Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders

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SUMMARY

1. To assess whether the reported slow breakdown of litter in tropical Cerrado streams is due to local environmental conditions or to the intrinsic leaf characteristics of local plant species, we compared the breakdown of leaves from *Protium brasiliense*, a riparian species of Cerrado (Brazilian savannah), in a local and a temperate stream. The experiment was carried out at the time of the highest litter fall in the two locations. An additional summer experiment was conducted in the temperate stream to provide for similar temperature conditions.

2. The breakdown rates (*k*) of *P. brasiliense* leaves in the tropical Cerrado stream ranged from 0.0001 to 0.0008 day⁻¹ and are among the slowest reported. They were significantly (F = 20.12, P < 0.05) lower than in the temperate stream (0.0046–0.0055). The maximum ergosterol content in decomposing leaves in the tropical Cerrado stream was 106 µg g⁻¹, (1.9% of leaf mass) measured by day 75, which was lower than in the temperate stream where maximum ergosterol content of 522 µg g⁻¹ (9.5% of leaf mass) was achieved by day 30. The ATP content, as an indicator of total microbial biomass, was up to four times higher in the tropical Cerrado than in the temperate stream (194.0 versus 49.4 nmoles g⁻¹). 3. Unlike in the temperate stream, leaves in the tropical Cerrado were not colonised by shredder invertebrates. However, in none of the experiments did leaves exposed (coarse mesh bags) and unexposed (fine mesh bags) to invertebrates differ in breakdown rates (F = 1.15, P > 0.05), indicating that invertebrates were unable to feed on decomposing P. brasiliense leaves.

4. We conclude that the slow breakdown of *P. brasiliense* leaves in the tropical Cerrado stream was because of the low nutrient content in the water, particularly nitrate (0.05 mgN L^{-1}), which slows down fungal activity and to the low density of invertebrates capable of using these hard leaves as an energy source.

Keywords: aquatic hyphomycetes, Cerrado, chemical composition, litter breakdown, shredders

Introduction

The litter breakdown of riparian leaves in lotic systems is being the intensely studied in the past 30 years in temperate regions, and it is now clear that allochthonous organic matter, originating in the riparian zone, is a main energy source to many low-order forested streams (e.g. Minshall, 1967; Kaushik & Hynes, 1971; Iversen, 1973; Hutchens & Wallace,

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2002). Forests produce large amounts of litter, with values typically below 1000 g m^{-2} year⁻¹ in temperate areas, and above that value in the tropics (reviewed by Abelho, 2001).

Litter entering forested streams is trapped in riffles and shallow areas, and incorporated into secondary production by bacteria, fungi and invertebrate consumers (Gessner, Chauvet & Dobson, 1999; Graca & Canhoto, 2006). The breakdown rates of leaves in streams are affected by external factors, such as temperature (Liski et al., 2003), physical abrasion (Heard et al., 1999), pH (Griffith & Perry, 1993), nutrient availability (Suberkropp & Chauvet, 1995) and presence of consumers (Graça, 2001). Leaf intrinsic factors can also be used to predict breakdown rates. These include leaf-nutrient content (Ostrofsky, 1997), hardness and the presence of defensive compounds (Canhoto & Graça, 1999; Osono & Takeda, 2001). The intrinsic properties of leaves are strongly related to local environmental conditions (Rier et al., 2002; Adams, Tuchamn & Moore, 2003). For example, plants from the South American savannah (known as Cerrado in Brazil) grow in sandstone-derived, arid soils with low nutrients (Oliveira & Marquis, 2002). The plants typically have hard leaves, which may herbivores present particular difficulties to (Fernandes, Gonçalves-Alvim & Carneiro, 2005; Moretti, Gonçalves & Callisto, 2007).

Most of the existing information about litter breakdown in aquatic systems comes from experiments carried out in temperate zones (Abelho, 2001; Graça, 2001). Furthermore, comparison of results gathered in different geographic areas and biomes can be questionable, as differences in decay rates can be attributed to differences in climate, but also differences in the quality of litter (Moretti et al., 2007). The only way to compare processes in different geographic areas directly is to use the same leaf substrate. Irons et al. (1994) used 10 species of leaves from North and Central America in a comparative litter breakdown experiment in streams of Costa Rica, Michigan and Alaska of U.S.A. They concluded that the importance of microbial breakdown increased towards the tropics because of increased temperature, whereas the breakdown attributed to invertebrate-feeding increased in the opposite direction. In a comparative experiment, Wantzen & Wagner (2006) reported that breakdown rates of Alnus glutinosa were faster in a Brazilian Cerrado stream than in a German temperate stream, and explained the difference in terms of temperature.

In a previous experiment, we compared the leaf breakdown rates of A. glutinosa in three streams located in Central Portugal (temperate climate), south Portugal (Mediterranean) and Brazil (tropical Cerrado) (Gonçalves, Graça & Callisto, 2006). Unlike Wantzen & Wagner (2006), we found that breakdown was the highest in the temperate and the lowest in both Mediterranean and tropical Cerrado streams. These differences were attributed to the lack of consumers in the Mediterranean and tropical Cerrado streams and to the low water nitrogen content in the tropical Cerrado stream. Nevertheless, it could be argued that the low breakdown rates of alder leaves in the tropical Cerrado could also be attributed to the exotic origin of the substrate. Here, we report another cross-climate experiment using leaves from riparian vegetation of Cerrado (Protium brasiliense) characterised by hard leaves. Based on our previous experiment, we predict that the factors 'nutrients' and 'presence of shredders' will override the temperature/climate effect and the origin of the leaf species.

Methods

The study was carried out in two streams from two climatic areas: temperate (fourth order) and tropical Cerrado (third order). The temperate stream was São João, Lousã Mountain, Portugal (40 °6'N, 8 °14'W; 230 m.a.s.l.). The riparian trees bordering the stream included *Castanea sativa* Miller, *Quercus* spp., *Pinus pinaster* Aiton, *Acacia dealbata* Link and *Eucalyptus globulus* Labill. The tropical Cerrado stream was Indaia, located at Serra do Cipó National Park (19°16.4'S and 43°31.2'W; 1,450 m.a.s.l.). The vegetation in the riparian zone was dense, represented mainly by *Augusta longifolia* Spreng, *Erytrhoxylum pelletarianum* St. Hil, *Miconia chartacea* Triana, *Miconia cyathanthera* Triana, *Myrcia guyanensis* (Aubl.) DC, *Ocotea lacifolia* (Schott) Mez. and *P. brasiliense* (Spreng) Engl.

To measure breakdown rates, we used the litter bag approach according to Bärlocher (2005). We used leaves of a native tropical Cerrado tree, *P. brasiliense*, common in the riparian zone. Senescent leaves of *P. brasiliense* were collected with nets placed at the base of a group of riparian trees. The leaves were air-dried, weighed into 3.0 ± 0.1 -g portions and allocated into litter bags (20×20 cm; 10 and 0.5-mm mesh

bags). A total of 96 bags was prepared (two mesh sizes \times 16 sampling dates – see below – \times three replicates). In the tropical Cerrado stream, we ran the experiment from May to July 2003, coinciding with a decrease in the temperature and rainfall (incubation periods of 1, 7, 15, 30, 45 and 75 days). In the temperate stream we ran the experiment in autumn when litter fall is the highest, temperatures decrease and rains increase (November–December 2002; incubation periods of 1, 7, 24 and 35 days), and in summer (August–September 2003; incubation periods of 1, 7, 15, 30, 45 and 63 days), the period of the lowest water level, the highest temperature and lower litter fall. We expected the climate in summer to be comparable with that from May to July in Cerrado.

Litter bags retrieved from the streams were dissembled in the laboratory and leaves gently washed with running tap water over a 0.125-mm sieve to remove sediments but retain invertebrates. From each bag five leaves were randomly taken and four discs cut from each leaf using a cork borer (12 mm). In this way, four sets of five discs were obtained.

One set of discs was used to measure fungal biomass in terms of the ergosterol content according to Gessner (2005a). The discs were preserved with methanol/KOH at -20 °C. Ergosterol extraction and saponification was achieved by heating the discs in methanol/KOH to 80 °C for 30 min. The extract was purified by fluxing through a solid-phase cartridge and ergosterol was eluted with isopropanol using a vacuum manifold (approximately 1 mL min⁻¹) and quantified by HPLC (DIONEX Summit P580, Germering, Germany). The results were expressed as mg ergosterol g⁻¹ leaf ash-free dry mass (AFDM) and percentage of leaf mass according to Gessner & Chauvet (1993).

The second set of discs was used to quantify total microbial biomass, in terms of ATP content. The ATP was extracted by transferring the discs to 5 mL 1.2 N H₂SO₄ containing 8 g L⁻¹ oxalic acid and 5 mL HEPES (0.05 M). Discs were homogenised and the ATP neutralised to pH 7.0–7.5, filtered (0.2 μ m) and stored at –20 °C. ATP reacts with the enzyme firefly bioluminescence, producing light, which can be measured with a (Turner designs TD-20/20, Sunnyvale, CA, U.S.A.) luminometer (Abelho, 2005).

The third set of discs was used to measure the sporulation rates by aquatic hyphomycetes during leaf breakdown. The discs were incubated in 100 mL

Erlenmeyer flasks with 25 mL of filtered water (Millipore filter GF/C, 0.45 μ m, Billerica, MA, U.S.A.) on an orbital shaker (100 rpm) for 48 h at 15 °C. The conidia suspensions were fixed with 2 mL of 37% formalin. The slides were prepared by adding 100 μ L of 0.5% Triton X-100 solution to the suspension to ensure a uniform distribution of conidia. The suspension was stirred and an aliquot was filtered (Millipore SMWP, pore size 5 μ m, Billerica, MA, U.S.A.). Filters were stained with cotton blue and phenol (0.05%), and spores were counted with a microscope (at 200× magnification). Discs were then dried, weighed, ashed and reweighed to determine their AFDM.

The final set of discs was used to quantify the mass of discs used for ergosterol and ATP content, assuming that discs cut from a contiguous area of the same leaf have the same mass. The discs were dried for 72 h at 60 °C, weighed, incinerated for 4 h at 550 °C, re-weighed and AFDM determined.

Leaf remains after retrieval of discs were dried (60 °C) to constant mass, weighed and, for selected dates, ground in a mill for nitrogen, phosphorus and lignin analyses. The lignin content was calculated according to Van Soest (1963): in the first step lignincellulose was extracted from leaves with an acidic detergent and then cellulose was dissolved in 72% H₂SO₄ at 5 °C. Nitrogen was analysed by the Kjeldahl method (Sarruge & Haag, 1974; Malavolta & Netto, 1989), whereby leaf material was digested with H_2SO_4 , the resulting NH_4^+ distilled under alkaline conditions and the NH₄⁺ condensate collected into a solution of H₃BO₃ and titrated with HCl. Phosphorus was analysed according to Malavolta & Netto (1989) and Miyazawa, Pavan & Bloch (1992) by spectrophotometry after $H_2PO_4^-$ binding with MoO_4^{2-} .

Invertebrates recovered from leaves were fixed in 70% alcohol, identified and classified into functional feeding groups (FFG) according to Tachet, Bournaud & Richoux (1980), Merritt & Cummins (1996), Perez (2003), Cummins, Merritt & Andrade (2005) and Costa, Ide & Simonka (2006). Chironomids were identified only to the family level, but not classified into feeding groups given the large number of unidentified species potentially belonging to any feeding group. On three occasions during each experiment, we measured the temperature, pH, water current, conductivity and oxygen concentration. A water sample was also collected for laboratory determination of total alkalinity by titration of 100 mL of water with H_2SO_4 , 0.00125 N to pH 4.5 (APHA (American Public Health Association), 1998), and in the temperate stream, PO₄ by the ascorbic acid method (APHA (American Public Health Association), 1998) and NO₃ with an ion analyser (Dionex[®]; Germering, Germany).

Breakdown rates (k) were calculated by the exponential decay model ($W_t = W_0 e^{-kt}$) and comparisons between streams, seasons and mesh sizes were performed by analysis of covariance (ANCOVA, followed by Tukey test). The numbers of invertebrates in decaying leaves accumulated through time and therefore ANCOVA was also used to test the differences among experiments. Ergosterol, sporulation and ATP data were used as dependent variables to compare microbial colonisation among streams and between mesh types. Each variable was independently subjected to a mixed within-block ANOVA, using stream and mesh size as between-block factors and sampling time as the within-block factor, followed by Tukey test (Zar, 1996). The relationships between fungal biomass, total microbial biomass and mass loss were investigated by Pearson's correlation (Zar, 1996).

Results

Environmental parameters

The waters were neutral in the temperate stream and acidic in the tropical Cerrado stream. Oxygen was near saturation in both streams. In the tropical Cerrado stream, alkalinity was higher and conductivity was lower than in the temperate stream (Table 1), whereas the temperature of the temperate stream in summer was similar to the tropical Cerrado. The phosphate-P content was highly variable in the temperate stream, and nitrate-N was higher in the temperate than in the tropical Cerrado stream (0.05 mg L⁻¹). Cerrado and temperate streams differed in flow, particularly in autumn in Portugal, when the flow peaked at $4.18 \text{ m}^{-3} \text{ s}^{-1}$. On one occasion the flow in the Cerrado stream was reduced nearly to zero.

Leaf breakdown

Leaf breakdown of *P. brasiliense* in the temperate stream was 2.8–3.5 times higher (autumn coarse bag k = 0.0057 g day⁻¹; summer fine bag k = 0.0046 g day⁻¹)

Table 1 Water quality characteristics of the studied streams

Parameters	Temperate autumn	Temperate summer	Tropical Cerrado
pН	(6.7–7.4)	(6.1–7.2)	(4.6-6.0)
Oxygen (mg L^{-1})	(11.4–12.8)	(9.0-10.2)	8.6 ⁺
Alkalinity (mg CaCO ₃ L^{-1})	4.1 ⁺	_	21.6 ⁺
Electrical conductivity (μS cm ⁻¹)	(39–40)	(46–53)	(5–7)
Temperature (°C)	(12.4–7.5)	(14.4–19.2)	(14.5–16.5)
Phosphate-P ($\mu g L^{-1}$)	0.06^{+}	9.65 ⁺	2.50*
Nitrate-N (mg L^{-1})	1.06 ⁺	0.90 ⁺	0.05*
Water flow $(m^3 s^{-1})$	(1.17-4.18)	(0.10-0.17)	(0.001-0.18)

Maximum and minimum values are showed into parentheses. [†]Data measurement only in the beginning of the experiment and ^{*}from Callisto *et al.*, 2004)

Table 2 Decay rates (*k*) and exponential correlation coefficients (R^2) for *Protium brasiliense* in coarse and fine mesh bags in the tropical Cerrado and temperate (autumn and summer) streams

Site	Season	Mesh size	-k (day ⁻¹)	R^2
Temperate	Autumn	Coarse	0.0057	0.70
-		Fine	0.0047	0.27
	Summer	Coarse	0.0055	0.95
		Fine	0.0046	0.96
Tropical	Autumn	Coarse	0.0021	0.42
Cerrado		Fine	0.0016	0.14

than in the tropical Cerrado stream (*k* values, 0.0021– 0.016 g day⁻¹; Table 2 and Fig. 1). However, the fits of some regressions were very low; that was the case for leaves in fine mesh bags in autumn in the temperate and in the Cerrado streams (Table 2). Breakdown rates were significantly lower in the tropical Cerrado than in the temperate stream (ANCOVA, F = 20.12; P < 0.05). There were no differences between the autumn and summer experiments in the temperate stream. Mesh size did not affect breakdown rates in the three experimental sets (Tukey test, P > 0.005).

During a breakdown, the lignin content of leaves increased from 26.3% to a maximum of 44.7%, nitrogen decreased from 2.3 to <1% and phosphorus decreased from 0.60% to a minimum 0.01% (Table 3). Loss of both N and P was particularly high already after 1 day in the water.

Microbial Community

As leaves incubated in fine and coarse mesh bags did not differ in the ergosterol content (ANOVA, F = 0.41;



Table 3 Chemical composition of *Protium brasiliense* leavesduring breakdown in terms of percentage of dry mass in the TEand TR streams in autumn and summer

Site	Day	N (%)	P (%)	Lignin (%)
Until incubation	0	2.31	0.060	26.3
TEa	1	0.68	0.010	32.9
TEa	35	0.84	0.025	38.2
TEs	7	0.69	0.020	34.8
TEs	63	1.44	0.025	44.7
TRa	8	0.91	0.010	33.7
TRa	75	1.17	0.015	38.7

TE, temperate; TR, tropical Cerrado; a, autumn; s, summer; N, nitrogen; P, phosphorus.

P = 0.5), we pooled the values of all bags for the same sampling date. The highest fungal biomass was measured during summer in the temperate stream



(up to 522 µg ergosterol g⁻¹ by day 30; corresponding to 9.5% of leaf mass), while in autumn the maximum value in this stream was 149 µg g⁻¹ (day 35; approximately 2.7% of leaf mass). The maximum value in the tropical Cerrado was 106 µg g⁻¹ (approximately 1.9% of leaf mass) measured by day 75 (Fig. 2). The ergosterol content was significantly higher in the temperate stream during summer than in the other experiments (ANOVA, F = 16.3; P = 0.000001).

Coarse and fine mesh bags in the tropical Cerrado stream differed in ATP content (ANOVA, F = 5.76; P = 0.02), but no differences were found in the temperate stream (Tukey test, P < 0.05) and therefore these data were pooled (n = 3 + 3 = 6). Significant differences in total microbial biomass (ATP) were found among coarse and fine mesh bags



Fig. 2 Ergosterol content (mean \pm SE; n = 6) of *Protium brasiliense* leaves during breakdown in temperate (autumn and summer) and tropical Cerrado streams.

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Fig. 3 ATP contents (mean \pm SE; n = 6 to temperate and n = 3 to tropical) of *Pro-tium brasiliense* leaves during breakdown in temperate (autumn and summer) and tropical Cerrado streams. C = coarse mesh bags; F = fine mesh bags.

in tropical Cerrado, and temperate (autumn and summer) streams (ANOVA, F = 62.75; P = 0.00001), but we did not find differences between seasons in the temperate stream (Tukey test, P < 0.05). The highest ATP content was measured in the tropical Cerrado by day 7 (194 and 152 nmoles g⁻¹, respectively in coarse and fine mesh bags). The maximum value recorded in the temperate stream (49.43 nmoles g⁻¹) occurred in autumn by day 24 (Fig. 3).

As fine and coarse mesh bags did not differ in sporulation rates (ANOVA, F = 0.46; P = 0.5), we pooled the values from the same sampling data. The highest sporulation values were recorded in the temperate stream during summer by day 30 (4100 conidia mg⁻¹ leaf AFDM day⁻¹); and in autumn by day 35 (970 conidia mg⁻¹ leaf AFDM day⁻¹). In the tropical Cerrado, the maximum sporulation rates reached 7 conidia mg⁻¹ leaf AFDM day⁻¹ (Fig. 4). The three data sets differed significantly in terms of spore production (ANOVA, F = 14.7; P = 0.000001).

The ergosterol and ATP content were correlated in the temperate stream but not in the tropical Cerrado. ATP and ergosterol were generally also inversely correlated with the remaining mass of leaves (Table 4).

Invertebrates

Although fine mesh bags were supposed to exclude invertebrates from feeding on leaves, some were found inside the bags. However, invertebrates colonising fine mesh bags were always smaller than those colonising coarse mesh bags. The density of invertebrates in coarse mesh bags was similar among the three experimental sets (ANCOVA, F = 1.15; P = 0.3) (Fig. 5). In the temperate stream, gatherer-collectors comprised 24–73% of all invertebrates colonising leaves, whereas shredders represented 10–38%. In the tropical Cerrado, filterer-collectors and predators comprised respectively 44% and 25% of the invertebrates, with shredders being represented by only 1%.



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	Temperate summer		Temperate autumn			Tropical Cerrado			
	r	Р	п	r	Р	п	r	Р	п
Mass × ergosterol	-0.46	0.04	36	-0.75	0.0001	19	-0.43	0.031	25
Mass \times ATP Mass \times Esporulation ATP \times ergosterol	-0.48 -0.26 0.36	0.003 0.96 0.034	36 30 36	-0.52 -0.59 0.41	0.023 0.007 0.08	19 19 19	-0.04 -0.01 0.26	0.85 0.96 0.21	25 25 25

Table 4 Pearson's correlation betweenleaf mass remaining and microbial colo-nisation during *Protium brasiliense*leafbreakdown

r, Pearson's correlation coefficient; P, value of probability; n, number of cases.

The main taxa colonising the leaves in all streams were the Chironomidae (Table 5).

Discussion

Leaf breakdown

Breakdown rates of *P. brasiliense* in the tropical Cerrado stream were slow, according to the classification of Petersen & Cummins (1974), lower than in the temperate stream and lower than those reported for other leaf species in temperate (Ostrofsky, 1997), Mediterranean (Maamri, Chergui & Pattee, 1997; Casas & Gessner, 1999) and tropical African and American streams (Mathuriau & Chauvet, 2002; Dobson *et al.*, 2003; Crowl *et al.*, 2006). Indeed, the breakdown rates in Cerrado were among the two lowest values listed among the 180 referred to in Abelho (2001). These slow rates could be related mainly to two interdependent factors: intrinsic leaf characteristics and environmental/climate factors.

A first factor could be the physical and chemical properties of *P. brasiliense*. The leaf nutrient content, especially nitrogen, has been shown to be a good predictor of breakdown rates, with a fast breakdown of nitrogen-rich leaves (Kaushik & Hynes, 1971; Canhoto & Graça, 1996; Cornelissen, 1996). Senescent leaves of *P. brasiliense* had 2.31% N, which is within the range of values reported for 47 species by Flindt & Lillebø (2005) whereas the 0.60% of P was in the lower range of 28 species mentioned by the same authors. The nutrient content of *P. brasiliense* leaves is therefore within the range of the published data.

Lignin can also be used to predict breakdown (Osono & Takeda, 2001; Gonçalves, Santos & Esteves, 2004). Leaves of *P. brasiliense* contain 26% of lignin, which is in the upper range of values reported by Gessner (2005b). Lignin is a structural constituent conferring toughness to leaves, protecting them from herbivory and microbial infections, and providing waterproofing properties to plant cell walls; it is particularly difficult to biodegrade and limits the degradation of other plant cell compounds. The high



Fig. 5 Cumulative invertebrate numbers in decomposing leaves of *Protium brasiliense* in streams from temperate and tropical Cerrado streams.

Table 5 Density (individuals g^{-1} ash-free dry mass) and proportion of the more abundant invertebrates that colonised leaves of *Protium brasiliense* in the three experiments

Site (experimental conditions)	Taxa	Number (%)
Temperate autumn	Chironomidae	60 (73)
-	Oligochaeta	13 (15)
	Rhyacophilidae	2 (2)
Temperate summer	Chironomidae	41 (52)
	Hidracarina	11 (13)
	Sericostomatidae	10 (12)
	Hydroptilidae	5 (6)
	Leptophlebiidae	5 (6)
Tropical Cerrado	Chironomidae	18 (63)
•	Copepoda	6 (19)
	Cladocera	3 (10)
	Leptophlebiidae	2 (7)

lignin content in *P. brasiliense* and other Cerrado plants could be a consequence of the water and nutrient stress of the Cerrado (Goodland & Ferri, 1979), and may explain the reported low herbivory in this system (Flower & Duarte, 1991; Salatino, 1993). Leaf toughness could therefore be an explanation for the slow breakdown rates observed in the tropical Cerrado streams reported in this and other related studies (Gonçalves *et al.*, 2006; Moretti *et al.*, 2007).

However, our results suggest environment also plays an important role in determining leaf breakdown rates, which were lower in the Cerado than in the temperate stream. The same result was obtained in a previous experiment with the temperate species *A. glutinosa*, a fast-decomposing substrate (Abelho, 2001) and a high-quality food for shredders (Gonçalves *et al.*, 2006). Nevertheless, as contrary results were found by Wantzen & Wagner (2006), generalisations about the low leaf litter breakdown rates in Cerrado streams are not yet possible, especially when Cerrado includes different vegetation physiognomies (Oliveira & Marquis, 2002) that can influence this process differently.

An important external factor influencing litter breakdown is nutrient availability in water (mainly N and P), as shown in tropical (Schoenlein-Crusius *et al.*, 1999; Rosemond *et al.*, 2002) and temperate streams (Suberkropp & Chauvet, 1995; Gulis, Ferreira & Graça, 2006) and under laboratory conditions (Gulis & Suberkropp, 2003a). In our study, concentrations of nitrogen and phosphorus were 0.050 mg L⁻¹-N and 2.5 µg L⁻¹-P. For comparison, Suberkropp & Chauvet (1995) measured very low breakdown rates of leaves in a soft water stream in North America (0.007– 0.024 mg L⁻¹-N and 8–11 μ g L⁻¹-P). Although the phosphate content of the Cerrado stream was within the autumn–summer variations of the temperate stream, nitrate was lower in the Cerrado stream. It is therefore plausible that the low nutrient content of tropical Cerrado stream can contribute to the slow breakdown of the allochthonous organic matter. Whether or not the low nutrient content of the studied stream is characteristic of other Cerrado rivers is uncertain, as the published chemical data are scarce.

Microbial community

Ergosterol values were low when compared with other studies in temperate and tropical systems (Abelho, 2001; Mathuriau & Chauvet, 2002). Data suggest that aquatic fungi colonised leaves of P. brasiliense with difficulty. Leaves of A. glutinosa exposed to the same stream in a parallel experiment yielded much higher ergosterol values by the end of the experiment (310–420 μ g g⁻¹; Gonçalves *et al.*, 2006), but were also comparatively low compared with values reported from other systems. Data for sporulation rates corroborated the ergosterol values: sporulation rates were higher in the temperate stream than in the Cerrado, and within the range in the literature for other leaves (e.g. Mathuriau & Chauvet, 2002; Gulis & Suberkropp, 2003b; Ferreira & Graça, 2006). In the Cerrado stream, the sporulation rates were almost zero.

The ATP contents in *P. brasiliense* (present data) and in A. glutinosa (Gonçalves et al., 2006) were consistently higher in the tropical Cerrado than in the temperate stream. The ATP content may reflect not just the fungal biomass but the entire set of microbes colonising leaves. These include bacteria and protozoa, which in some situations could be important in terms of biomass (Suberkropp, Gessner & Chauvet, 1993). It is generally accepted that bacteria are important colonisers of fine particulate organic matter (Findlay et al., 2002); in this context, the high ATP content could be partially caused by the accumulation of fine particulate organic matter at the surface of decomposing leaves, or because of the presence of a biofilm with autotrophic properties. Microbial data therefore suggest unfavourable conditions for microbial breakdown of leaves.

Invertebrates

Protium brasiliense (this experiment) and A. glutinosa (Gonçalves et al., 2006) were poorly colonised by invertebrates. Wantzen & Wagner (2006) also reported that leaves of the endemic Chrysophyllum marginatum were not consumed by invertebrates during leaf breakdown and that shredder invertebrates were rare in another Cerrado stream. However, unlike our study, Wantzen & Wagner (2006) observed mining Chironomids feeding on decomposing leaves and accelerating the rate of litter breakdown. In our experiments, as no differences in the breakdown rates between coarse and fine mesh bags were observed in the three experimental runs, we conclude that the breakdown of *P. brasiliense* is mainly a microbiological and physical process. This result contrasts with the experiment using A. glutinosa (Gonçalves et al., 2006) in which differences between coarse and fine mesh bags were observed in the temperate, but not in the tropical Cerrado stream.

In terms of FFG, our results from invertebrates recovered from leaves and the results of Wantzen & Wagner (2006) of invertebrates recovered from the benthos were comparable: on an average, collectors, predators and shredders corresponded to 44%, 25% and 1% of total macroinvertebrates in our experiment and to 53%, 36% and 3% in Wantzen & Wagner (2006). Wantzen & Wagner (2006) found Phylloicus sp. to be consistently the only true shredder found in the Cerrado stream, and it was relatively rare. This species is known as a selective shredder in the tropics (Graça et al., 2001; Rincón & Martínez, 2006). However, it should be recognised that the ecology of the freshwater invertebrates of Cerrado is virtually unknown and that genera or families may differ in their trophic ecology across ecosystems with generalist strategies possibly selected for more strongly in the tropics (e.g. Cheshire, Boyero & Pearson, 2005).

Overall, and pending further study, we can provisionally characterise the leaf breakdown process in tropical Cerrado streams in the following way. Litter input can be comparable with that of temperate zones (Wantzen & Wagner, 2006). Leaves subsidising tropical Cerrado streams have a hard texture and are decomposed slowly, because of the very low nutrient content of waters, which slows fungal colonisation. Shredder invertebrates are apparently scarce, probably caused by low quality of food resources, reinforced by the spates washing the leaf litter from the stream-bed. Breakdown rates are therefore very slow and catalysed mainly by microbial activity. Physical abrasion may also be important during the rainy season, but we did not evaluate this factor, as our study was carried out during the dry season. Many other topics related to litter breakdown still need to be investigated, including the life history of true shredders, the capability of other invertebrates to feed on decomposing leaves, the presence and abundance of mining shredders (as reported Wantzen & Wagner, 2006), and the variability of leaf litter input to streams.

Acknowledgments

The first author received a scholarship from CNPq during his PhD in the Graduate Programme in Ecology, Conservation and Management of Wildlife in UFMG, CAPES and IMAR during his stay in Portugal. This study received financial support from CNPq (Proc 472328/01-8), FAPEMIG (Proc 1085/03), PADI-Project AWARE Foundation and from the Portuguese Science Foundation (POCTI/1999/BSE/ 32389). The authors thank the logistical support offered by IBAMA and US Fish & Wildlife Service. Thanks also to Verónica Ferreira, Cláudia Mieiro, Nuno Coimbra, Juliana França, Rafael Ligeiro and Joana D'Arc de Paula for their assistance in the field and laboratory. We also thank the valuable comments Prof. Roger Jones and two anonymous referees.

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(Manuscript accepted 26 February 2007)