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Leaf-litter breakdown in 3 streams in temperate, Mediterranean, and tropical Cerrado climates

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Abstract. The objectives of our study were to assess leaf-litter breakdown in 3 streams in 3 climates and to determine the contributions of associated microbial and invertebrate communities to the process. We incubated leaves of *Alnus glutinosa* in 1 stream in each of 3 climate zones: temperate (mountains of Central Portugal), Mediterranean (South Portugal), and tropical Cerrado (Minas Gerais, Brazil). Leaf-litter breakdown rates (/d) were faster in temperate (k = 0.023–0.017) than in tropical (k = 0.014) or Mediterranean (k = 0.014–0.009) streams. Leaf-litter breakdown rates (/degree day) also were higher in the temperate stream (k = 0.0018–0.0032) and similar between the other 2 streams (k = 0.008–0.0012). Colonization of leaves by aquatic hyphomycetes was faster in the temperate stream (maximum = 421 μg ergosterol/g of leaf by day 24) than in the tropical Cerrado or Mediterranean streams. However, peak ergosterol content was highest in the tropical Cerrado stream (573 μg/g on day 75). Ergosterol content was lowest in the Mediterranean stream (maximum = 341 μg/g on day 7). Total microbial biomass (as ATP) was higher in the tropical Cerrado stream (maximum = 531 nmoles/g on day 75) than in the Mediterranean (maximum = 108 nmoles/g on day 92) and temperate (maximum = 93 nmoles/g on day 7) streams. These results suggest either that not all microorganisms associated with leaves were involved in leaf-litter breakdown or that other less efficient microorganisms than fungi were involved in leaf-litter breakdown in the tropical stream. Leaves exposed to invertebrates (coarse-mesh bags) decomposed significantly faster than leaves protected from invertebrate feeding (fine-mesh bags) only in the temperate stream. This result suggests that invertebrates were important mediators of leaf-litter breakdown only in the temperate stream. A larger proportion of invertebrates recovered from decomposing leaves were shredders in the temperate stream (nearly 5%) than in the Mediterranean (1%) and tropical Cerrado (0%) streams. Leaf-litter processing rates increased with discharge and NO_3 concentration in the water. Our results suggest that the positive effect of temperature on breakdown rates of allochthonous organic matter in streams can be overridden by nutrient content in the water and the presence of invertebrate shredders.

Key words: Savannah stream, Cerrado, aquatic hyphomycetes, litter breakdown, decomposition, leaf litter.

Food webs in forested headwater streams are sustained by organic matter from the riparian zone. Therefore, litter decomposition is a key process linking nutrient cycling, energy transfer, and trophic interactions (Benfield 1997, Wallace et al. 1997, Rosemond et al. 2002). In general, leaf-litter breakdown includes leaching of soluble compounds, microbial decomposition and conditioning, and feeding by aquatic invertebrates. The relative importance of microbes and invertebrates in leaf-litter breakdown seems to vary across systems (Eggert and Wallace 2003, reviews by Graça 2001, Abelho 2001).

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Among microorganisms, aquatic hyphomycetes are the most important decomposers in terms of biomass and activity (Baldy and Gessner 1997, Pascoal and Cassio 2004). Microbial communities enhance the quality of litter for invertebrate consumers because they have the capacity to break down structural compounds such as cellulose and lignin. They also take nutrients from the water column and incorporate them into the biofilm, thereby increasing leaf nutrient content, especially N and P (Graça 1993, Gessner et al. 1999, Haapala et al. 2001). Litter consumption by invertebrates is affected by intrinsic characteristics of leaves and by microbial colonization of leaves (Robinson et al. 1998, Graça 2001).

Most studies on leaf processing have been done in temperate deciduous forests (e.g., reviews by Abelho 2001, Graça 2001), but the few studies in tropical streams have shown that litter breakdown also can be affected by land use (Mathuriau and Chauvet 2002, Chara 2003) and nutrient availability (Pearson and Connolly 2000, Rosemond et al. 2002). The significance of invertebrates and microbial communities in detrital processing in the tropics remains unclear. Some studies suggest that an important difference between temperate and tropical systems is the low abundance of shredders in the tropics (e.g., Irons et al. 1994, Wantzen et al. 2002, Gonçalves et al. 2004, Wantzen and Wagner 2006 and references therein), whereas others have suggested that the apparent lack of shredders in tropical studies reflects sampling deficiencies that cause very large consumers, particularly crabs (Wantzen et al. 2002, Dobson 2004, Crowl et al. 2006), to be undersampled or missed altogether. Several recent studies have shown that invertebrate shredders have an important role in litter processing in some tropical systems (e.g., Crowl et al. 2001, 2006, Cheshire et al. 2005) and that tropical invertebrate shredders are selective feeders (Rincón and Martínez 2006).

Tropical savannah (Cerrado) covers 1,783,200 km^2 in central Brazil. Cerrado is the 2nd-largest biome in Brazil (after the tropical rain forest) and accounts for 21% of the area of Brazil, but it is under agricultural pressure (Sano and Almeida 1998). Cerrado has a pronounced dry season, and it supports a unique array of ~10,000 drought- and fire-adapted species of plants, including 4400 endemics (Giulietti 1997). Streams are frequently bordered by riparian trees, but studies of organic-matter dynamics in Cerrado systems are extremely scarce (but see Ciglasch et al. 2004). Wantzen and Wagner (2006) showed that litter input in Cerrado streams is comparable to input in the temperate zone, but shredders are scarce. Streams in the Cerrado generally have extremely low conductivity and buffering capacity (Wantzen 2003).

The Mediterranean climate is characterized by dry summers and mild winters. Many streams and rivers have surface flow only in winter, after the first rains, because of the precipitation regime and topography. In summer, streams can be reduced to perennial pools, and some stream sections dry completely. Leaf-litter breakdown studies are scarce in Mediterranean-climate streams, but studies carried out in South Europe and North Africa (e.g., Casas and Gessner 1999, Chergui and Pattee 1991a) have shown that some shredders typical of temperate zones are absent in Mediterranean zones, although their trophic niche may be occupied by other consumers such as gastropods.

Thus, the role of aquatic invertebrate consumers in leaf-litter processing is unclear outside temperate systems. The goal of our study was to compare leaf-litter breakdown and the roles of microorganisms and invertebrates in 3 streams located in different climate zones: temperate, Mediterranean, and tropical Cerrado. We tested 2 hypotheses: 1) Leaf-litter breakdown rates should be fastest in the tropical stream and slowest in the temperate stream because decomposition is largely a biotic process and temperature is a major factor affecting metabolism. 2) Streams from the 3 geographic areas do not differ in terms of shredder contribution to leaf-litter breakdown. This null hypothesis was tested because the data regarding the importance of shredders in litter processing is controversial.

Study Area

The temperate stream was São João, located in the Lousã Mountains, central Portugal (lat 40°6′N, long 8°14′W) at 230 m asl. The experimental stretch was in a 4th-order stream (4 m wide, 15 cm deep) and was shaded by a diverse canopy of riparian trees including *Castanea sativa* Miller, *Quercus* spp., *Pinus pinaster* Aiton, *Acacia dealbata* Link, and *Eucalyptus globulus* Labill.

The Mediterranean stream was Oeiras, located in southern Portugal ~5 km above the town of Almodôvar (lat 37°28′N, long 8°1′W) at 270 m asl. The experimental stretch was in a 4th-order intermittent stream (6 m wide, 50 cm maximum depth in pools) with no flow during summer. The riparian vegetation was sparse and mainly composed of shrubs of *Nerium oleander* L.

The tropical Cerrado stream was Indaiá, located in the Serra do Cipó National Park, south of the Espinhaço Cordillera, State of Minas Gerais, Brazil (lat 19°16.4′S, long 43°31.2′W) at 1450 m asl. The experimental reach was in a 3rd-order stream (2.5 m wide, 30 cm deep). The stream is in the high Cerrado, which is dominated by grasses and shrubs. The vegetation in the riparian zone is dense and mainly consists of *Augusta longifolia* (Spreng), *Erythroxylum*

pelleterianum St. Hil, *Miconia chartacea* Triana, *Miconia cyathanthera* Triana, *Myrcia guyanensis* (Aubl.) DC, *Ocotea lacifolia* (Schott) Mez., and *Protium brasiliense* (Spreng) Engl. Rains occur from October to April.

Methods

Leaf-litter breakdown experiments

The fine- and coarse-mesh-bags approach was used to assess the role of invertebrates and microbes in leaf processing. This method can be criticized as unnatural, and some invertebrates are not prevented from entering fine-mesh bags (e.g., Boulton and Boon 1991), but it is a standard, widely accepted method used to differentiate microbial from invertebrate litter processing. Leaves of alder (*Alnus glutinosa*) were used because information about decomposition of this species in the Northern Hemisphere is readily available and its breakdown is fast (Abelho 2001). Freshly fallen leaves were collected from the ground under a group of trees near Coimbra, Portugal, in autumn 2002 and stored dry in boxes. Air-dried leaves were weighed into 3.0 ± 0.1 g sets and enclosed in 20 × 20-cm bags (10-mm [coarse] and 0.5-mm [fine] mesh).

Leaf-litter breakdown experiments ran from November to December 2002 in the temperate stream (incubation periods of 1, 7, 24, and 35 d), from October 2002 to January 2003 in the Mediterranean stream (incubation periods of 1, 7, 15, 30, 51, and 92 d), and from May to July 2003 in the Cerrado stream (incubation periods of 1, 7, 15, 30, 45, and 75 d). Dates were selected to coincide with the period of peak litter fall in all zones, and the experiments were adjusted to run until ~75% of the initial leaf mass was lost.

Three leaf bags were retrieved from each stream on each sampling date. Leaf bags were transferred to plastic zip-lock bags and transported to the laboratory in ice chests. In the laboratory, the bags were disassembled, and the leaves were washed gently with running tap water over a sieve (120-μm mesh) to remove debris and invertebrates. The invertebrates retained on the sieve were preserved in alcohol for later identification and classification into functional feeding groups according to Merritt and Cummins (1996) and Tachet et al. (1987).

Three sets of 5 leaf disks were cut from the leaves in each bag with a cork borer (7-mm diameter). The discs were obtained from contiguous areas of the leaves, avoiding the main ribs. One set of disks was used for determination of fungal biomass (mass of ergosterol, see below), the 2nd was used for determination of ATP content (see below), and the 3rd was used for measurement of ash-free dry mass (AFDM; oven dried at 60°C to constant mass, weighed, burned at 500°C for 4 h, and reweighed). Leaf material remaining after removal of the disks was dried, weighed, burned in a muffle furnace, and weighed again to determine AFDM. The mass of the disks, which was estimated by multiplying the AFDM of the 3rd set of disks × 3, was added to the mass of leaf material remaining after removal of the disks.

The breakdown rate coefficient (k) was determined using the exponential decay model $W_t = W_0 e^{-kt}$, where W_t is the final mass of leaf material, W_0 is the initial mass of leaf material, and t is time (d). Calculations also were made using time expressed as degree days (dd; computed from values of mean water temperatures).

Microorganisms

Fungal biomass.—Fungal biomass was evaluated by ergosterol content. Sets of disks were preserved in 10 mL of methanol and stored in the dark at 4°C. Ergosterol was extracted by lipid extraction and saponification at 80°C for 30 min followed by purification of the crude extract by solid-phase extraction. The extracted lipids, including ergosterol, were partitioned into a nonpolar phase, evaporated to dryness, resuspended in methanol, and filtered. Ergosterol was quantified with HPLC (DIONEX Summit P580), and values were expressed as μg ergosterol/g leaf litter AFDM (Gessner 2005).

Microbial biomass.—ATP content was used as an indicator of total microbial biomass. ATP was extracted from sets of disks with 5 mL of 1.2 N H_2SO_4 containing 8 g/L oxalic acid and 5 mL HEPES buffer. The sample was homogenized, centrifuged, neutralized with NaOH, and stored frozen. ATP was quantified by the firefly bioluminescence method according to Abelho (2005).

Environmental variables

Temperature, pH, conductivity, and dissolved O_2 (DO) were measured with field meters at the beginning, middle, and end of each experiment. Current velocity (Braystoke BFM002 current meter, Valeport, Totnes, UK) was measured at a uniform section of the site, and discharge was calculated from velocity, stream width, and depth measurements. Water (1 L) was collected from the temperate and Mediterranean sites in clean plastic bottles and transported to the laboratory in an ice chest. In the laboratory, the water was filtered (0.48-μm glass-fiber filter) and used for determination of alkalinity (titration to pH 4.5; APHA 1998) and NO_3 and PO_4 content (Dionex® ion analyzer, Sunnyvale, California). Data for the tropical Cerrado stream were taken from Callisto et al. (2004).

Data analysis

Analysis of covariance (ANCOVA) was used to test for differences in leaf-litter breakdown rates between coarse- and fine-mesh bags and among the 3 streams. Tukey's tests were used to compare slopes among treatments. The assumption was made that invertebrate colonization increased linearly with time. ANCOVA was used to compare the rate of colonization of leaf bags in the 3 streams, after testing for normality (Komogorov–Smirnov) and log(x +1) transformation of invertebrate densities. Ergosterol and ATP data were used as dependent variables to compare microbial colonization among streams and between litter-bag mesh sizes. Each variable was log transformed and 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 (Zar 1996).

Results

Leaf-litter breakdown

Leaf-litter breakdown rates were faster in the temperate ($k = \sim 0.030$–0.017) than in the tropical Cerrado ($k = \sim 0.014$) or Mediterranean ($k = \sim 0.014$–0.009) streams in both coarse- and fine-mesh bags (ANCOVA, $F = 8.66$, $p = 0.0001$; Table 1, Fig. 1A). Leaf-litter breakdown rates differed between coarse- and fine-mesh bags in the temperate stream, but not in the Mediterranean or tropical Cerrado streams (Tukey's test: $p < 0.05$). When leaf-litter breakdown rates were corrected for temperature (i.e., calculated using dd), leaf-litter breakdown rates were faster in the temperate stream than in the Mediterranean stream, and faster in the Mediterranean stream than in the tropical Cerrado stream (ANCOVA: $F = 4.80$, $p = 0.0006$, Tukey's test: $p < 0.05$; Table 1, Fig. 1B).

Microorganisms

Fungal biomass.—Leaves had initial values of 12 μg ergosterol/g, suggesting some microbial colonization before incubation in the streams. Ergosterol content did not differ between leaves incubated in fine- and coarse-mesh bags (ANOVA: $F = 0.36$, $p = 0.55$), so data from both bags were pooled. Colonization of leaves by aquatic hyphomycetes (evaluated by ergosterol content) was fastest in the temperate stream, and ergosterol content reached 421 μg/g on the 24th day of incubation (Fig. 2). In the Mediterranean stream, ergosterol content reached 341 μg/g by day 7, but decreased afterwards. In the tropical Cerrado stream, ergosterol content increased slowly and consistently and reached 573 μg/g by day 75. Ergosterol content differed significantly among streams (ANOVA: $F = 8.32$, $p = 0.001$), and ergosterol content was higher in the temperate stream than in the Mediterranean or tropical Cerrado streams (Tukey's test: $p < 0.05$).

TABLE 1. Leaf-litter breakdown rates (k) and correlation coefficients (R^2) for *Alnus glutinosa* leaves in coarse- (10 mm) and fine- (0.5 mm) mesh bags in the temperate, Mediterranean, and tropical Cerrado streams. Exponential regressions were calculated for leaf mass loss relative to time expressed as days or degree days (dd).

		Time (d)		Time (dd)	
Location	Mesh	k	R^2	k	R^2
Temperate	Coarse	0.0295	0.976	0.0032	0.998
	Fine	0.0166	0.974	0.0018	0.975
Mediterranean	Coarse	0.0137	0.791	0.0012	0.704
	Fine	0.0093	0.812	0.0008	0.905
Tropical Cerrado	Coarse	0.0136	0.960	0.0009	0.960
	Fine	0.0143	0.906	0.0009	0.919

Microbial biomass.—ATP content of leaves (an indicator of total microbial biomass) did not differ between leaves incubated in fine- and coarse-mesh bags (ANOVA: $F = 0.21$, $p = 0.65$), so data from both bags were pooled. ATP content was highest in the tropical Cerrado stream (531 nmoles/g by day 75; Fig. 3). In the Mediterranean and temperate streams, maximum values were 108 nmoles/g (on day 92) and 93 nmoles/g (on day 7), respectively. ATP content differed significantly among streams (ANOVA: $F = 26.10$, $p = 0.0001$), and was higher in the tropical Cerrado stream than in the temperate or Mediterranean streams (Tukey's test: $p < 0.05$).

Invertebrates

Fine-mesh bags did not prevent invertebrates from colonizing leaves. However, invertebrates colonizing fine-mesh bags (mainly Chironomidae and Oligochaeta) were very small (Table 2). Colonization of leaves by invertebrates was greater in the temperate stream than in the Mediterranean and tropical Cerrado streams (ANCOVA: $F = 8.81$, $p = 0.00033$; Fig. 4). Chironomids were the most abundant invertebrates colonizing leaves in all 3 streams. Shredders made up ~5% of the taxa in the temperate stream (Nemouridae, Lepidostomatidae, Leuctridae), ~1% in the Mediterranean stream (Leuctridae, Nemouridae [within Others category]), and they were absent from the tropical Cerrado stream (Table 2).

Environmental variables

Streams were similar in terms of width, but they differed markedly in chemical characteristics and discharge (Table 3). The pH of temperate and

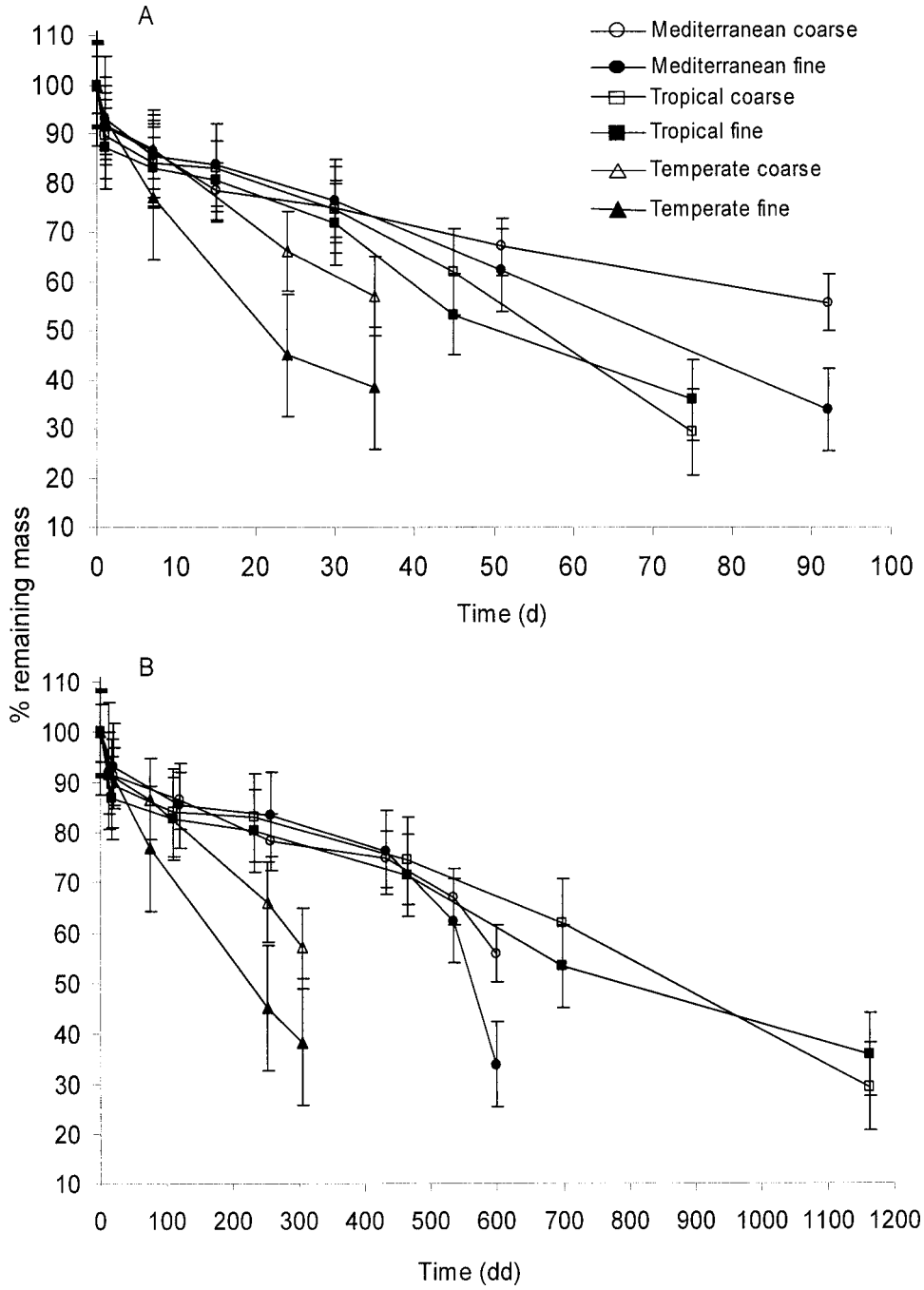

FIG. 1. Mean (±1 SE) mass loss relative to time expressed as days (A) and degree days (dd; B) of *Alnus glutinosa* leaves during leaf-litter breakdown experiments in 3 streams from temperate, Mediterranean, and tropical Cerrado climates.

Mediterranean streams was neutral, whereas the pH of the tropical Cerrado stream was low. The alkalinities of the tropical Cerrado and Mediterranean streams were similar, whereas the alkalinity of the temperate stream was comparatively low. The conductivities of the tropical Cerrado and temperate streams were similar (6–39 μS/cm), but the conductivity of the Mediterranean stream was much higher (401 μS/cm). NO_3 content of the temperate stream was higher than that of the tropical Cerrado stream (0.70 vs 0.05 mg/L N-NO_3), whereas PO_4 content of the tropical Cerrado stream was higher than that of the temperate stream (<0.01 vs 2.50 μg/L P-PO_4; Table 3). Discharge values reflected the seasonality of the systems (range 0.001 to 4.18 m^3/s across streams). The experiment coincided with the period of high precipitation in the temperate

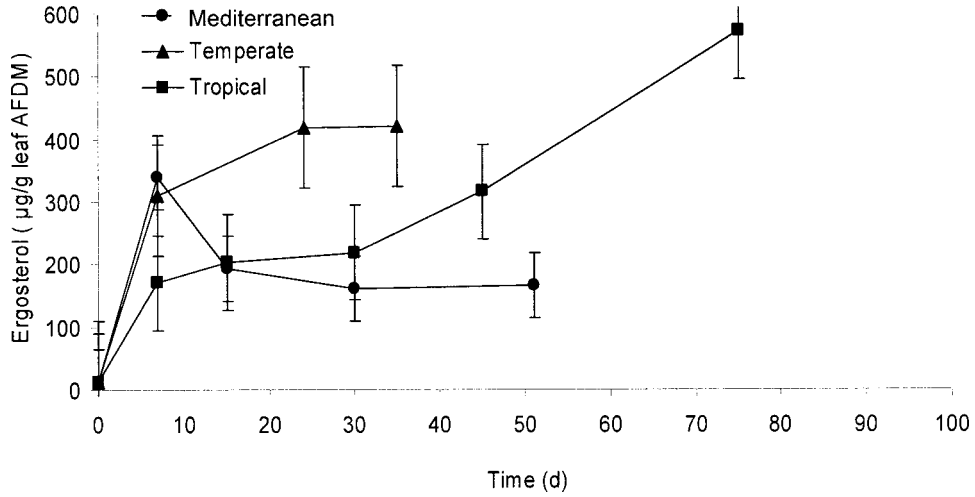

FIG. 2. Mean (±1 SE) ergosterol content of *Alnus glutinosa* leaves during leaf-litter breakdown experiments in 3 streams from temperate, Mediterranean, and tropical Cerrado climates (values from fine- and coarse-mesh bags pooled; $n = 6$). AFDM = ash-free dry mass.

and Mediterranean streams and with the period of low precipitation in the tropical Cerrado stream.

Discussion

Leaf-litter breakdown rates

Values of k in the temperate stream were similar to those previously obtained with *A. glutinosa* in the same temperate stream (our study: $k = 0.0166$ in fine-mesh bags; Canhoto and Graça 1996: $k = 0.0161$ in fine-mesh bags). Values of k obtained in the Mediterranean and tropical Cerrado streams (0.0093 to 0.0143) were in the lower range of values reported for this species in temperate regions ($k = 0.0123$; see review by Abelho 2001). In contrast to our results, Irons et al. (1994) reported higher decomposition rates of *Alnus crispa* in streams in Costa Rica than in Alaska and Michigan. In the Colombian Andes, Mathuriau and Chauvet (2002) reported very high leaf-litter breakdown rates for 2 tropical leaf species ($k = 0.0235$ to 0.0651; mean water temperature = 19°C). Similar to our results, Chara (2003) reported low decay rates for leaves of some native species (e.g., $k = 0.0008$ to 0.0645) in streams in the Colombian Andes (1500 m asl).

However, leaf-litter breakdown rates are strongly dependent on leaf chemistry and physical properties

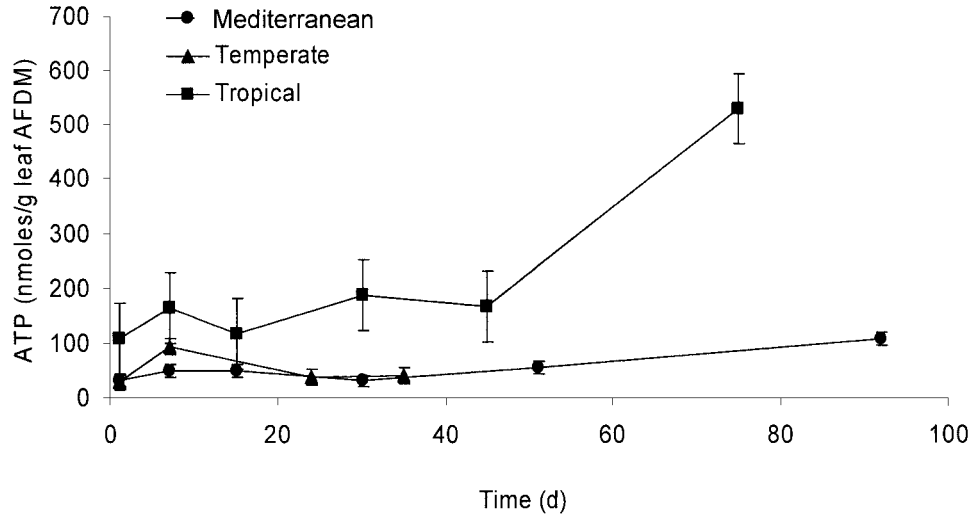

FIG. 3. Mean (±1 SE) ATP content of *Alnus glutinosa* leaves during leaf-litter breakdown experiments in 3 streams from temperate, Mediterranean, and tropical Cerrado climates (values from fine- and coarse-mesh bags pooled; $n = 6$). AFDM = ash-free dry mass.

TABLE 2. Mean densities and relative abundances of invertebrates colonizing leaves of *Alnus glutinosa* in coarse- (10 mm) and fine- (5 mm) mesh bags in 3 streams in temperate, Mediterranean, and tropical Cerrado climates. Shr = shredder, AFDM = ash-free dry mass.

Location	Mesh	Taxon	Density (no./g leaf AFDM)	Relative abundance (%)
Temperate	Coarse	Chironomidae	320	79
		Baetidae	13	3
		Empididae	9	2
		Hydropsychidae	9	2
		Nemouridae (Shr)	9	2
		Lepidostomatidae (Shr)	5	1
		Leuctridae (Shr)	4	1
		Others	271	10
	Fine	Chironomidae	141	74
		Oligochaeta	14	7
		Acarina	14	7
		Baetidae	6	3
		Nemouridae (Shr)	2	1
		Lepidostomatidae (Shr)	1	1
		Others	13	7
Mediterranean	Coarse	Chironomidae	333	58
		Oligochaeta	175	30
		Elmidae	28	5
		Others	37	7
	Fine	Chironomidae	81	40
		Oligochaeta	61	30
		Copepoda	26	13
		Ostracoda	13	6
		Others	23	11
Tropical Cerrado	Coarse	Chironomidae	209	88
		Copepoda	18	7
		Cladocera	7	3
		Others	4	2
	Fine	Chironomidae	386	96
		Cladocera	12	3
		Others	5	1

(e.g., Ostrofsky 1997), so comparison of rates among studies using different leaf types should be interpreted with caution. We were able to isolate the effects of climate (or geography) by comparing breakdown rates of the same leaf species using the same methods across streams in different climates (locations). If temperature were the main factor determining leaf-litter breakdown rates, then we would expect leaf-litter breakdown rates to be fastest in the tropical Cerrado and slowest in the temperate stream. Contrary to our initial hypothesis, the fastest leaf-litter breakdown rates for *A. glutinosa* were obtained in the temperate stream, and lower rates were obtained in the Mediterranean and the tropical Cerrado streams. Similar results were reported by Chergui and Pattee (1991b) who found that leaves of *Salix* sp. did not decompose faster in a Mediterranean stream (water temperature = 20°C) than streams in more temperate climates. The temperature effect apparently was overridden by other factors in our 3 streams. We suggest that 2 of these factors could have been: 1) microbial colonization, constrained by flow-related conditions and water chemistry, and 2) invertebrate feeding activities, constrained by biogeographical factors.

Microbial colonization

Fungal colonization, judged by ergosterol content, was very fast in the temperate stream (24 d). Fungal colonization was too slow in the tropical Cerrado stream to reach levels comparable to those on leaves in the temperate stream within 45 d, although ergosterol values measured at the end of the experiment in the tropical Cerrado stream were higher than those obtained for the temperate stream. Leaf-litter breakdown rates were fast in the temperate stream, slower in the tropical Cerrado stream, and slowest in the Mediterranean stream where leaf ergosterol content also was lowest. The observation that the only stream where fungi accumulated quickly (the temperate stream) also was the stream with fast leaf-litter breakdown rates is consistent with the notion that

TABLE 3. Mean (range) values of environmental variables in 3 streams in temperate, Mediterranean, and tropical Cerrado climates.

Variables	Temperate	Mediterranean	Tropical
pH	7.0	7.1	5.3
	(6.7–7.4)	(11.4–12.8)	(4.6–6.0)
Dissolved O_2 (mg/L)	12.1	10.7	8.6
	(11.4–12.8)	(7.8–13.6)	–
Alkalinity (μg $CaCO_3$/L)	4.1	34.4	21.6
	–	(26.9–41.9)	–
Conductivity (μS/cm)	39.5	401	6
	(39–40)	(398–404)	(5–7)
Temperature (°C)	10.6	13.3	15.5
	(12.4–7.5)	(6.5–20.0)	(14.5–16.5)
PO_4-P (μg/L)	<0.01	0.12	2.50
NO_3-N (mg/L)	0.702	0.240	0.050
Discharge (m^3/s)	2.7	1.1	0.1
	(1.17–4.18)	(0.13–2.72)	(0.001–0.18)

aquatic hyphomycetes are the microorganisms that play a key role in the breakdown of leaves in streams.

If total microbial biomass, estimated from ATP content, increases with no equivalent increase in ergosterol, the implication is that microorganisms other than fungi are accumulating biomass in decomposing leaves. Bacteria could be an important component of the microbiota, but their role in the initial stages of leaf-litter breakdown is controversial (e.g., Baldy and Gessner 1997, McArthur and Tuckfield 1997, Hieber and Gessner 2002, Pascoal and Cassio 2004), at least in temperate streams. High ATP also could be attributed to other microorganisms such as Oomycetes (lacking ergosterol) and protozoans, but their participation in leaf decomposition is unclear (but see Ribblett et al. 2005). Total microbial biomass was greatest on leaves in the tropical Cerrado stream, but the microorganisms involved seem to have had limited capabilities to decompose alder leaves because leaf-litter breakdown was slow in this stream. In a similar experiment in another Cerrado stream, and also using leaves of *A. glutinosa*, Wantzen and Wagner (2006) reported breakdown rates three times faster ($k = 0.035$) than in our experiment. They also found that breakdown rates were ~4x faster in the Cerrado than in a temperate stream in Germany. We do not know why the 2 studies differed, but the differences could partly be explained by differences in temperature (lower in our study) and water chemistry, particularly N (no data provided by Wantzen and Wagner 2006).

Flow-related conditions.—Overall microbial colonization (ATP and ergosterol content) was low in the Mediterranean stream, consistent with the low leaf-litter breakdown rates obtained. However, aquatic

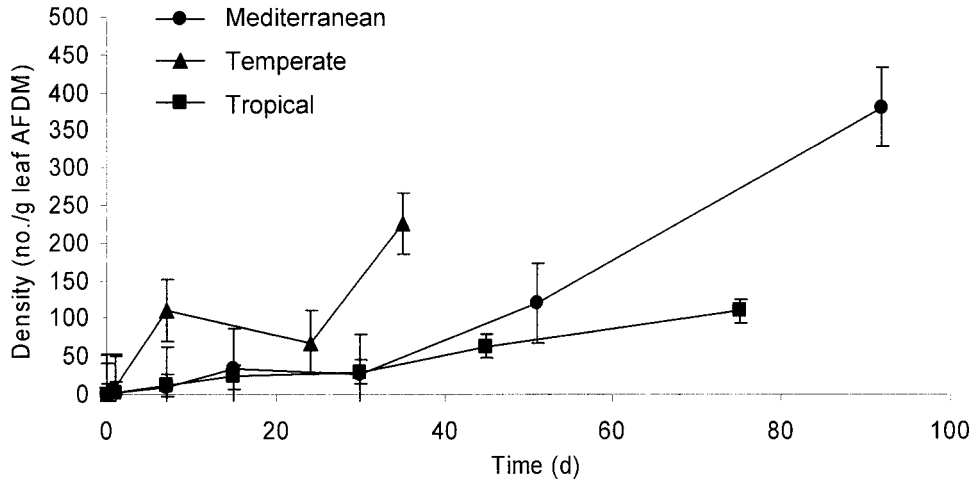

FIG. 4. Mean (±1 SE) density of invertebrates in *Alnus glutinosa* leaves during leaf-litter breakdown experiments in 3 streams from temperate, Mediterranean, and tropical Cerrado climates. AFDM = ash-free dry mass.

hyphomycetes colonized leaves in other Mediterranean streams (e.g., Chergui 1990, Chergui and Pattee 1990, 1991a, b, Maamri et al. 1998). Leaf-litter breakdown rates in North African streams are slower in intermittent than in permanent sites, and they are slower during the drought period than the wet period (Maamri et al. 2001). Moreover, microbial respiration is lower in intermittent than in permanent stream sites in North Africa (Maamri et al. 1999), and the activity and diversity of aquatic hyphomycetes decreases during the summer (Maamri et al. 1998). Absence of flow is not favorable to aquatic hyphomycetes, which need turbulence for reproduction (Chergui and Pattee 1990, Suberkropp 1998). Our experiment took place at the beginning of autumn when leaves had started falling, but rain was still rare. The study section of the stream had only temporary flow, and therefore, the current was very slow. Thus, it is plausible that the low rate of microbial colonization in the Mediterranean stream was related to the timing of our experiment.

Environmental factors.—Three environmental factors (DO, NO_3, and discharge) varied across the 3 streams in a manner consistent with leaf-litter breakdown rates. Low DO slows decomposition of leaf litter (e.g., Pascoal and Cassio 2004). However, DO probably did not affect leaf-litter breakdown rates in our experiments because differences among streams were certainly related to differences in temperature, which affected the solubility of O_2 in the water. Aquatic hyphomycete growth, decomposition activity, and sporulation rates are stimulated by turbulence or water motion (Chergui and Pattee 1990, Suberkropp 1998), and nutrients are important in controlling growth, biomass, and decomposition by aquatic hyphomycetes (Suberkropp and Chauvet 1995, Rosemond et al. 2002, Chadwick and Huryn 2003). NO_3 content and discharge were both high in the temperate stream and could have acted synergistically to promote high leaf-litter breakdown rates. NO_3 content was low in the tropical Cerrado stream, and discharge was extremely low in the Mediterranean stream, and these factors may have constrained microbial colonization. Our results suggest that N was a more important factor than P in controlling leaf-litter breakdown rates, but manipulative experiments will be needed to address the relative importance of each nutrient in the experimental streams (e.g., Gulis and Suberkropp 2003).

Differences in flow also could have caused differences in rates of physical fragmentation of leaves. We can not rule out this factor, but Ferreira et al. (2006) reported that current velocities up to 1.2 m/s have a minor role in litter fragmentation in laboratory channels and in temperate streams, probably because leaves are protected inside the coarse-mesh bags.

Invertebrate feeding activities

The influence of shredders on leaf-litter breakdown is well-known in temperate zones (e.g., Graça 2001, Haapala et al. 2001, Hieber and Gessner 2002), but poorly documented in other areas. The proportion of shredders in the coarse-mesh bags in the temperate stream was low (our study: 5%, Graça et al. 2001 in the same stream: 20–24%, Fleituch 2001 in a different temperate stream: 22–45%), but shredders still were significantly more abundant in the temperate stream than in the Mediterranean and tropical Cerrado streams. The results were consistent with the low proportion of shredders and high proportion of predators observed in another Cerrado stream (Wantzen and Wagner 2006).

In our study, leaf-litter breakdown was faster in coarse- than in fine-mesh bags in the temperate stream, but leaf-litter breakdown rates did not differ between coarse- and fine-mesh bags in the Mediterranean and tropical Cerrado stream. Our results indicate that invertebrates played a role in leaf-litter breakdown in the temperate stream, but not in the Mediterranean or tropical Cerrado streams. Irons et al. (1994) suggested that the role of microbes in decomposition should increase at lower latitudes, and the paucity of shredders in the Mediterranean and tropical Cerrado systems indicates that the microbial community was mainly responsible for leaf-litter breakdown in these systems.

Some chironomids, the most abundant group of invertebrates in the 3 geographic zones, feed on leaves and may contribute to decomposition (Grubbs et al. 1995, Wantzen and Wagner 2006). Leaf-litter breakdown rates did not differ between coarse- and fine-mesh bags in the tropical Cerrado stream in our study, so chironomid activities appear to have been unimportant for leaf-litter breakdown.

Biogeographical factors.—Shredders often are rare or absent in Mediterranean systems (Essafi et al. 1994, Maamri et al. 1997, but see Chergui and Pattee 1991a, b). Other studies in southern Portugal have reported the same paucity of shredders that we observed in the Mediterranean stream (e.g., Graça et al. 2004). This general lack of shredders could be related to the extreme variations in flow conditions found in these streams. In many Mediterranean areas, precipitation occurs only in winter and, during most of the year, the streams are reduced to disconnected pools. These conditions are not favorable to Plecoptera and many Trichoptera, rheophilic groups containing a large number of shredders.

Shredders also are often rare or absent in some tropical systems (Mathuriau and Chauvet 2002, Mouton and Magalhães 2003, but see Cheshire et al. 2005). The low number of shredders in the tropical Cerrado stream in our study is consistent with reports by Wantzen (2003) for other streams in the tropical Cerrado. However, Wantzen and Wagner (2006) reported that mining chironomids were able to consume alder leaves quickly in another Cerrado stream and speculated that shredders could either occur briefly in a habitat when food is available or be localized in small areas of the stream where leaves can persist. Unlike in other tropical systems (e.g., Covich et al. 2003, Crowl et al. 2006), crabs, shrimps, and fishes feeding on litter were absent from the tropical Cerrado stream in our study (Callisto et al. 2001). Thus, it is unlikely that our conclusion that shredders were not important to leaf-litter breakdown in the tropical Cerrado stream was a methods problem caused by coarse-mesh bags excluding consumers of large detritus (as suggested by Dobson 2004).

In summary, rates of leaf-litter breakdown and the relative importance of organisms involved in decomposition differed among the 3 climate/geographical regions. In the tropical Cerrado stream, litter breakdown was low, presumably because of lack of nutrients and invertebrate shredders. In the Mediterranean stream, decomposition also was slow, probably because of slow water movement and an absence of invertebrate shredders. Water temperature was lower in the temperate stream, but breakdown of leaves proceeded faster in the temperate stream than in the other 2 climates. The temperate stream had comparatively high nutrient content, faster water movement, and invertebrate shredders. The contrasting results between our study and Wantzen and Wagner (2006) suggest that local environmental conditions could be as important as large geographic factors in determining litter breakdown rates.

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Literature Cited

ABELHO, M. 2001. From litterfall to breakdown in stream: a review. TheScientificWorld 1:658–680.

ABELHO, M. 2005. Extraction and quantification of ATP as a measure of microbial biomass. Pages 223–229 *in* M. A. S. Graça, F. Bärlocher, and M. O. Gessner (editors). Methods to study litter decomposition. A practical guide. Springer, Dordrecht, The Netherlands.

APHA (AMERICAN PUBLIC HEALTH ASSOCIATION). 1998. Standard methods for the examination of water and wastewater. 20th edition. American Public Health Association, American Waterworks Association,and Water Environment Federation, Washington, DC.

BALDY, V., AND M. O. GESSNER. 1997. Towards a budget of leaf litter decomposition in a first order woodland stream. Life Sciences 320:747–758.

BENFIELD, E. F. 1997. Comparison of litterfall input to streams. Pages 104–108 *in* J. R. Webster and J. L. Meyer (editors). Stream organic matter budgets. Journal of the North American Benthological Society 16.

BOULTON, A. J., AND P. I. BOON. 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., M. GOULART, A. O. MEDEIROS, P. MORENO, AND C. A. ROSA. 2004. Diversity assessment of benthic macroinvertebrates, yeasts, and microbiological indicators along a longitudinal gradient in Serra do Cipó, Brazil. Brazilian Journal of Biology 64:1–12.

CALLISTO, M., P. MORENO, AND F. A. R. BARBOSA. 2001. Habitat diversity and benthic functional trophic groups at Serra do Cipó. Revista Brasileira de Biologia 61:259–266.

CANHOTO, C., AND M. A. S. GRAÇA. 1996. Decomposition of *Eucalyptus globulus* leaves and 3 native leaf species (*Alnus glutinosa*, *Castanea sativa* and *Quercus faginea*) in a Portuguese low order stream. Hydrobiologia 333:79–85.

CASAS, J. J., AND M. O. GESSNER. 1999. Leaf litter breakdown in a mediterranean stream characterised by travertine precipitation. Freshwater Biology 41:781–793.

CHADWICK, M. A., AND A. D. HURYN. 2003. Effects of a whole-catchment N addition on stream detritus processing. Journal of the North American Benthological Society 22: 194–206.

CHARA, J. D. 2003. Interaction between biodiversity and land use in low order stream catchments of the Colombian Andes. PhD Thesis, University of Stirling, Stirling, Scotland.

CHERGUI, H. 1990. The dynamics of aquatic hyphomycetes in

an eastern Moroccan stream. Archiv für Hydrobiologie 118:341–352.

CHERGUI, H., AND E. PATTEE. 1990. The processing of leaves of trees and aquatic macrophytes in the network of the River Rhône. Internationale Revue der gesamten Hydrobiologie 75:281–302.

CHERGUI, H., AND E. PATTEE. 1991a. An experimental study of the breakdown of submerged leaves by hyphomycetes and invertebrates in Morocco. Freshwater Biology 26:97–110.

CHERGUI, H., AND E. PATTEE. 1991b. Breakdown of allochthonous leaves in the catchment of the lower Moulouya River in Morocco. Acta Oecologica 12:543–560.

CHESHIRE, K., L. BOYERO, AND R. C. PEARSON. 2005. Food webs in tropical Australian streams: shredders are not scarce. Freshwater Biology 50:748–769.

CIGLASCH, H., J. LILIENFEIN, K. KAISER, AND W. WITAKE. 2004. Dissolved organic matter under native cerrado and *Pinus caribaea* plantation in Brazilian savannah. Biochemistry 67:157–182.

COVICH, A. P., T. A. CROWL, AND F. N. SCATENA. 2003. Effects of extreme low flows on freshwater shrimps in a perennial tropical stream. Freshwater Biology 48:1199–1206.

CROWL, T. A., W. H. MCDOWELL, A. P. COVICH, AND S. L. JOHNSON. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. Ecology 82:775–783.

CROWL, T. A., V. WELSH, T. HEARTSILL-SCALLEY, AND A. P. COVICH. 2006. Effects of different types of conditioning on rates of leaf-litter shredding by *Xiphocaris elongata*, a Neotropical freshwater shrimp. Journal of the North American Benthological Society 25:198–208.

DOBSON, M. 2004. Freshwater crabs in Africa. Freshwater Forum 21:3–26.

EGGERT, S. L., AND J. B. WALLACE. 2003. Litter breakdown and invertebrate detritivores in a resource-depleted Appalachian stream. Archiv für Hydrobiologie 156:315–338.

ESSAFI, K., H. CHERGUI, E. PATTEE, AND J. MATHIEU. 1994. The breakdown of dead leaves buried in the sediment of a permanent stream in Morocco. Archiv für Hydrobiologie 130:105–112.

FERREIRA, V. J. L., M. A. S. GRAÇA, J. P. LIMA, AND R. GOMES. 2006. Role of physical fragmentation and invertebrate activity in the breakdown of leaves. Archiv für Hydrobiologie (in press).

FLEITUCH, T. 2001. Beech leaf breakdown and POM storage along an altitudinal stream gradient. International Review of Hydrobiology 86:515–525.

GESSNER, M. O. 2005. Ergosterol as a measure of fungal biomass. Pages 189–195 *in* M. A. S. Graça, F. Bärlocher, and M. O. Gessner (editors). Methods to study litter decomposition. A practical guide. Springer, Dordrecht, The Netherlands.

GESSNER, M. O., E. CHAUVET, AND M. DOBSON. 1999. A perspective on leaf litter breakdown in stream. Oikos 85:377–384.

GIULIETTI, A. M. 1997. Flora: diversidade, distribuição geográfica e endemismo. Pages 84–100 *in* G. W. Fernandes (editor). Serra do Cipó: ecologia e evolução. Edição Vozes, Rio de Janeiro, Brazil.

GONÇALVES, J. F., A. M. SANTOS, AND F. A. ESTEVES. 2004. The influence of the chemical composition of *Typha domingensis* and *Nymphaea ampla* detritus on invertebrate colonization during decomposition in a Brazilian coastal lagoon. Hydrobiologia 527:125–137.

GRAÇA, M. A. S. 1993. Patterns and processes in detritus-based stream systems. Limnologica 23:107–114.

GRAÇA, M. A. S. 2001. The role of invertebrates on leaf litter decomposition in streams – a review. International Review of Hydrobiology 86:383–393.

GRAÇA, M. A. S., R. C. F. FERREIRA, AND C. N. COIMBRA. 2001. Litter processing along a stream gradient: the role of invertebrates and decomposers. Journal of the North American Benthological Society 20:408–420.

GRAÇA, M. A. S., P. PINTO, R. CORTES, N. COIMBRA, S. OLIVEIRA, M. MORAIS, M. J. CARVALHO, AND J. MALO. 2004. Factors affecting macroinvertebrate richness and diversity in Portuguese streams: a two-scale analysis. International Review of Hydrobiology 89:151–164.

GRUBBS, S. A., R. E. JACOBSEN, AND K. W. CUMMINS. 1995. Colonization by Chironomidae (Insecta, Diptera) on 3 distinct leaf substrates in an Appalachian mountain stream. Annales de Limnologie 31:105–118.

GULIS, V., AND K. SUBERKROPP. 2003. Interactions between stream fungi and bacteria associated with decomposing leaf litter and different levels of nutrient availability. Aquatic Microbial Ecology 30:149–157.

HAAPALA, A., T. MUOTKA, AND A. MARKKOLA. 2001. Breakdown and macroinvertebrate and fungal colonization of alder, birch, and willow leaves in a boreal forest stream. Journal of the North American Benthological Society 20: 395–407.

HIEBER, M., AND M. O. GESSNER. 2002. Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. Ecology 83:1026–1038.

IRONS, J. G., M. W. OSWOOD, R. J. STOUT, AND C. M. PRINGLE. 1994. Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshwater Biology 32: 401–411.

MAAMRI, A., F. BÄRLOCHER, E. PATTEE, AND H. CHERGUI. 2001. Fungal and bacterial colonisation of *Salix pedicellata* leaves decaying in permanent and intermittent streams in eastern Morocco. International Review of Hydrobiology 86:337–348.

MAAMRI, A., E. CHAUVET, H. CHERGUI, F. GOURBIERE, AND E. PATTEE. 1998. Microbial dynamics on decaying leaves in a temporary Moroccan river. I. Fungi. Archiv für Hydrobiologie 144:41–59.

MAAMRI, A., H. CHERGUI, AND E. PATTEE. 1997. Leaf litter processing in a temporary northeasten Moroccan river. Archiv für Hydrobiologie 140:513–531.

MAAMRI, A., E. PATTEE, X. GAYTE, AND H. CHERGUI. 1999. Microbial dynamics on decaying leaves in a temporary Moroccan river. II. Bacteria. Archiv für Hydrobiologie 144:157–175.

MATHURIAU, C., AND E. CHAUVET. 2002. Breakdown of leaf litter

in a neotropical stream. Journal of the North American Benthological Society 21:384–396.

MCARTHUR, J V., AND R. C. TUCKFIELD. 1997. Information length: spatial and temporal parameters among stream bacterial assemblages. Journal of the North American Benthological Society 16:347–357.

MERRITT, R. W., AND K. W. CUMMINS (EDITORS). 1996. An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt, Dubuque, Iowa.

MOULTON, T. P., AND S. A. P. MAGALHÃES. 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.

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.

PASCOAL, C., AND F. CASSIO. 2004. Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Applied and Environmental Microbiology 70:1–8.

PEARSON, R. G., AND N. M. CONNOLLY. 2000. Nutrient enhancement, food quality and community dynamics in a tropical rainforest stream. Freshwater Biology 43:31–42.

RIBBLETT, S. G., M. A. PALMER, AND D. W. COATS. 2005. The importance of bacterivorous protists in the decomposition of stream leaf litter. Freshwater Biology 50: 516–526.

RINCÓN, J., AND I. MARTÍNEZ. 2006. Food quality and feeding preferences of *Phylloicus* sp. (Trichoptera:Calamoceratidae). Journal of the North American Benthological Society 25:208–215.

ROBINSON, C. T., M. O. GESSNER, AND J. V. WARD. 1998. Leaf breakdown and associated macroinvertebrates in alpine glacial streams. Freshwater Biology 40:215–228.

ROSEMOND, A. D., C. M. PRINGLE, A. RAMÍREZ, M. J. PAUL, AND J. L. MEYER. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnology and Oceanography 47:278–289.

SANO, S. M., AND S. P. ALMEIDA (EDITORS). 1998. Cerrado: ambiente e flora. EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária)-CPAC, Planaltina, Distrito Federal, Brazil.

SUBERKROPP, K. 1998. Microorganisms and organic matter decomposition. Pages 120–143 *in* R. J. Naiman and R. E. Bilby (editors). River ecology and management: lessons from the Pacific Coastal ecoregion. Springer–Verlag, New York.

SUBERKROPP, K., AND E. CHAUVET. 1995. Regulation of leaf breakdown by fungi in streams: influences of water chemistry. Ecology 76:1433–1445.

TACHET, H., M. BOURNAUD, AND P. RICHOUX (EDITORS). 1987. Introduction à L'étude des macroinvertébrés des eaux douces (Systématique élémentaire et aperçu écologique). Université Lyon, Lyon, France.

WALLACE, J. B., S. L. EGGERT, J. L. MEYER, AND J. R. WEBSTER. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104.

WANTZEN, K. M. 2003. Cerrado streams – characteristics of a threatened freshwater ecosystem type on the tertiary shields of Central South America. Amazonia Limnologia et Oecologia Regionalis Systemae Fluminis Amazonas 17:481–502.

WANTZEN, K. M., AND R. WAGNER. 2006. Detritus processing by invertebrate shredders: a Neotrophical–temperate comparison. Journal of the North American Benthological Society 25:216–233.

WANTZEN, K. M., R. WAGNER, R. SÜTFELD, AND W. J. JUNK. 2002. How do plant-herbivore interactions of trees influence coarse detritus processing by shredders in aquatic ecosystems of different latitudes? Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie 28:815–821.

ZAR, J. H. 1996. Biostatistical analysis. 3rd edition. Prentice–Hall, Upper Saddle River, New Jersey.

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