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Leaf breakdown in two tropical streams: Differences between single and mixed species packs

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Abstract

We assessed leaf breakdown of five native riparian species from Brazilian Cerrado (*Myrcia guyanensis*, *Ocotea* sp., *Miconia chartacea*, *Protium brasiliense*, and *Protium heptaphyllum*), incubated in single and mixed species packs in two headwater streams with different physico-chemical properties in the Espinhaço Mountain range (Southeastern Brazil). Leaves were placed in plastic litter bags (15 cm × 20 cm, 10 mm mesh size) and the experiments were carried out during the dry seasons of 2003 and 2004. Leaf nitrogen and phosphorus contents were similar in all species, but polyphenolic contents were different ($P<0.001$). *M. guyanensis* showed higher polyphenolics content (8.48% g^{-1} dry mass) and leaf toughness. Individually, higher breakdown rates were found in *M. guyanensis* at Indaiá stream ($k = 0.0063 \pm 0.0005\,d^{-1}$) and in *Ocotea* sp. at Garcia stream ($k = 0.0088 \pm 0.0006\,d^{-1}$). However, *P. brasiliense* and *P. heptaphyllum* showed lower breakdown rates at Indaiá and Garcia streams (Indaiá: $k = 0.0020 \pm 0.0002$ and $0.0019 \pm 0.0001\,d^{-1}$; Garcia: $k = 0.0042 \pm 0.0001$ and $0.0040 \pm 0.0002\,d^{-1}$). Single and mixed breakdown processes of each species were not statistically different on both streams. However, all species showed higher breakdown rates at Garcia stream ($P<0.01$). These results suggest that leaf breakdown is not altered when litter benthic patches are composed by a mixture of species in the same proportions that they occur on riparian leaf falls.

Keywords: Breakdown rates; Leaf toughness; Nutrients; Polyphenolics; Brazilian Cerrado

Introduction

Allochthonous organic matter input is the main energy source to aquatic communities in streams where the stream bed is shaded by riparian vegetation (Minshall, 1967; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980; Webster & Meyer, 1997). Studies on the decomposition process of organic detritus in lotic ecosystems have received considerable attention since the late 1960s, due to their role in the energetic metabolism of these environments (Abelho, 2001). However, equivalent studies on tropical streams are scarce and little is known about the processing of organic matter in these ecosystems. Recent studies at lower latitudes include those of Mathuriau and Chauvet (2002) and Chará (2003) in Colombia, Dobson, Mathooko, Ndegwa, and M'erimba (2003) in Kenya, Abelho, Cressa, and Graça (2005) in Venezuela, and Moulton and Magalhães (2003), Wantzen, Rosa, Neves, and Da Cunha (2005), and Gonçalves, França, Medeiros, Rosa, and Callisto (2006b) in Brazil.

Leaf breakdown rates may vary in a wide scale of magnitude among species (Cornelissen, 1996; Petersen &

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Cummins, 1974). They are influenced by internal (leaf physical and chemical characteristics) and external factors (e.g., water chemical composition, temperature, discharge, dissolved oxygen, and biotic communities) (Abelho, 2001; Kaushik & Hynes, 1968; Webster & Benfield, 1986). Lower breakdown rates are associated to leaf toughness, which is influenced by lignin and tannin contents (Hoorens, Aerts, & Stroetenga, 2003; Ostrofsky, 1997; Pereira, Graça, & Molles, 1998). These traits may affect leaf breakdown due to their influence on the activity of decomposing microorganisms and invertebrate shredders (Canhoto & Graça, 1996; Gessner & Chauvet, 1994; Rosemond, Pringle, Ramírez, Paul, & Meyer, 2002).

In headwaters, leaf breakdown is a critical process that affects availability of food to aquatic webs (Cummins, Wilzbach, Gates, Perry, & Talaiferro, 1989; Minshall, 1967; Wallace, Eggert, Meyer, & Webster, 1997). Studying the breakdown of a single species is not sufficient to understand the energy flow through the ecosystem (Hoorens et al., 2003), especially in tropical ecosystems where riparian corridors are composed of many tree species. In such environments, leaves are found in mixed species packs on the stream beds. As leaf structure and chemical composition may differ considerably among species (Webster & Benfield, 1986), species composition of mixed leaf packs may affect the breakdown rate of each species individually (Leff & McArthur, 1989; Petersen & Cummins, 1974). Thus, the breakdown of mixed packages may result from the characteristics of all the component species (additive effects) or be determined by a dominant species in the process (key-species) (Hoorens et al., 2003; Swan & Palmer, 2004; Wardle, Bonner, & Nicholson, 1997). In Savannah-like ecosystems such as the Brazilian Cerrado, a main trait of the vegetation is its sclerophylly, with species differing in toughness, lignin, and tannin content (Madeira, Ribeiro, & Fernandes, 1998; Marques, Garcia, & Fernandes, 1999). Therefore, studies in such environment should be important in an attempt to bridge the gap existing in our understanding of the decomposition process in a more broad scale.

Knowing that Brazilian Cerrado leaves may differ in their chemical and physical characteristics, we hypothesized that leaf breakdown rates would be different in single and mixed species leaf packs. We expected that leaf processing of different species would affect each other when incubated on mixed packages, altering the individual breakdown rates. So, the main goal of this study was to assess the breakdown rates of five native Brazilian Cerrado riparian leaves (*Myrcia guyanensis* Aubl., *Ocotea* sp. Aubl., *Miconia chartacea* Triana, *Protium brasiliense* Engl., and *Protium heptaphyllum* March.) when incubated in single and mixed species leaf packs. To verify the influence of stream characteristics on leaf breakdown in tropical ecosystems, experiments were conducted in two headwater streams that present different landscape cover, use, and water properties.

Material and methods

Study area

The Cerrado Biome of Tropical South America covers about 2 million km^2, representing ca. 22% of the land surface of Brazil, plus small areas in eastern Bolivia and northwestern Paraguay (Oliveira & Marquis, 2002). The riverine forests following the drainage throughout the Cerrado Biome cover probably less than 10% of its total area but harbor an enormous floristic and faunal diversity (Da Silva & Bates, 2002). Some databases contain more than 600 species of trees for riverine forests of the Cerrado Biome and it is certain that they contain more, once there are still relatively few surveys of these forests (Fonseca, Sousa-Silva, & Ribeiro, 2001; Oliveira & Marquis, 2002).

Indaiá and Garcia streams belong to the headwaters of the Doce river basin and are located in the southern Espinhaço Mountain range (Southeastern Brazil), a water divisor of the Brazilian basins of São Francisco and Doce rivers (Fig. 1). According to the Koppen Climate Classification System, the climate type of this area is Tropical of Altitude (Cwb) with cool summers and dry winters. During the experiments (dry season), monthly means of precipitation and air temperature at study streams ranged from 5 to 20 mm and 18 to 22 °C (www.simge.mg.gov.br).

Indaiá stream is located in the Serra do Cipó National Park (33,800 ha), a well-preserved landscape consisting of rocky grasslands at higher elevations and highly diverse riverine forests in the valleys and canyons. The experiment was carried out in a third order reach (19° 16.4′S–43° 31.2′W), at the altitude of 1459 m a.s.l, where waters present total N and total P contents of 0.19 and 0.02 mg L^{-1}, respectively. The riparian corridor at the study site is 7 m wide, shading most of the stream bed, and is mainly composed of *Augusta longifolia* (Spreng) Rehder (Rubiaceae), *Erythroxylum pelletarianum* St. Hil (Erythroxylaceae), *M. chartacea*, *Miconia cyathanthera* Triana (Melastomataceae), *M. guyanensis* (Myrtaceae), *Ocotea* sp. (Lauraceae), and *P. brasiliense* (Burseraceae) (Gonçalves, França, & Callisto, 2006a).

Garcia stream is located in Serra do Ouro Branco, where the landscape consists of grasslands, grazing areas, and forest fragments of different areas. The study was carried out in a third order reach (20° 21′S–43° 41′W) inside a forest fragment, at the altitude of 1300 m a.s.l. In this reach, waters present higher nutrient contents (total N: 1.0 mg L^{-1}, total P: 0.026 mg L^{-1}) and the stream bed is totally shaded by the riparian

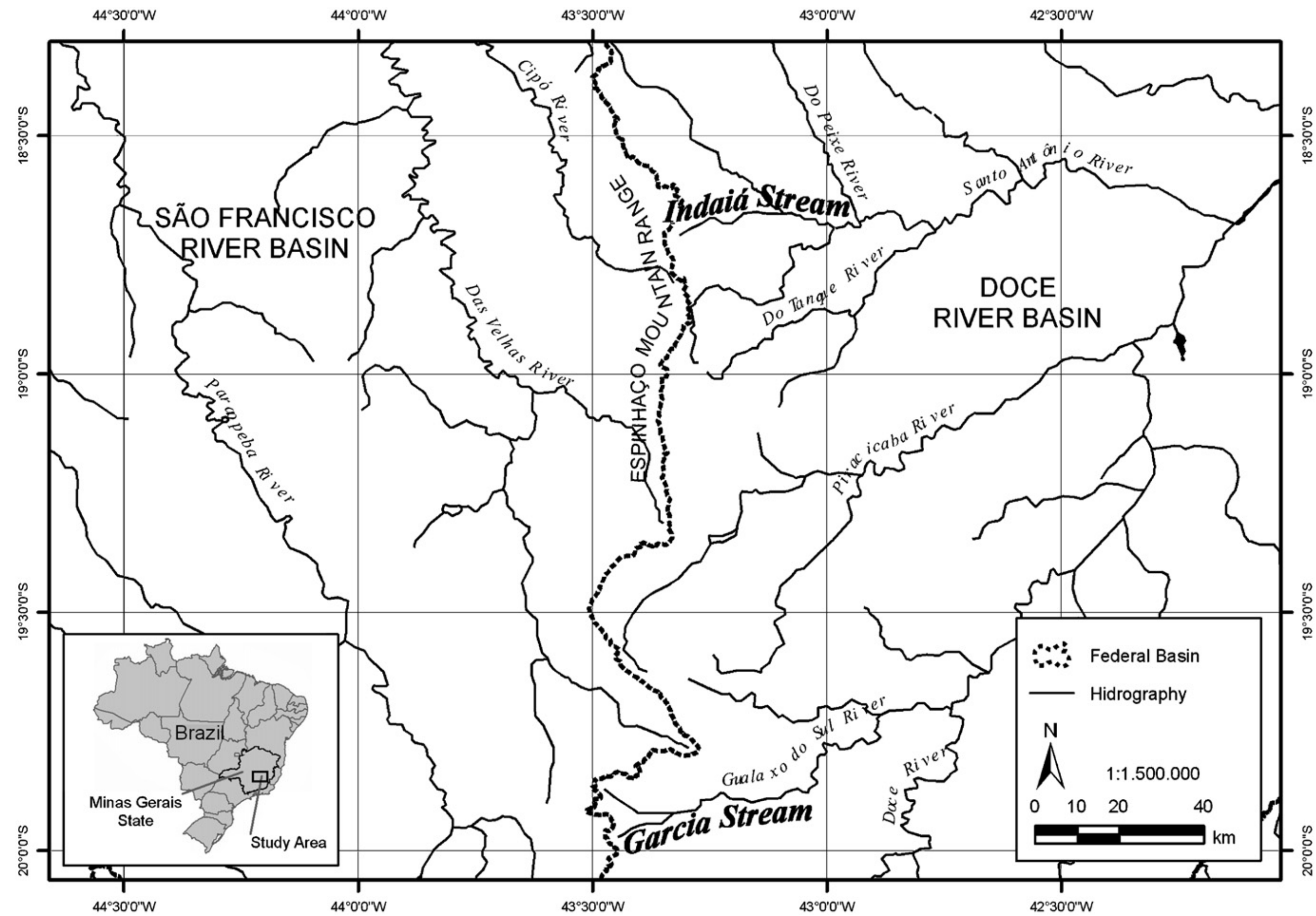

Fig. 1. Map of the study area, showing the location of Indaiá and Garcia streams in the Espinhaço Mountain range (Southeastern Brazil) (dotted line indicates Espinhaço Mountain range watershed).

vegetation, mainly composed of *Vochysia tucanorum* Mart. (Vochysiaceae), *Leandra scabra* DC., *Miconia corallina* Spring (Melastomataceae), *P. heptaphyllum* (Burseraceae), *Alibertia concolor* K. Schum (Rubiaceae), *Myrcia detergens* Miq., and *Myrceugenia ovata* O. Berg (Myrtaceae) (F.O. Silva, pers. obs.).

Leaf sampling

The species used in this experiment were chosen because they are the five most abundant in the leaf fall (vertical input) of Indaiá stream during all the year (Gonçalves et al., 2006a). All of them are native of the Cerrado Biome, showing adaptations to the harsh conditions (e.g., water stress, high solar radiation, and herbivory) and are very common on the riparian zones of headwater streams (Fonseca et al., 2001).

Leaves were collected from the riparian vegetation of the two margins of Indaiá stream using four plastic nets ($1\,m^2$, 10 mm mesh size) approximately 1.5 m height. From February 2003 to March 2004, leaves were collected every month on the nets and then transported into plastic bags to the laboratory. Then, they were air dried, sorted by species, and stored at room temperature. Species identification was carried out at the Institute of Biological Sciences Herbarium, Federal University of Minas Gerais.

Stream characterization

On each sampling date, width and depth of the study reaches were taken. Stream flow was measured using a flowmeter (Global Water, Gold River, CA, USA) while other abiotic parameters (pH, dissolved oxygen, electrical conductivity, and temperature) were measured *in situ* using a multi-probe Horiba model U-10 (Horiba, Irvine, CA, USA). On the beginning of the experiment, water nitrogen and phosphorus contents in the Garcia stream were determined according to "Standard Methods for the Examination of Water and Wastewater" (APHA, 1992). Data on the nitrogen and phosphorus contents of the water in the Indaiá stream were taken from Callisto, Goulart, Medeiros, Moreno, and Rosa (2004).

Leaf analyses

Leaves of all studied species were ground for the analyses of total polyphenolics [Folin–Denis method according to Martin and Martin (1982) and Bärlocher and Graça (2005)], total nitrogen [Kjeldahl method according to Sarruge and Haag (1974) and Malavolta and Netto (1989)], and total phosphorus (Malavolta & Netto, 1989; Miyazawa, Pavan, & Bloch, 1992). Leaf toughness was estimated using a device that measures the force needed to tear apart a leaf sample (see Graça & Zimmer, 2005). To determine the mean resistance of each species on time 0, leaf disks were cut, avoiding leaf veins, from three leaves of each species using a cork borer (three disks per leaf).

Experimental design

The experiments were carried out in the dry seasons (May–September) of 2003 (Indaiá stream) and 2004 (Garcia stream). Single and mixed leaf packs, composed by all studied species, were placed into plastic litter bags (15 cm × 20 cm, 10 mm mesh size). Single leaf packs consisted of 1.0 ± 0.005 g (air dry mass) while mixed leaf packs consisted of 2.0 ± 0.005 g. Proportions of each component species of the mixed leaf packs were determined from the leaf fall data of these species at Indaiá stream (Gonçalves et al., 2006a). Then, the biomass of each species used in the mixed leaf packs was determined: *M. guyanensis* (0.58 g), *Ocotea* sp. (0.20 g), *M. chartacea* (0.20 g), *P. brasiliense* (0.32 g), and *P. heptaphyllum* (0.70 g).

A total of 144 litter bags (24 of each single species and 24 containing the mixed species package) was incubated on the stream bed of each stream, tied to the marginal vegetation and submerged stones, under similar flow and turbulence conditions. Four replicates of each treatment were collected randomly after 7, 15, 30, 60, 90, and 120 days of incubation. On each sampling date, the samples were placed individually in plastic bags and transported in a cool box to the laboratory, where the leaves were washed to remove associated debris and invertebrates. Leaves of the different species were sorted and dried at 60 °C for 72 h to determine the leaf dry mass remaining. Approximately 5.0 g of each leaf species (four replicates per species), which had not been used in any experiment, were dried at 60 °C until they reached constant mass to calculate a conversion factor air dry mass/oven dry mass to estimate initial oven dry mass of the leaves used in the experiment.

Statistical analysis

Breakdown rates were estimated using the exponential decay model $W_t = W_o e^{-kt}$, where W_t is the remaining mass at time t, W_o is the initial mass, and k is the breakdown rate (Webster & Benfield, 1986). Regression lines of ln transformed data were compared by analysis of covariance (ANCOVA; Zar, 1999) to look for differences in breakdown rates between treatments. Leaf characteristics were compared by analysis of variance (ANOVA) followed by a Tukey test whenever the null hypothesis was rejected at $P < 0.05$ (Zar, 1999). Statistical analyses were performed using the Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA).

Results

Streams and leaf characteristics

During the experiments, the water temperature of the two streams was similar (Table 1). The Indaiá stream showed lower values of dissolved oxygen (4.1 mg L^{-1}), low discharge (0.23 $m^3 s^{-1}$), and acid waters (pH 5.9).

Leaf nitrogen and phosphorus contents were low and similar in all plant species (Table 2). *M. guyanensis* presented higher total polyphenolic content and leaf toughness. *Ocotea* sp. showed lower polyphenolic

Table 1. Water properties of Indaiá and Garcia streams (range was calculated from seven measures taken during the experimental periods)

Parameters	Indaiá stream		Garcia stream	
	Mean	Range	Mean	Range
Width (m)	2	1–3	4	2–5
Depth (cm)	35	15–60	21	15–25
Water discharge ($m^3 s^{-1}$)	0.23	0.19–0.32	0.51	0.37–0.69
Temperature (°C)	16.1	14.4–18.2	16.5	13.8–19.0
Dissolved oxygen (mg L^{-1})	4.1	3.1–7.7	8.6	4.9–10.2
pH	5.9	5.4–6.4	7.6	7.0–7.9
Electrical conductivity (μS cm^{-1}) at 25 °C	76	50–110	220	180–350

Table 2. Percentages of nitrogen, phosphorus, and polyphenolics in leaves (%g^{-1} dry mass) and toughness values (g) (mean ± SE, $n = 3$)

	Nitrogen	Phosphorus	Polyphenolics*	Toughness
Myrcia guyanensis	0.95±0.07	0.030±0.000	8.48±0.22[a]	869.90±65.96
Ocotea sp.	1.11±0.06	0.030±0.000	6.31±0.16[c]	590.17±6.60
Miconia chartacea	0.88±0.03	0.027±0.003	7.31±0.28[b]	481.66±63.66
Protium brasiliense	0.98±0.11	0.027±0.003	6.37±0.17[c]	731.95±80.90
Protium heptaphyllum	0.92±0.03	0.023±0.003	7.83±0.11[a,b]	576.87±167.31
F	1.611	1.167	30.264	1.776
P	0.246	0.382	< 0.001	0.210

*Percentages of polyphenolics with identical superscript letters are not statistically different ($P > 0.05$).

content while lower toughness values were found on *M. chartacea*. Only polyphenolic content was significantly different between the species studied (ANOVA; Table 2).

Breakdown rates

Indaiá stream: During the 120 days of incubation, *M. guyanensis* and *Ocotea* sp. showed the faster breakdown rates in the single species leaf packs ($k = 0.0063 \pm 0.0005$ and $0.0043 \pm 0.0003\,d^{-1}$, 49% and 57% of mass remaining, respectively) whereas *P. brasiliense* and *P. heptaphyllum* presented the slower rates (respectively, $k = 0.0020 \pm 0.0002$ and $0.0019 \pm 0.0001\,d^{-1}$, 80% and 81% of mass remaining; Fig. 2). Leaf breakdown of *M. chartacea* was $k = 0.0033 \pm 0.0003\,d^{-1}$, with 64% of dry mass remaining at the end of the experiment. The breakdown rate of each species between single and mixed leaf packs did not differ statistically (ANCOVA, $P > 0.05$; Fig. 2), although *P. brasiliense* presented the slowest rate when incubated in mixed packs.

Garcia stream: Breakdown rates in the single species leaf packs were faster in *Ocotea* sp. and *M. guyanensis*, with 34% and 53% of mass remaining, respectively ($k = 0.0088 \pm 0.0006$ and $0.0053 \pm 0.0003\,d^{-1}$; Fig. 2). *P. brasiliense* and *P. heptaphyllum* leaf material decomposed slowly, with 63% and 66% of the mass remaining, respectively ($k = 0.0042 \pm 0.0001$ and $0.0040 \pm 0.0002\,d^{-1}$). *M. chartacea* leaf material showed intermediate breakdown rates with 56% of mass remaining at the end ($k = 0.0051 \pm 0.0002\,d^{-1}$). With the exception of *Ocotea* sp., breakdown rates tended to be slightly faster in the mixed packs, but the difference was not significant in any case (ANCOVA, $P > 0.05$; Fig. 2).

Indaiá vs. Garcia: Except for *Ocotea* sp., which decomposed always faster in single leaf packs, breakdown of the leaves followed the same general pattern in both streams. Breakdown rates of all species were significantly different in the Indaiá and the Garcia streams (ANCOVA, $P < 0.01$), being higher for leaves incubated in the Garcia stream (Fig. 2).

Discussion

Few studies assessed the importance of mixed-litter breakdown in aquatic ecosystems, specially in the tropics. In our study, breakdown rates did not change when species were incubated in mixed leaf packs, rejecting the prediction of our hypothesis. These results corroborate the ones obtained by Leff and McArthur (1989) in a decomposition experiment with mixed leaf packs in a temperate stream. However, in a similar study in Maryland (USA), Swan and Palmer (2004) found differences between single and mixed treatments both in the summer and autumn. According to these authors, the high predictability of mixture effects on breakdown rates (additive effects) during autumn may be a consequence of the slow leaf processing observed at this season, since low temperatures reduce decomposers activity. In a highly seasonal environment such as the Cerrado, leaves may decompose at different rates in the summer and winter. Our study that was carried out in the dry season (autumn–winter) found low breakdown rates in both treatments. This trend was probably a result of the studied species leaf quality, which may also have influenced decomposers activity (Gonçalves et al., 2006b), once temperature does not seem to be a limiting factor to leaf processing in Brazilian Cerrado streams (Gonçalves, Graça, & Callisto, 2006c).

A possible explanation for the non-difference between single and mixed treatments could be the similarity of the five leaf species in terms of toughness, nitrogen, and phosphorus contents. Only polyphenolic content differed significantly among species, but this difference apparently was not sufficient to influence breakdown process since these compounds are quickly removed from the leaf tissues by leaching (Abelho, 2001; Boulton & Boon, 1991; Webster & Benfield, 1986). Chapman, Whittaker, and Heal (1988) and Wardle et al. (1997)

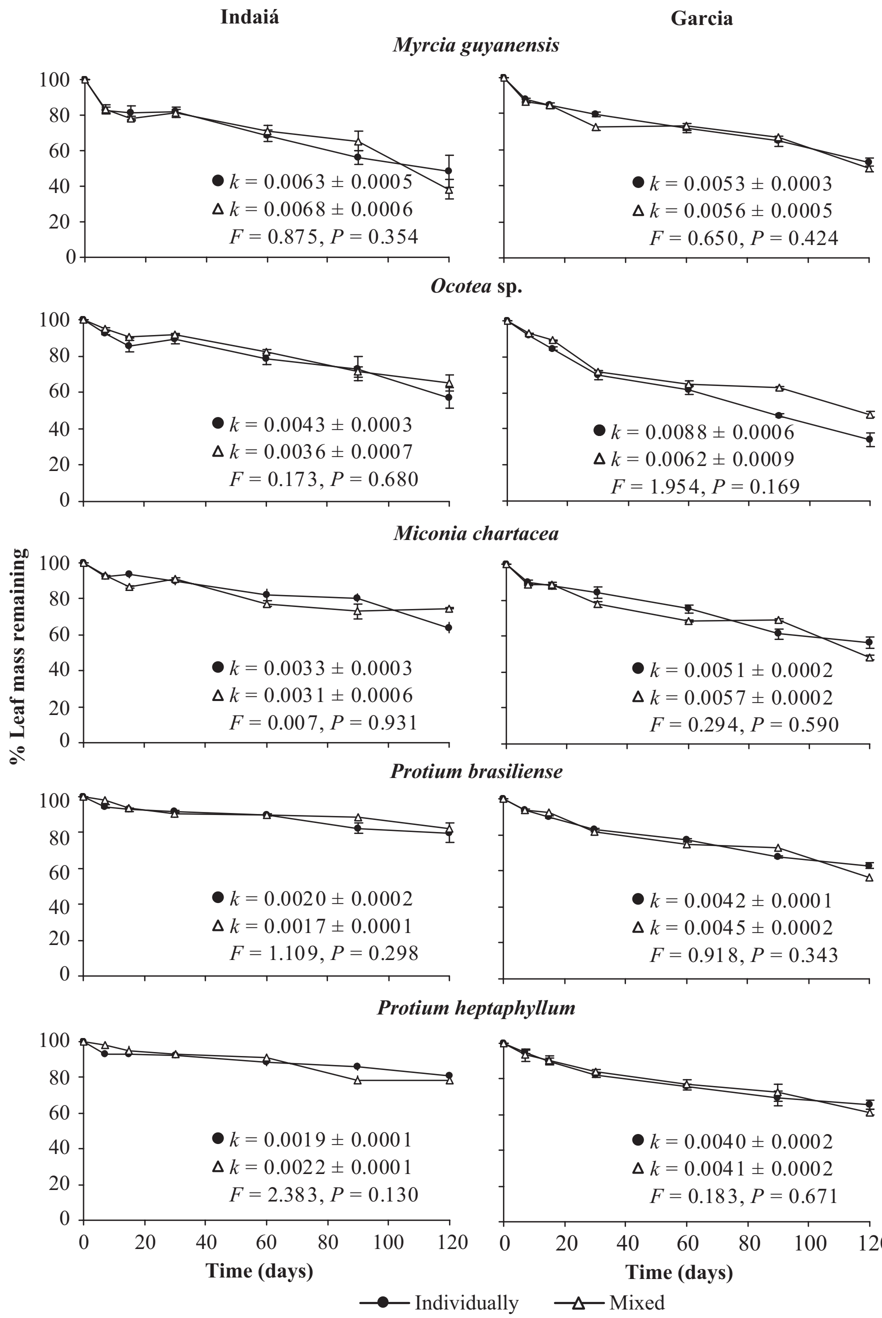

Fig. 2. Leaf mass remaining and breakdown rates ($-k\,d^{-1}$) of the studied species when incubated individually and in mixed species packs at Indaiá and Garcia streams (mean ± SE, $n = 4$).

suggested that the breakdown of one given species is different when incubated in mixed species leaf packs, and that leaf species richness is not directly related to breakdown rates. Moreover, Hoorens et al. (2003) concluded that initial differences on chemical characteristics of each component species are not useful to explain interactions on mixed packages in terrestrial environments.

Our results of water chemistry indicate that both streams can be considered oligotrophic (Wetzel, 1993), and the differences in water nutrient contents may be correlated with landscape factors, once Indaiá stream is located inside a National Park and Garcia is close to some little farms and roads. Except for *M. guyanensis*, all species showed faster breakdown rates at Garcia stream, probably due to the higher values of water discharge, dissolved oxygen, and dissolved nutrients presented by this stream. Previous studies developed by Gonçalves et al. (2006c) and Gonçalves, Jr., Graça, and Callisto (in press) at tropical Cerrado, temperate and Mediterranean streams found slower breakdown rates in Indaiá stream, which also presented lower water nutrient contents. Studies developed on temperate streams showed that the growth and the activity of aquatic hyphomycetes are stimulated by water nutrient content (Rosemond et al., 2002; Suberkropp & Chauvet, 1995) and water turbulence (Suberkropp, 1998).

The Brazilian Cerrado presents acidic soils and low contents of mineral nutrients, but high contents of aluminum and iron (Fonseca et al., 2001). Cerrado tree species also have leaves with thick cuticles and high contents of structural and inhibitory compounds that makes their breakdown difficult (Marques, Garcia, Resende, & Fernandes, 2000; Oliveira, Meirelles, & Salatino, 2003; Wantzen et al., 2005; Wantzen & Wagner, 2006). The five species of the present study had low nutrient contents (nitrogen and phosphorus) and high polyphenolics and toughness values, compared with temperate leaves (see Canhoto & Graça, 1996; Ostrofsky, 1997), which probably contributed to the generally slow decomposition rates found in this study. The fast breakdown rates of *M. guyanensis* were probably due to a fast leaching of chemical compounds in the first 7 days of incubation (Boulton & Boon, 1991; Suberkropp, Godshalk, & Klug, 1976). On the other hand, the fast breakdown of *Ocotea* sp. may be related to lower contents of inhibitory compounds, which facilitate microbial colonization and turn the leaves more palatable to aquatic invertebrates (Mathuriau & Chauvet, 2002; Rosemond et al., 2002).

P. brasiliense and *P. heptaphyllum* seem to have developed different strategies against herbivory, despite belonging to the same genus. While *P. brasiliense* is tougher, *P. heptaphyllum* produced more chemical inhibitory compounds (polyphenolics). In our study, the result of these strategies seems to exert the same effect on the breakdown rates of both species. According to Canhoto and Graça (1996), total or partial removal of cuticles, which normally are thick and waxy, is needed to hasten polyphenolics solubilization, hyphomycetes colonization, and decomposition.

Gessner and Chauvet (1994) suggested that breakdown studies must be directed to understand the relationship between detritus chemical nature and breakdown rates. In other words, research should identify the biological mechanisms by which detritus quality affects breakdown rates. The content of polyphenolics, defensive compounds produced by plant tissues, varies with leaf species, age, and degree of decomposition (Bärlocher & Graça, 2005). Ostrofsky (1997) did not find a clear correlation between polyphenolic content and breakdown rates, concluding that breakdown rates are best explained by a combination of factors related to nutritional quality, refractory capacity, and residual inhibitors.

In conclusion, our results suggest that the breakdown rates of some Brazilian Cerrado tree species are not dependent on the diversity of leaves present in the benthic litter patches of the streams. However, in the large picture of tropical ecosystems, this information may be of great importance due to the constant human impacts that riparian zones are suffering, specially in the developing countries. We expect that our study may be useful to a better understanding of the patterns that determine organic matter processing in tropical streams and contributes with some information to the conservation of Brazilian Cerrado Biome that is one of the 25 terrestrial hotspots (Myers, Mittermeier, Mittermeier, Fonseca, and Kent, 2000) and is considered the largest, the richest, and possibly the most threatened tropical savannah of the whole world (Diniz-Filho, Bini, Bastos, Vieira, & Vieira, 2005).

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References

Abelho, M. (2001). From litterfall to breakdown in streams: A review. *The Scientific World*, *1*, 656–680.

Abelho, M., Cressa, C., & Graça, M. A. S. (2005). Microbial biomass, respiration, and decomposition of *Hura crepitans* L. (Euphorbiaceae) leaves in a tropical stream. *Biotropica*, *37*, 397–402.

APHA. (1992). *Standard methods for the examination of water and wastewater* (18th ed.). Washington, DC: American Public Health Association.

Bärlocher, F., & Graça, M. A. S. (2005). Total phenolics. In M. A. S. Graça, F. Bärlocher, & M. O. Gessner (Eds.), *Methods to study litter decomposition: A practical guide* (pp. 45–48). Dordrecht: Springer.

Boulton, A. J., & Boon, P. I. (1991). A review of methodology used to measure leaf litter decomposition in lotic environments: Time to turn over an old leaf? *Australian Journal of Marine and Freshwater Research*, *42*, 1–43.

Callisto, M., Goulart, M., Medeiros, A. O., Moreno, P., & Rosa, C. A. (2004). Diversity assessment of benthic macroinvertebrates, yeasts, and microbiological indicators along a longitudinal gradient in Serra do Cipó, Brazil. *Brazilian Journal of Biology*, *64*, 743–755.

Canhoto, C., & Graça, M. A. S. (1996). Decomposition of *Eucalyptus globulus* leaves and three native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. *Hydrobiologia*, *333*, 79–85.

Chapman, K., Whittaker, J. B., & Heal, O. W. (1988). Metabolic and faunal activity in litters of tree mixtures compared with pure stands. *Agriculture, Ecosystems and Environment*, *24*, 33–40.

Chará, J. D. (2003). *Interactions between biodiversity and land use in low-order stream catchments of the Colombian Andes*. Ph.D. thesis, Institute of Aquaculture, University of Stirling, Stirling, Scotland.

Cornelissen, J. H. C. (1996). An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology*, *84*, 573–582.

Cummins, K. W., Wilzbach, M. A., Gates, D. M., Perry, J. B., & Talaiferro, W. B. (1989). Shredders and riparian vegetation. *Bioscience*, *39*, 24–30.

Da Silva, J. M. C., & Bates, J. M. (2002). Biogeographic patterns and conservation in the South American Cerrado: A tropical savannah hotspot. *Bioscience*, *52*, 225–233.

Diniz-Filho, J. A. F., Bini, L. M., Bastos, R. P., Vieira, C. M., & Vieira, L. C. G. (2005). Priority areas for anuran conservation using biogeographical data: A comparison of greedy, rarity and simulated annealing algorithms to define reserve networks in Cerrado. *Brazilian Journal of Biology*, *65*, 251–261.

Dobson, M., Mathooko, J. M., Ndegwa, F. K., & M'erimba, C. (2003). Leaf litter processing rates in a Kenyan highland stream, the Njoro River. *Hydrobiologia*, *519*, 207–210.

Fonseca, C. E. L., Sousa-Silva, J. C., & Ribeiro, J. F. (2001). *Cerrado: Caracterização e recuperação de matas de galeria*. Planaltina: Embrapa Cerrados.

Gessner, M. O., & Chauvet, E. (1994). Importance of stream microfungi in controlling breakdown rates of leaf litter. *Ecology*, *75*, 1807–1817.

Gonçalves, J. F., Jr., França, J., & Callisto, M. (2006a). Dynamics of allochthonous organic matter in a tropical brazilian headstream. *Brazilian Archives of Biology and Technology*, *49*, 967–973.

Gonçalves, J. F., Jr., França, J. S., Medeiros, A. O., Rosa, C. A., & Callisto, M. (2006b). Leaf breakdown in a tropical stream. *International Review of Hydrobiology*, *91*, 164–177.

Gonçalves, J. F., Jr., Graça, M. A. S., & Callisto, M. (2006c). Leaf-litter breakdown in 3 streams in temperate, Mediterranean and tropical Cerrado climates. *Journal of the North American Benthological Society*, *25*, 344–355.

Gonçalves, J. F., Jr., Graça, M. A. S., & Callisto, M. (in press). Litter decomposition in a Cerrado – savannah stream is retarded by leaf toughness, low dissolved water nutrient levels and low densities of shredders. *Freshwater Biology*.

Graça, M. A. S., & Zimmer, M. (2005). Leaf toughness. In M. A. S. Graça, F. Bärlocher, & M. O. Gessner (Eds.), *Methods to study litter decomposition: A practical guide* (pp. 109–113). Dortrecht: Springer.

Hoorens, B., Aerts, R., & Stroetenga, M. (2003). Does initial litter chemistry explain litter mixture effects on decomposition? *Oecologia*, *137*, 578–586.

Kaushik, N. K., & Hynes, H. B. N. (1968). Experimental study on the role of autumn shed leaves in aquatic environments. *Journal of Ecology*, *56*, 229–243.

Leff, L. G., & McArthur, J. V. (1989). The effect of leaf pack composition on processing: A comparison of mixed and single species packs. *Hydrobiologia*, *182*, 219–224.

Madeira, J. A., Ribeiro, K. T., & Fernandes, G. W. (1998). Herbivory, tannins and sclerophylly in *Chamaecrista linearifolia* (Fabaceae) along an altitudinal gradient. *Brazilian Journal of Ecology*, *2*, 24–29.

Malavolta, E., & Netto, A. V. (1989). *Nutrição Mineral, Calagem, Cessagem e Adubação dos Citros*. Piracicaba: Associação Brasileira para Pesquisa do Potássio e do Fosfato.

Marques, A. R., Garcia, Q. S., & Fernandes, G. W. (1999). Effects of sun and shade on leaf structure and sclerophilly of *Sebastiana mirtilloides* (Euphorbiaceae) from Serra do Cipó, Brazil. *Boletim de Botânica da Universidade de São Paulo*, *18*, 21–27.

Marques, A. R., Garcia, Q. S., Resende, J. L. P., & Fernandes, G. W. (2000). Variations in leaf characteristics of two species of *Miconia* in the Brazilian cerrado under different light intensities. *Tropical Ecology*, *41*, 47–60.

Martin, J. S., & Martin, M. M. (1982). Tannin assay in ecological studies: Lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents in mature foliage of six oak species. *Oecologia*, *54*, 205–211.

Mathuriau, C., & Chauvet, E. (2002). Breakdown of leaf litter in a neotropical stream. *Journal of the North American Benthological Society*, *21*, 384–396.

Minshall, G. W. (1967). Role of allochthonous detritus in the trophic structure of a woodland springbrook community. *Ecology*, *48*, 139–149.

Miyazawa, M., Pavan, M. A., & Bloch, M. F. (1992). *Análise Química de Tecido Vegetal*. Londrina: Instituto Agronômico do Paraná, Circular 74.

Moulton, T. P., & Magalhães, S. A. P. (2003). Responses of leaf processing to impacts in streams in Atlantic Rain Forest, Rio de Janeiro, Brazil – A test of the biodiversity-ecosystem functioning relationship? *Brazilian Journal of Biology*, *63*, 87–95.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853–858.

Oliveira, P. S., & Marquis, R. J. (2002). *The cerrados of Brazil: Ecology and natural history of netropical Savanna*. New York: Columbia University Press.

Oliveira, A. F. M., Meirelles, S. T., & Salatino, A. (2003). Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. *Anais da Academia Brasileira de Ciências*, *75*, 431–439.

Ostrofsky, M. L. (1997). Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *Journal of the North American Benthological Society*, *16*, 750–759.

Pereira, A. P., Graça, M. A. S., & Molles, M. (1998). Leaf litter decomposition in relation to litter physico-chemical properties, fungal biomass, arthropod colonization, and geographical origin of plant species. *Pedobiologia*, *42*, 316–327.

Petersen, R. C., & Cummins, K. W. (1974). Leaf pack processing in a woodland stream. *Freshwater Biology*, *4*, 343–368.

Rosemond, A. D., Pringle, C. M., Ramírez, A., Paul, M. J., & Meyer, J. L. (2002). Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography*, *47*, 278–289.

Sarruge, J. R., & Haag, H. P. (1974). *Análises Químicas em Plantas*. Piracicaba: Departamento de Química, USP/ESALQ.

Suberkropp, K. (1998). Microorganisms and organic matter decomposition. In R. J. Naiman, & R. E. Bilby (Eds.), *River ecology and management: Lessons from the Pacific coastal ecoregion* (pp. 120–143). Berlin: Springer.

Suberkropp, K., & Chauvet, E. (1995). Regulation of leaf breakdown by fungi in streams: Influences of water chemistry. *Ecology*, *76*, 1433–1445.

Suberkropp, K., Godshalk, G. L., & Klug, M. J. (1976). Changes in the chemical composition of leaves during processing in a woodland stream. *Ecology*, *57*, 720–727.

Swan, C. M., & Palmer, M. A. (2004). Leaf diversity alters litter breakdown in a Piedmont stream. *Journal of the North American Benthological Society*, *23*, 15–28.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*, 130–137.

Wallace, J. B., Eggert, S. L., Meyer, J. L., & Webster, J. R. (1997). Multiple trophic levels of a forest stream linked to terrestrial litter inputs. *Science*, *277*, 102–104.

Wantzen, K. M., Rosa, F. R., Neves, C. O., & Da Cunha, C. N. (2005). Leaf litter addition experiments in riparian ponds with different connectivity to a Cerrado stream in Mato Grosso, Brazil. *Amazoniana*, *18*, 387–396.

Wantzen, K. M., & Wagner, R. (2006). Detritus processing by invertebrate shredders: A neotropical–temperate comparison. *Journal of the North American Benthological Society*, *25*, 216–232.

Wardle, D. A., Bonner, K. I., & Nicholson, K. S. (1997). Biodiversity and plant litter: Experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos*, *79*, 247–258.

Webster, J. R., & Benfield, E. F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *17*, 567–594.

Webster, J. R., & Meyer, J. L. (1997). Organic matter budgets for streams: A synthesis. Stream organic matter budgets. *Journal of the North American Benthological Society*, *16*, 141–161.

Wetzel, R. G. (1993). *Limnologia*. Lisboa: Fundação Calouste Gulbenkian.

Zar, J. H. (1999). *Biostatistical analysis*. Upper Saddle River: Prentice Hall.