams regarding decomposition of *Picramnia sellowii* leaves in low-order streams from Marmelos Stream Basin, southeastern Brazil.

Environmental variables	Reference	Impaired	*t*-test		
			t	df	p
Ammonia (µg/L)	58.02 ± 22.25	151.40 ± 64.78	–2.93	4	0.036
Electrical conductivity (µS/cm)	26.60 ± 2.88	45.65 ± 6.08	–4.46	4	0.011
Nitrate (µg/L)	202.86 ± 23.27	465.16 ± 258.66	–2.02	4	0.129
Nitrite (µg/L)	1.69 ± 0.32	7.13 ± 4.49	–15.86	4	< 0.001
Dissolved oxygen (mg/L)	7.88 ± 0.83	4.77 ± 1.52	–4.46	4	0.003
pH	6.66 ± 0.13	6.59 ± 0.12	–1.23	4	0.303
Temperature (°C)	17.77 ± 0.53	22.05 ± 1.36	–4.65	4	0.001
TIA (ha)	0.00 ± 0.00	29.85 ± 7.41	-	-	-
Turbidity (NTU)	2.52 ± 2.93	10.17 ± 5.90	–4.49	4	0.011
Water velocity (m/s)	0.01 ± 0.01	0.03 ± 0.02	–1.17	4	0.306

$T = 6.47$, $df = 4$, $p = 0.003$). The simple linear regression showed positive influence of the fungal biomass on the leaf breakdown rate (R^2=0.70, $F_{(1,4)} = 12.56$, $p = 0.024$). We recorded a negative relationship between urbanization and fungal biomass ($R^2 = 0.88$, $F_{(1,4)} = 36.88$, $p = 0.004$).

3.3. Aquatic invertebrates in leaf detritus

In the reference streams we counted 750 individuals distributed in 28 taxa, versus 408 individuals distributed in 11 taxa observed in the impaired streams (Table 2). In the reference streams the average abundance of shredders (*Triplectides* (Leptoceridae) and *Phylloicus* (Calamoceratidae)) was 2.88 ± 2.52 (mean ± SD) and the biomass was 2.60 ± 2.31 mg. No shredders were found in the impaired streams. The regression indicated positive influence of the shredder biomass ($R^2 = 0.78$, $F_{(1,4)} = 18.96$, $p = 0.012$) but non influence of shredder abundance ($R^2 = 0.35$, $F_{(1,4)} = 3.64$, $p = 0.129$) on the leaf breakdown rate. We recorded a negative relationship between urbanization and shredder biomass ($R^2 = 0.61$, $F_{(1,4)} = 8.89$, $p = 0.041$).

Table 2. Abundance (mean ± standard deviation) of invertebrates associated with *Picramnia sellowii* leaves during the decomposition experiment in the reference and impaired streams in low-order streams from Marmelos Stream Basin, southeastern Brazil.

Taxa	Reference	Impaired
Coleoptera		
Dryopidae	0.00	0.11 ± 0.33
Elmidae	1.55 ± 1.74	0.00
Diptera		
Ceratopogonidae	0.77 ± 1.71	0.00
Chironomidae	63.78 ± 57.95	26.44 ± 68.78
Empididae	0.11 ± 0.33	0.00
Ephemeroptera		
Caenidae	0.00	0.22 ± 0.66
Leptophlebiidae	5.77 ± 5.11	0.00
Leptohyphidae	0.44 ± 1.01	0.00
Odonata		
Gomphidae	0.11 ± 0.33	0.00
Megapodagrionidae	1.44 ± 1.33	0.11 ± 0.33
Plecoptera		
Gripopterygidae	0.55 ± 0.88	0.00
Perlidae	0.22 ± 0.44	0.00
Trichoptera		
Calamoceratidae*	1.77 ± 2.48	0.00
Helichopsichidae	1.55 ± 2.60	0.00
Hydroptilidae	0.33 ± 1.00	0.11 ± 0.33
Hydropsychidae	0.11 ± 0.33	0.00
Leptoceridae*	1.11 ± 1.53	0.00
Odonteceridae	0.33 ± 1.00	0.00
Polycentropodidae	0.55 ± 1.01	0.00
Crustacea		
Copepoda	0.33 ± 1.00	0.00
Gastropoda	0.77 ± 2.33	4.66 ± 7.31
Hirudinea	0.11 ± 0.33	9.88 ± 19.24
Oligochaeta		
Aelosomatidae	0.11 ± 0.33	0.00
Enchytraeidae	0.11 ± 0.33	0.00
Naididae	1.33 ± 2.96	3.78 ± 7.05

* = families of shredder invertebrates.

4. Discussion

Studies of the influence of alterations in the natural landscape caused by urbanization on the microorganisms and shredders biomass, and consequently on the leaf litter breakdown, have produced different results. Suberkropp et al. (2010) and Krauss et al. (2011), for example, reported a positive effect of human changes on breakdown rates, while Lecerf and Chauvet (2008) and Moulton et al. (2009) provided evidence of a negative effect. Our results are in line with those of the second group of authors, finding a negative effect of urbanization on the leaf breakdown of *P. sellowii* by decrease in decomposition rate.

Deforestation along the margins reduces the plant cover over streams, increasing the solar radiation and water temperature (Khoi and Suetsugi, 2013), and also causes more sediment to enter because of the lower soil stability along the banks (Gilvear et al., 2002; Wang et al., 2012). The increase in water temperature can accelerate the leaching of soluble compounds and favor microbial activity, positively influencing the leaf breakdown (Fernandes et al., 2012). However, we observed that urbanization (increase in electrical conductivity, water temperature, turbidity and NO_2; and decrease in dissolved oxygen) had a negative effect on the leaf breakdown of *P. sellowii*, probably due to the fact that these streams contain lower fungal biomass and no invertebrate shredders. Ferreira and Chauvet (2011) stress that the activity of many species of invertebrates and fungi is inhibited or prevented by increased water temperature. Further, according to those authors lower water temperatures in streams are associated with higher fungal and invertebrates biomass and faster leaf breakdown rates.

The greater entry of sediment on impaired streams in this study caused the burial of most of the litter bags. According to Pascoal et al. (2005), this leads to hypoxia in the detritus and reduces the abrasive action of the current and the leaf area available for colonization by microbes, factors that probably delay leaf breakdown in these environments. The higher turbidity values, presence of a fine layer of mud on the litter and the dark coloration of the leaves observed when the litter bags were removed from the impaired streams all indicates that the entrance of sediment contributed to the slower leaf breakdown in these streams.

We also found that higher concentrations of dissolved oxygen in the reference streams probably contributed to leaf breakdown, by having a positive effect on the fungal and shredders biomass. The limitation of oxygen excludes some species of aquatic fungi (Pascoal and Cassio, 2004; Medeiros et al., 2009) and causes negative effects on the fungal biomass (Pascoal et al., 2003) and sensitive shredders (Couceiro et al., 2007). The results of these studies confirm the importance of dissolved oxygen on the leaf breakdown rate.

Various studies have shown that enrichment of streams with nutrients can accelerate the leaf litter breakdown, by stimulating the growth of microorganisms (Pascoal and Cassio, 2004; Lecerf and Chauvet, 2008; Imberger et al., 2010). However, like in our study, Medeiros et al. (2009) found an inverse relationship between the fungal biomass and the presence of nutrients. Besides this, the enrichment of nutrients in polluted streams also promotes reduced presence of sensitive shredders (Lecerf et al., 2006; Couceiro et al., 2007). We did not observe any of these invertebrates in the impaired streams.

The leaf breakdown rate of *P. sellowii* was greater in the reference streams, possibly related to the abundance and biomass of invertebrate shredders. Neatrour et al. (2004) also observed a positive relation between leaf breakdown rates and shredders biomass in low-order streams. The fauna in the impaired streams was dominated by Oligochaeta and Chironominae as observed in another studies in aquatic environments impacted by urbanization effects (Henriques-de-Oliveira et al., 2007). These organisms that mainly feed on fine particulate organic matter (Walsh et al., 2005), so their presence probably may not directly influence the leaf breakdown of *P. sellowii*.

Our results indicate that the selected environmental variables influenced the activity of the fungi and shredders on leaf breakdown of *P. sellowii* in the studied streams. Besides this, we can confirm the importance of the information obtained for future studies of anthropic impacts, since clear differences were observed in the leaf mass loss between the reference and impaired streams.

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