Oecologia (2008) 155:311–323
DOI 10.1007/s00442-007-0913-x

ECOSYSTEM ECOLOGY - ORIGINAL PAPER

Do secondary compounds inhibit microbial- and insect-mediated leaf breakdown in a tropical rainforest stream, Costa Rica?

Marcelo Ardón · Catherine M. Pringle

Received: 26 July 2006 / Accepted: 26 October 2007 / Published online: 30 November 2007

Abstract We examined the hypothesis that high concentrations of secondary compounds in leaf litter of some tropical riparian tree species decrease leaf breakdown by inhibiting microbial and insect colonization. We measured leaf breakdown rates, chemical changes, bacterial, fungal, and insect biomass on litterbags of eight species of common riparian trees incubated in a lowland stream in Costa Rica. The eight species spanned a wide range of litter quality due to varying concentrations of nutrients, structural and secondary compounds. Leaf breakdown rates were fast, ranging from 0.198 d^{-1} (*Trema integerrima*) to 0.011 d^{-1} (*Zygia longifolia*). Processing of individual chemical constituents was also rapid: cellulose was processed threefold faster and hemicellulose was processed fourfold faster compared to similar studies in temperate streams. Leaf toughness ($r = -0.86$, $P = 0.01$) and cellulose ($r = -0.78$, $P = 0.02$) were the physicochemical parameters most strongly correlated with breakdown rate. Contrary to our initial hypothesis, secondary compounds were rapidly leached (threefold faster than in temperate studies), with all species losing all secondary compounds within the first week of incubation. Cellulose was more important than secondary compounds in inhibiting breakdown. Levels of fungal and bacterial biomass were strongly correlated with breakdown rate (fungi $r = 0.64$, $P = 0.05$; bacteria $r = 0.93$, $P < 0.001$) and changes in structural compounds (lignin $r = -0.55$, $P = 0.01$). Collector–gatherers were the dominant functional group of insects colonizing litterbags, in contrast to temperate studies where insect shredders dominate. Insect biomass was negatively correlated with breakdown rate ($r = -0.70$, $P = 0.02$), suggesting that insects did not play an important role in breakdown. Despite a wide range of initial concentrations of secondary compounds among the eight species used, we found that secondary compounds were rapidly leached and were less important than structural compounds in determining breakdown rates.

Keywords Decomposition · Tannins · Lignin · Ergosterol · Fungi · Bacteria · Collector–gatherers

Introduction

Leaf litter chemistry affects its persistence, quality and availability as a resource to consumers in stream ecosystems (Webster and Benfield 1986; Lecerf et al. 2005). Leaves of different species are known to decompose at different rates, and much effort has been devoted to determining which physical and chemical characteristics are the main drivers of decomposition rate (Petersen and Cummins 1974; Webster and Benfield 1986). Leaf litter chemical constituents reported to slow breakdown rate in temperate streams include: (1) high concentrations of structural compounds such as lignin (Triska and Sedell 1976; Gessner and Chauvet 1994); (2) low nitrogen content (Melillo et al. 1983); (3) high lignin-to-nitrogen and

Communicated by Mercedes Bustamante.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-007-0913-x) contains supplementary material, which is available to authorized users.

M. Ardón · C. M. Pringle
Institute of Ecology, University of Georgia,
Athens, GA 30602, USA

M. Ardón (✉)
Department of Biology, Duke University,
P.O. Box 90338, Durham, NC 27708, USA
e-mail: mla5@duke.edu; marcelo.ardon@gmail.com

carbon-to-nitrogen ratios (Enriquez et al. 1993); and (4) high concentrations of tannins (Ostrofsky 1997; Driebe and Whitman 2000).

Classic leaf breakdown studies in temperate streams have examined both initial and subsequent changes in leaf litter chemistry and associated biota, providing important insights into the breakdown process (Kaushik and Hynes 1971; Petersen and Cummins 1974; Triska et al. 1975; Suberkropp et al. 1976). Variation in leaf litter quality among species creates a "continuum of processing rates" (different species decompose at different rates, Petersen and Cummins 1974), which is important in providing resources for stream consumers throughout the year (Webster and Benfield 1986). Studies in temperate streams have shown that fungi and insect shredders are the principal drivers of decomposition rates (Suberkropp et al. 1976; Gessner and Chauvet 1994; Hieber and Gessner 2002). Tropical streams, on the other hand, have not received as much attention. Given the physical and biological differences between tropical and temperate streams, there is good reason to believe that the drivers of leaf breakdown might differ between these two regions.

The quality of leaves entering tropical streams represent a wide range of chemistry, due to the high plant species diversity and the tendency for many tropical plants to be better chemically defended against herbivores than temperate species (Coley and Aide 1991). Previous studies have suggested that high concentrations of phenolics and tannins in leaves of tropical species inhibit colonization by insects (Janzen 1975; Wantzen et al. 2002) and microbes (Stout 1989) and thus retard leaf litter breakdown (Stout 1989). However, very little is known regarding the absolute and relative importance of leaf secondary compounds in determining breakdown in tropical streams. The available literature provides conflicting evidence regarding the role of secondary compounds in leaf breakdown in tropical streams. Stout (1989), using new and published data, showed negative correlations between initial tannin concentration and breakdown rates of leaves decomposing in tropical streams. Irons et al. (1994) suggested that differences in tannin concentration led to difference in breakdown rates among ten species of temperate and tropical species decomposing in a Costa Rican stream. Wantzen et al. (2002) found evidence that phenolics in tropical species decreased leaf decomposition rates in a temperate stream. On the other hand, Campbell and Fuchshuber (1995) incubated both tropical and temperate species in a temperate stream, and found that initial concentrations of tannins only inhibited decomposition among closely related *Eucalyptus* species.

While previous studies have measured initial chemistry and leaf breakdown of three or less species in tropical streams (e.g., Rosemond et al. 1998; Mathuriau and Chauvet 2002; Gonçalvez et al. 2006; Ardón et al. 2006; Stallcup et al. 2006), to our knowledge no study has examined breakdown rates, chemical changes of leaves, and biota colonizing decomposing leaves of species covering a broad range of litter quality. Our previous work suggested that initial concentrations of cellulose and lignin within three species were more important than secondary compounds in retarding leaf breakdown (Ardón et al. 2006). Here, we expand on this work by providing the first data on changes in leaf chemistry and associated biota on decomposing leaves from eight native species representing a wide range of quality in a tropical stream. Information on the changes in leaf chemistry during breakdown can increase our understanding of the direct and indirect role of secondary compounds in leaf breakdown.

The role of detritivores has also been hypothesized to differ between temperate and tropical streams (Irons et al. 1994). Based on a latitudinal comparison of leaf breakdown and a literature review, Irons et al. (1994) hypothesized that due to the lack of insect shredders in tropical systems, leaf breakdown rates in the tropics were driven more by microbial breakdown than insect shredding, compared to temperate streams. Some studies in tropical streams have supported this hypothesis by excluding insects using litterbags of different mesh size (O'Connor et al. 2000; Rosemond et al. 2002; Wright and Covich 2005). However, data on changes in fungal and bacterial biomass on decomposing tropical leaves are notably scarce (but see: Mathuriau and Chauvet 2002; Rosemond et al. 2002; Ardón et al. 2006; Gonçalvez et al. 2006).

We examined the hypothesis that high concentrations of secondary compounds retard leaf breakdown dynamics by decreasing microbial and insect colonization. Our objectives were to: (1) compare leaf litter breakdown rates among eight tropical riparian tree species spanning a wide range of initial chemistry; (2) document changes in litter carbon and nutrient (nitrogen and phosphorus) content, as well as structural (lignin, cellulose, and hemicellulose) and secondary (condensed tannins, total phenolics, and hydrolysable tannins) compounds during breakdown; (3) examine if changes in fungal, bacterial and insect biomass on leaves are correlated with chemical changes in leaves; and (4) compare chemical changes of decomposing leaves in this study to classic studies of leaf breakdown conducted in temperate streams.

Methods

Study site

This study was conducted at La Selva Biological Station, Costa Rica (10°26′N, 84°01′W). The 1,536 ha reserve is

the lowland terminus of the last protected unbroken biological corridor, spanning an altitudinal gradient from 35 to 2,906 m above sea level, on the Caribbean slope of Central America. La Selva receives 4,000 mm of rain a year, with more than 400 mm a month from May to December (Sanford et al. 1994).

Due to dense canopy cover (>90%), streams are heavily shaded, resulting in predominantly detritus-based food webs (Pringle et al. 1993; Rosemond et al. 2002). We conducted experiments in the Sabalo, a third-order stream on the eastern edge of La Selva. The Sabalo drains primary forest to the west and pasture/grasslands to the east. Substrata consist primarily of small cobbles and gravel. Stream water temperature is relatively constant throughout the year (24–27 °C), and pH values range from 5.5 to 6 (Ramírez and Pringle 1998). This stream has been described in more detail elsewhere (Pringle and Hamazaki 1997; Ramírez and Pringle 1998; Rosemond et al. 1998).

Measurements of stream physical and chemical characteristics

During the four-month study period, two water samples were taken monthly for analyses of NO_3-N, NH_4-N and soluble reactive phosphorus (SRP). Samples were filtered (0.45 μm filters, Millipore, Billerica, MA, USA) and kept frozen until analyses at the University of Georgia. Phosphorus was measured as SRP using the molybdenum-blue technique (APHA 1998). NO_3-N and NH_4-N were measured using the cadmium reduction and phenate methods, respectively (APHA 1998). Temperature, pH and conductivity were measured biweekly using handheld meters (Hanna Instruments, Woonsocket, RI, USA). Daily maximum and minimum water temperatures were also recorded. Flow velocity over the leaf litterbags was measured biweekly using a Marsh-McBirney velocity meter (Frederick, MD, USA). Gauge height was recorded monthly and used to calculate discharge based on staging equations (Pringle, unpublished data).

Leaf breakdown experiment

We conducted two separate leaf breakdown experiments in the Sabalo stream. We selected four sites which had similar flow velocities and channel characteristics along a 1 km stream reach. Because of tractability (i.e., limitation on how many leaf litterbags one researcher could process immediately), we ran two separate experiments. From 26 February to 26 June of 2003 we examined leaf breakdown of six species: *Trema integerrima* (Beurl) Standl (family Ulmaceae), *Zygia longifolia* (Humb. & Bonpl. ex Willd.) Britton & Rose (Fabaceae), *Ficus insipida* Willd. (Moraceae), *Castilla elastica* Sessé ex Cerv. (Moraceae), *Terminalia oblonga* (Ruiz & Pav.) Steud. (Combretaceae),and *Luehea seemannii* Triana & Planch (Tiliaceae). From 25 February to 26 June of 2004 we repeated the experiment using leaves from two new species [*Carapa nicaraguensis* C. DC. (Meliaceae) and *Simira maxonii* (Standl.) Steyerm. (Rubiaceae)], along with two species that had also been included in the 2003 experiment (*Zygia longifolia*, and *Ficus insipida*). From now on, all species will be referred to by their genus. The second experiment was run using two new species so that we could expand the range of initial leaf chemistry. We repeated the use of two species (one fast-decaying species and one slow-decaying species) from our first experiment to account for possible differences in breakdown rates due to changes in physical or chemical conditions between years. We collected freshly fallen leaves from at least ten different individual trees of each of the eight species; leaves were air-dried for three days and stored in an air-conditioned room until use. We created 5-g litterbags using plastic mesh bags (22 × 40 cm) with a coarse mesh (5 mm) to allow access by stream fauna (Benfield 2006). We mixed leaves collected from different individual trees before placing them into bags. Litterbags were anchored to the streambed using metal stakes (Benfield 2006).

We collected one litterbag per site on day 0 and six predetermined dates for each species. Published breakdown rates of three species (*Trema*, *Ficus*, and *Zygia*; Irons et al. 1994; Rosemond et al. 1998) and knowledge of the initial chemistry of all eight species enabled us to preselect leaf collection retrieval dates for each species in order to cover most of the breakdown process (i.e., up to 90% mass loss). We overestimated the decomposition rate for *Simira* and stopped collection at 60% mass loss. Day "0" samples were brought back to the laboratory immediately to account for handling losses and determine initial concentrations of chemical constituents. On each collection date we removed one litterbag per species per site from the stream with a fine mesh net, placed it into an individual plastic bag, and transported it back to the laboratory for immediate processing. Leaves were rinsed over a 300 μm sieve to remove sediments and insects. Leaves were dried for 24–36 h at 40 °C, weighed, and ground. A subsample (1 g) was ashed at 500 °C for 1 h and reweighed to determine ash free dry mass (AFDM).

Leaf toughness was measured on day 0 samples using a pressure set on a Pesola spring scale (Pesola AG, Baar, Switzerland). Leaves were clamped between two Plexiglas plates with a 4 mm diameter hole. The number of grams necessary to punch a 1.7 mm diameter rod completely through the leaf provided an index of toughness (Kursar and Coley 2003). Five replicate punches were made on each leaf with care to avoid the veins, and five leaves from each litterbag were examined.

Subsamples were taken for chemical analyses on day "0" and three collection dates selected for each species to represent early, middle and late stages of breakdown. Subsamples were ground to fine powder and refrigerated at 4 °C until analyzed. We estimated cellulose, hemicellulose, and lignin by sequential neutral detergent/acid detergent digestion on an ANKOM 200 fiber analyzer (ANKOM Fiber Technologies, Fairport, NY, USA). Three separate analyses were conducted for phenolics: condensed tannins were estimated as proanthocyanidins using the *n*-butanol/HCL methods (Bate-Smith 1975; Schultz and Baldwin 1982), hydrolysable tannins were estimated using a potassium-iodate technique developed by Bate-Smith (1977) and modified by Schultz and Baldwin (1982), and total phenolics were estimated with the Folin-Denis assay (Swain 1979). All assays produced colorimetric reactions in proportion to phenolic concentration, which were compared to standards made from bulk samples used in the experiments. Standards for tannin analysis were made by sequential washes of bulk samples by acetone extraction, to avoid problems associated with using commercial standards (Appel et al. 2001). Because these assays rely on different reactions, condensed tannins and hydrolysable tannins can be higher than total phenolics (Schultz and Baldwin 1982). While there are clear limitations on the results provided by these assays (Martin and Martin 1982), they do provide a relative measure of the concentration of these compounds in the leaves during the breakdown process. Leaf carbon and nitrogen content were determined using a Carlo Erba NA 1500 CHN Analyzer (Carlo Erba, Milan, Italy). For phosphorus analysis, ground leaf material was weighed into acid-washed and pre-ashed ceramic crucibles, ashed at 500 °C, acid-digested, and analyzed spectrophotometrically (ascorbic acid method, APHA 1998).

On three collection dates for each species (selected to represent early, middle and late stages of breakdown), we sampled fungal, bacterial, and insect biomass. Insects were preserved in 10% formalin and later identified to the lowest possible taxonomic level (genus in most cases; family for Chironomidae) using available literature (M. Springer, unpublished data; Roldán 1996). Biomass for insects was estimated using length-mass regressions (Benke et al. 1999). Fungal biomass was estimated using ergosterol (Suberkropp and Weyers 1996). Forty disks (1.2 cm diameter) were punched from randomly selected leaves from each litterbag immediately after collection on three dates. Thirty disks were stored in methanol for ergosterol analysis, five disks were stored in 2% buffered formalin solution for bacterial enumeration, and the five remaining disks were dried for 24–36 h at 40 °C, weighed, ignited at 500 °C for 1 h, and reweighed to determine AFDM. Ergosterol was extracted from leaf disks in alkaline methanol by refluxing for 30 min, partitioning into pentane, drying and redissolving in methanol. The amount of ergosterol present was determined by comparing absorbance at 282 nm with known quantities of ergosterol after separation from other lipids by high-performance liquid chromatography (Suberkropp and Weyers 1996). Ergosterol was converted to fungal biomass using the ratio of 5.5 μg ergosterol per 1 mg fungal dry mass (Gessner and Chauvet 1993).

Bacterial biomass was calculated by staining bacteria with DAPI (Porter and Feig 1980). To dislodge bacterial cells from leaf disks, samples were sonicated in an ice bath for 10 min (HT 150 Sonicator, VWR Scientific Inc., West Chester, PA, USA). Following sonication, 2 mL subsamples were placed in a 12-port Millipore vacuum filter manifold and stained with DAPI (final concentration 10 μg L^{-1}) for 10 min in the dark. Samples were filtered through black polycarbonate membrane filters (0.22 μm, Poretics) backed with a 0.45 μm Millipore cellulose nitrate filters and rinsed with 2 ml of 2% buffered formalin. Filters were mounted on glass slides with Cargille type FF nonfluorescent immersion oil. Bacteria were counted using 1000× epifluorescent microscopy (BH-2, Olympus, Tokyo, Japan). At least ten grids per filter (20–30 cells per grid) were counted. Biovolumes were estimated using geometric shapes (Bratbak 1985; Wetzel and Likens 2000), and total carbon by multiplying biovolumes by 5.6×10^{-13} g C μm^{-3} (Bratbak 1985).

Statistical analyses

Litterbag breakdown rates were estimated by linear regression of natural log-transformed percent AFDM remaining versus day (negative exponential model; Benfield 2006). Analysis of covariance (ANCOVA) was used to test for differences in breakdown rates among leaves of the eight species. We used analysis of variance (ANOVA) followed by post hoc Tukey tests to examine differences in stream physical characteristics, initial litter chemistry, fungal, bacterial and insect biomass among species. We tested for normality using Shapiro–Wilk *W* test; logarithmic or arcsine square-root transformations were used when needed to meet assumptions of normality. We used Pearson correlation analyses to examine relationships between breakdown rates, invertebrate, bacterial and fungal biomass, and leaf litter chemistry (Gessner and Chauvet 1994). When analyzing relationships between initial leaf chemistry and breakdown we conducted the analyses with eight species (using only the 2003 data for *Ficus* and *Zygia*) and with 10 species (using the 2003 and 2004 data for both *Ficus* and *Zygia*). The main conclusions were the same with both analyses, so we are only presenting the results of analyses with eight species. All analyses were performed on Statistical Analysis Systems (SAS 1999). In order to compare the processing of individual chemical constituents in our study to

Table 1 Chemical and physical characteristics of the Sabalo study stream, La Selva Biological Station, during experiments

	2003		2004	
	Mean	Range	Mean	Range
Discharge ($m^3\ s^{-1}$)	0.79*	0.48–1.14	1.20*	0.95–1.56
Flow velocity ($m\ s^{-1}$)	0.14*	0.08–0.25	0.45*	0.14–1.17
Temperature (°C)	26.20	25.40–27.20	24.83	24.10–26.10
pH	5.89	5.71–6.13	5.90	5.26–7.23
Conductivity (µS/cm)	65.13	47.40–80.10	65.61	47.20–85.00
PO_4-P ($\mu g\ L^{-1}$)	4	1–12	6	1–27
NO_3-N ($\mu g\ L^{-1}$)	102	33–247	134	96–197
NH_4-N ($\mu g\ L^{-1}$)	20	0–36	3	0–29

Discharge $n = 4$; water chemistry, flow velocity, pH, temperature and conductivity $n = 8$

* Significantly different between years ($P < 0.05$)

Table 2 Breakdown rate coefficients for eight common riparian tree species incubated in the Sabalo study stream

Species	Breakdown rate k (day^{-1})	Standard error	r^2	Days to 95% mass loss
2003				
Trema integerrima	0.198a	0.0170	0.81	15
Ficus insipida	0.079b	0.0039	0.93	38
Terminalia oblonga	0.039c	0.0029	0.93	77
Castilla elastica	0.038c	0.0018	0.94	79
Luehea seemannii	0.033c	0.0045	0.83	91
Zygia longifolia	0.011d	0.0014	0.86	272
2004				
Ficus insipida	0.111e	0.0120	0.86	27
Simira maxonii	0.048bc	0.0044	0.90	62
Carapa nicaraguensis	0.023cd	0.0020	0.92	130
Zygia longifolia	0.014d	0.0017	0.86	214

Different letters denote significant differences using ANCOVAs

studies in temperate streams, we used Data Thief software (http://www.datathief.org/) to extract data from figures conducted in temperate studies. Using at least four points from each graph in each study, we were able to estimate decay coefficients of the chemical constituents by linear regression of the natural log-transformed concentration versus day (Benfield 2006).

Results

Site characteristics

Rainfall was higher in 2004 than in 2003; the daily average during the study period in 2003 was 10.3 mm while in 2004 it was 17.3 mm ($P = 0.013$). The difference was driven mostly by rain during March of each year. Daily rainfall in March 2003 was 3.95 mm while in March 2004 it was 13.94 mm ($P < 0.005$). Litterbags were subject to higher flow velocity in 2004 ($1.20\ m\ s^{-1}$) than in 2003 ($0.79\ m\ s^{-1}$, Table 1; $P = 0.001$).

Mass loss

There was an order of magnitude variation in breakdown rate among the eight species [Table 2, see also "Electronic supplementary material" (ESM) Fig. S1]. Breakdown rates were fastest for *Trema* ($k = 0.198\ d^{-1}$, 15 days to 95% mass loss), and slowest for *Zygia* ($k = 0.011\ d^{-1}$, 214 days to 95% mass loss; Table 2). We observed a faster breakdown rate for *Ficus* in 2004 than in 2003 (k in 2003 = $0.079\ d^{-1}$, k in 2004 = $0.11\ d^{-1}$; $P = 0.02$). Breakdown rates for *Zygia* did not differ between the two years (k in 2003 = $0.011\ d^{-1}$, k in 2004 = $0.014\ d^{-1}$; $P = 0.15$).

Chemical changes in leaves during breakdown

Initial leaf litter chemistry differed greatly among the eight species used in both experiments (Table 3). We observed wide variation in initial lignin concentration [5.57–30.28% dry mass (DM)], phosphorus (0.06–0.16% DM), total phenolics (0.97–32.33% DM), and condensed tannins (0.48–23.20% DM). Breakdown rate was correlated with leaf toughness and initial concentrations of structural compounds (leaf toughness $r = -0.86$, $P = 0.01$; cellulose $r = -0.78$, $P = 0.02$; carbon $r = -0.76$, $P = 0.02$; Table 4). Breakdown was also correlated, although marginally significantly, with lignin ($r = -0.66$, $P = 0.07$) and condensed tannins ($r = -0.58$, $P = 0.06$).

Nutrient content

Initial carbon concentration was highest in *Zygia* (47.5% DM) and lowest in *Trema* leaf litter (34.1% DM, Table 3). Initial leaf litter nitrogen concentration was highest in *Simira* (2.05% DM) and lowest in *Carapa* (0.91% DM; Table 3, "ESM" Fig. S2). Only *Trema*, *Ficus* and *Castilla* had net decreases in the concentration of N during decomposition ("ESM" Fig. S2). *Castilla* had the highest initial phosphorus concentration (0.11% DM), whereas *Zygia* had the lowest (0.06% DM; Table 3, "ESM" Fig. S3). Phosphorus concentration tended to increase in all eight species ("ESM" Fig. S3)

Structural compounds

Initial concentrations of cellulose were lowest in *Trema* (12.2% DM), and highest in *Zygia* (24.5% DM; Table 3,

Table 3 Initial concentration (percent dry mass) of chemical constituents and leaf toughness (g) in eight species of common riparian trees at La Selva Biological Station (mean ± SD)

Species	Nitrogen	Carbon	Phosphorus	Lignin	Cellulose	Hemicellulose	Total phenolics	Hydrolysable tannins	Condensed tannins	Leaf toughness
2003										
Trema integerrima	1.61 (0.11)	34.08 (0.55)	0.10a (0.010)	5.57a (1.36)	12.26 (0.70)	10.10a (1.22)	0.97 (0.28)	2.96a (0.24)	0.80a (0.11)	12 (2)
Ficus insipida	1.27 (0.03)	35.03 (0.64)	0.08b (0.006)	8.33a (1.55)	19.07a (1.34)	14.67b (1.29)	11.78a (5.23)	2.66a (0.29)	0.48a (0.22)	120a (26)
Terminalia oblonga	1.36 (0.14)	39.22a (0.46)	0.12a (0.014)	6.96a (1.61)	17.50a (0.41)	13.89b (1.27)	11.46a (2.69)	11.37 (2.01)	12.85b (7.52)	138a (9)
Castilla elastica	2.03a (0.20)	40.44a (1.05)	0.16 (0.025)	13.40b (1.44)	19.47a (1.67)	21.96 (2.73)	12.29a (7.45)	9.44 (3.02)	11.29b (4.68)	152 (16)
Luehea seemannii	1.27 (0.15)	44.18ab (0.10)	0.11a (0.010)	16.33b (0.81)	19.85a (0.70)	15.46b (1.59)	7.42 (2.81)	13.99 (4.61)	13.65b (3.51)	124a (19)
Zygia longifolia	1.87a (0.09)	46.45b (0.54)	0.06b (0.002)	28.36c (1.09)	24.58b (1.61)	17.52 (0.82)	10.04a (1.29)	7.82c (0.85)	8.12c (0.50)	232b (15)
2004										
Ficus insipida	1.40 (0.06)	39.81a (1.06)	0.08b (0.004)	8.20a (1.55)	19.54a (1.20)	15.05b (1.22)	13.86a (5.23)	2.45b (4.46)	0.49a (0.18)	118 (8)
Simira maxonii	2.05a (0.14)	44.91ab (0.83)	0.11a (0.010)	15.33b (3.12)	22.93b (0.60)	14.62b (0.40)	4.26 (0.68)	6.64b (1.38)	2.80 (0.54)	134a (10)
Carapa nicaraguensis	0.91 (0.26)	41.71a (0.43)	0.08b (0.002)	18.34b (4.71)	17.98a (2.82)	7.57a (2.17)	32.33 (11.80)	34.88 (10.60)	23.20 (5.64)	204b (4)
Zygia longifolia	1.87a (0.06)	47.48b (0.69)	0.06b (0.009)	30.28c (3.37)	24.50b (2.21)	17.30a (1.90)	10.43a (0.87)	7.75c (0.65)	8.10c (0.49)	230b (10)

Means with the same letters are not significantly different

Table 4 Pearson correlation coefficients between exponential breakdown rate (k) and initial concentrations of leaf litter chemical constituents ($n = 8$)

Parameter	r	P
Carbon	−0.76	0.02
Nitrogen	−0.03	0.93
Phosphorus	0.01	0.96
C:N	−0.38	0.34
C:P	−0.37	0.35
Lignin	−0.66	0.07
Hemicellulose	−0.36	0.36
Cellulose	−0.78	0.02
Condensed tannins	−0.58	0.06
Hydrolysable tannins	−0.46	0.24
Total phenolics	−0.50	0.20
Leaf toughness	−0.86	0.01

Fig. 1). Cellulose concentrations declined in all species (Fig. 1). Lignin concentrations were highest in *Zygia* (30.2% DM) and lowest in *Trema* (5.5% DM; Table 3, "ESM" Fig. S4). Lignin concentrations tended to increase in *Ficus* and *Trema*, while *Zygia* and *Carapa* showed an initial increase followed by a decline ("ESM" Fig. S4).

Secondary compounds

Concentrations of condensed tannins were lowest in *Ficus* (0.48% DM) and highest in *Carapa* (23.2% DM; Table 3,

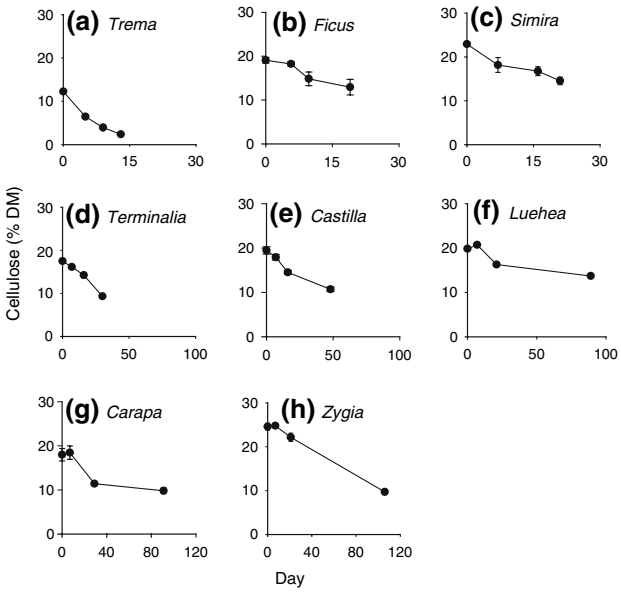

Fig. 1 Chemical changes in concentration of cellulose (percent dry mass ± 1 standard error) in decomposing leaves of eight species of common riparian trees in the Sabalo study stream. Note differences in x-axes

Fig. 2). Condensed tannin concentration declined rapidly in all eight species (Fig. 2). Total phenolics were highest in *Carapa* (32.3% DM) and lowest in *Trema* (0.97% DM; Table 3), and declined rapidly in all eight species ("ESM" Fig. S5). Hydrolysable tannins were highest in *Carapa* (34.8% DM) and lowest in *Ficus* (2.6% DM; Table 3).

Fungal, bacterial, and invertebrate biomass on the leaves

Fungi

Fungal biomass differed among species ($P < 0.001$) and time affected species differently (time × species interaction $P < 0.001$, Fig. 3). *Trema* and *Ficus* supported the highest fungal biomass (Fig. 3). Maximum fungal biomass on leaves was strongly and positively correlated with leaf litter breakdown rate ($r = 0.64$, $P = 0.05$; Table 5). Fungal biomass on leaves was negatively correlated to initial leaf toughness ($r = -0.78$, $P = 0.001$, Table 6a). Fungal biomass during the breakdown process was negatively correlated to changes in lignin concentration, lignin:N and lignin:P ratios during breakdown (lignin $r = -0.55$, $P = 0.016$; lignin:N $r = -0.55$, $P = 0.016$, lignin:P $r = -0.45$, $P = 0.057$; Table 6b).

Bacteria

Bacterial biomass differed among species ($P < 0.0001$) and increased over time ($P < 0.001$), though not at the same rate

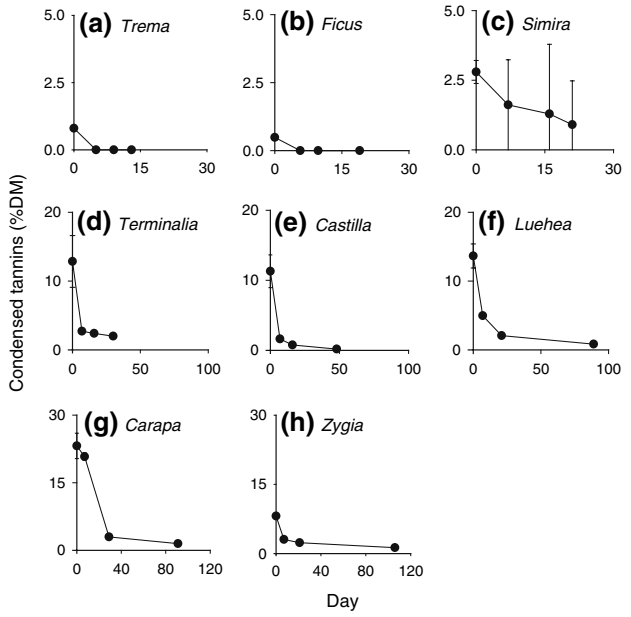

Fig. 2 Chemical changes in concentration of condensed tannins (percent dry mass ± 1 standard error) in decomposing leaves of eight species of common riparian trees in the Sabalo study stream. Note different scales on *x* and *y*-axes

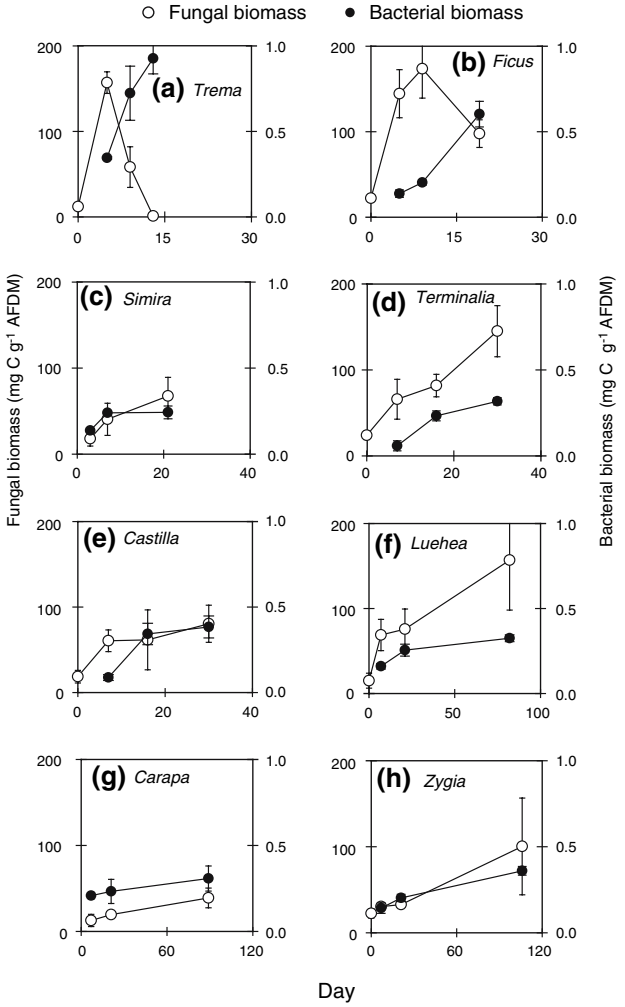

Fig. 3 Fungal and bacterial biomass on decomposing leaves of eight species of common riparian trees. Mean biomass ± 1 standard error. Fungal biomass is represented by *white circles* while bacterial biomass is shown by *black circles*. Note the different scales on *x* and *y*-axes

Table 5 Pearson correlation coefficients between breakdown rate (*k*) and maximum biomass of fungi, bacteria and insects ($n = 8$)

Parameter	r	P
Maximum fungal biomass	0.64	0.05
Maximum bacterial biomass	0.93	<0.0006
Maximum invertebrate biomass	−0.70	0.02

for all species (species × time interaction $P < 0.001$, Fig. 3). Similar to fungal biomass, *Trema* and *Ficus* supported the highest bacterial biomass. Maximum bacterial biomass was strongly positively correlated to leaf litter breakdown rate ($r = 0.93$, $P < 0.001$; Table 5). Bacterial biomass was negatively correlated with initial concentrations of cellulose ($r = -0.73$, $P = 0.03$) and carbon ($r = -0.80$, $P = 0.01$; Table 6a). Changes in carbon ($r = -0.73$, $P < 0.001$), C:P ($r = -0.49$, $P = 0.03$), and lignin:P ($r = -0.55$, $P = 0.01$)

Table 6 (a) Pearson correlation coefficients between maximum decomposer biomass and mean initial leaf chemical constituents ($n = 8$); and (b) Pearson correlations between decomposer biomass for each of three collection dates and mean concentrations of chemical constituents during breakdown sampled on same date

Parameter	Chemical constituent	r	P
(a) Initial chemistry			
Fungal biomass	Leaf toughness	−0.78	0.001
Bacterial biomass	Cellulose	−0.73	0.03
	Carbon	−0.80	0.01
Invertebrate biomass	Condensed tannins	0.74	0.03
(b) Chemical changes during breakdown			
Fungal biomass	Lignin	−0.55	0.016
	Lignin:N	−0.55	0.016
	Lignin:P	−0.45	0.057
Bacterial biomass	Carbon	−0.73	0.0005
	C:P	−0.49	0.03
	Lignin:P	−0.55	0.01
Invertebrate biomass	No variables		

8 species × 3 collection dates per species = 24

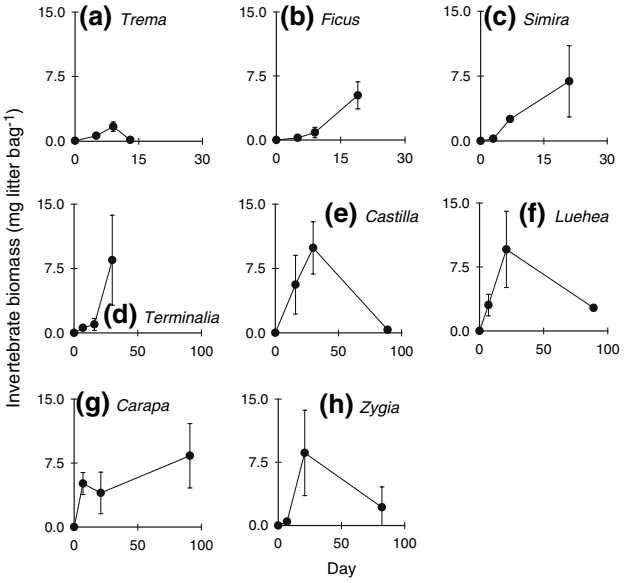

Fig. 4 Insect biomass on decomposition of eight species of common riparian trees in the Sabalo study stream. Mean biomass ± 1 standard error. Note different scale of x-axis

during the decomposition process also negatively affected bacterial biomass (Table 6b). Since these are some of the first estimates of bacterial biomass on decomposing leaves in a tropical stream, we recounted a third of the samples to make sure that our counts were consistent. Out of 15 comparisons, we only found two dates for which there were significant differences between counts. In those two cases we present the average of the two counts.

Insects

Assemblages were dominated by collector−gatherers ("ESM" Table S1). There were significant differences in insect biomass among species ($P = 0.01$), over time ($P = 0.01$) and their interaction (species × time, $P < 0.001$, Fig. 4). *Carapa* had the highest total biomass (biomass = 17.9 mg litterbag^{-1}) and *Trema* the lowest (biomass = 2.2 mg litterbag^{-1}). Insect biomass was negatively correlated with breakdown rate ($r = -0.70$, $P = 0.02$, Table 5) and positively correlated with condensed tannins ($r = 0.74$, $P = 0.03$, Table 6a). Insect biomass tended to decrease with increasing fungal and bacterial biomass, even though the relationship was not significant ($r = -0.35$, $P = 0.1$, data not shown).

Discussion

Our results did not support our initial hypothesis that secondary compounds inhibit microbial and invertebrate processing of leaves. Contrary to a previous hypothesis that high concentrations of secondary compounds in tropical leaves inhibit breakdown rate (Stout 1989; Wantzen et al. 2002), secondary compounds were rapidly leached (threefold faster than rates reported for temperate leaves, Table 7) and structural compounds (i.e., cellulose) were more important than secondary compounds in retarding leaf breakdown (Table 4). Structural compounds appear to retard leaf breakdown by inhibiting fungal and bacterial colonization of leaves (Table 6). In support of previous claims of the importance of microbes in tropical streams (Irons et al. 1994), bacteria and fungi were strongly correlated to breakdown rate in this study while insects played a small role in the overall breakdown process (Table 5).

What are the direct and indirect roles of secondary compounds in leaf breakdown?

Despite the wide range of initial concentrations of secondary compounds among the eight species, all species lost almost all secondary compounds within the first week of incubation in the stream (Fig. 2, "ESM" Fig. S5). Loss of these compounds was threefold faster in our tropical study stream compared to reports from temperate streams, (Table 7). This fast disappearance is partly due to the relatively high water temperature, which is known to enhance leaching of soluble compounds (Short and Ward 1980; Paul et al. 1983).

Rapid leaching rates of secondary compounds, and the lack of a correlation between concentrations of these compounds and breakdown rate, strongly suggest that second-

Table 7 Decay coefficients (r^2 in parentheses) for chemical constituents in studies conducted in temperate streams, compared to decay coefficients in decomposing leaf litter in this study

Species	*N*	Lignin	Cellulose	HC	Phenolics*	Source
Alnus rubra	−0.0003	−0.0068	−0.017			Triska et al. (1975)
	(0.02)	(0.48)	(0.98)			
Acer circinatum	0.0053	−0.0023	−0.004			Triska et al. (1975)
	(0.99)	(0.57)	(0.31)			
Acer macrophyllum	0.0026	−0.00156	−0.004			Triska et al. (1975)
	(0.70)	(0.05)	(0.88)			
Pseudotsuga menziesii and *Tsuga heterophylla*	0.0029	0.0013	−0.003			Triska et al. (1975)
	(0.70)	(0.12)	(0.98)			
Quercus alba	−0.0038	−0.0047	−0.0096	−0.01	−0.0084	Suberkropp et al. (1976)
	(0.91)	(0.93)	(0.98)	(0.99)	(0.41)	
Carya glabra	−0.0159	−0.0034	−0.0074	−0.01	−0.0105	Suberkropp et al. (1976)
	(0.98)	(0.94)	(0.70)	(0.61)	(0.62)	
Quercus petrea	0.0028	0.0050	−0.0044		−0.0182	Rosset et al. (1982)
	(0.43)	(0.61)	(0.94)		(0.90)	
Larix deciduas	0.0026	0.0029	−0.0002		−0.0176	Rosset et al. (1982)
	(0.73)	(0.44)	(0.04)		(0.72)	
Picea abies	0.0028	0.0019	0.0005		−0.0015	Rosset et al. (1982)
	(0.99)	(0.53)	(0.07)		(0.79)	
Acer negundo		−0.0025	−0.011	−0.018		Paul et al. (1983)
		(0.16)	(0.93)	(0.95)		
Platanus occidentalis		0.0024	−0.0087	−0.029		Paul et al. (1983)
		(0.80)	(1.00)	(0.28)		
Alnus glutinosa	−0.0015	−0.0009	−0.0039	−0.003		Chauvet (1987)
	(0.75)	(0.40)	(0.96)	(0.96)		
Populus nigra	−0.0027	−0.0012	−0.0031	−0.004		Chauvet (1987)
	(0.86)	(0.78)	(0.97)	(0.97)		
Salix alba	−0.0011	−0.0008	−0.0034	−0.004		Chauvet (1987)
	(0.39)	(0.38)	(0.93)	(0.93)		
Alnus glutinosa					−0.093	Bärlocher et al. (1995)
					(0.71)	
Eucalyptus globules					−0.065	Bärlocher et al. (1995)
					(0.98)	
Castilla elastica	−0.007	0.00	−0.011	−0.012	−0.046	This study
	(0.90)	(0.00)	(0.98)	(0.97)	(0.51)	
Carapa nicaraguensis	0.0015	0.0003	−0.0016	−0.007	−0.0126	This study
	(0.32)	(0.00)	(0.01)	(0.79)	(0.29)	
Ficus insipida	−0.0119	0.0186	0.012	−0.021	−0.0242	This study
	(0.52)	(0.51)	(0.98)	(0.92)	(0.18)	
Luehea seemannii	−0.0002	−0.0020	−0.0033	−0.004	−0.146	This study
	(0.02)	(0.27)	(0.74)	(0.84)	(0.98)	
Simira maxonii	0.0024	0.0148	−0.0080	−0.019	−0.043	This study
	(0.66)	(0.54)	(0.51)	(0.94)	(0.20)	
Terminalia oblonga	0.0108	−0.021	−0.0074	−0.021	−0.35	This study
	(0.85)	(0.79)	(0.47)	(0.95)	–	
Trema integerrima	−0.0172	0.067	−0.13	−0.125	−0.0100	This study
	(0.40)	(0.73)	(0.99)	(0.98)	–	

Table 7 continued

Species	*N*	Lignin	Cellulose	HC	Phenolics*	Source
Zygia longifolia	0.0012	−0.0031	−0.0134	−0.009	−0.1469	This study
	(0.97)	(0.78)	(0.99)	(0.99)	(0.93)	
Average temperate studies	−0.00053	−0.0027[a]	−0.0058	−0.0063	−0.0307	
Average this study	−0.003	−0.0017[a]	−0.0203	−0.0276	−0.0938	
Difference this study vs. temperate studies	5.6	0.62	3.5	4.3	3.1	

Blank spaces indicate data was not available

HC hemicellulose, *N* nitrogen

* We only included studies that used the Folin-Denis or the Folin-Ciocalteus methods

[a] Does not include species in which lignin concentrations increased during breakdown

ary compounds are unlikely to play a direct role in leaf breakdown among the tropical species we examined. Our results contradict previous suggestions that high concentrations of secondary compounds in tropical leaves slow leaf breakdown (Stout 1989; Wantzen et al. 2002). The few studies that have examined the role of secondary compounds in determining leaf breakdown in tropical streams have either involved transplant experiments between temperate and tropical streams with a few species from each site (i.e., two or less, Stout 1989; Irons et al. 1994), or incubation of three or more tropical species in temperate streams (Campbell and Fuchshuber 1995; Wantzen et al. 2002). By incubating native leaves in a tropical stream and following the chemistry of the leaves, we showed that tannins and phenolics are rapidly leached, and are thus unlikely to play a direct role in leaf breakdown.

It is plausible that secondary compounds might have played an indirect role in leaf breakdown by reacting with other leaf chemical constituents. In three species in our study (*Trema*, *Ficus*, and *Simira*), lignin increased during the decomposition process ("ESM" Fig S4). This phenomenon of increasing lignin during decomposition can results from interactions between litter-N and phenolics, which react to form recalcitrant compounds that behave like lignin in the sequential extraction method (see Suberkropp et al. 1976). In our study lignin showed a net increase only in the three fastest decomposing species (*Trema, Ficus*, and *Simira*), which also had high initial N content (Table 3), suggesting that N could have reacted with phenolics to form lignin-like compounds. As suggested by Suberkropp et al. (1976), future studies should examine the N content of the lignin fraction to determine if there is higher N content compared to the initial leaf N.

Structural compounds, particularly cellulose, were more important in determining leaf breakdown rate than secondary compounds (Table 4). Lignin was also correlated to breakdown rate, although the correlation coefficient was only marginally significant ($P = 0.07$). Previous studies in temperate streams have shown that structural compounds can be important in determining leaf breakdown (Gessner and Chauvet 1994). The persistence of both cellulose and lignin concentrations in leaves suggests that these compounds can play a direct role in determining leaf breakdown by inhibiting microbial and insect consumers processing the leaves.

The processing of cellulose and hemicellulose was slower relative to secondary compounds in our study (Figs. 1, 2; Table 7). However, when compared to studies conducted in temperate streams, cellulose and hemicellulose were processed much faster (3.5 and 4.3-fold faster respectively; Table 7) in our study. Our work suggests that processing of individual structural compounds in leaves is faster in our tropical study stream than in temperate streams (Table 7). Our results expand on previous studies that have reported rapid leaf breakdown in tropical streams versus temperate streams (Irons et al. 1994; Benstead 1996; Dudgeon and Wu 1999; Mathuriau and Chauvet 2002) by showing that the processing of cellulose and hemicellulose is also faster.

Do changes in secondary or structural compounds affect fungal, bacterial and insect biomass on leaves?

We did not find any evidence that initial concentrations of secondary compounds inhibited fungal, bacterial or insect biomass (Tables 5, 6). We did find a positive correlation between condensed tannins and invertebrate biomass (Table 6). However, rapid leaching of total phenolics, condensed tannins and hydrolyzable tannins in our leaves suggest that, regardless of initial concentrations, secondary compounds are unlikely to determine microbial or insect colonization of leaves. Previous laboratory studies have shown that secondary compounds decreased fungal biomass on leaves (Canhoto and Graça 1999) and feeding by insects (Rincón and Martínez 2006). Our results indicate that in the field, secondary compounds might not persist in the leaves for long enough to limit microbial or insect processing. Other studies in temperate streams have

also found that invertebrates are not affect by secondary compounds due to the rapid leaching that occurs once leaves enter the stream (Tuchman et al. 2002; Rier et al. 2005).

In contrast to secondary compounds, our results indicate that both initial concentrations and changes in structural compounds during breakdown are important in determining fungal and bacterial biomass (Table 6). Kinetic limitation of fungi due to the presence of recalcitrant structural compounds, in particular lignin, has been shown in previous studies (Triska and Sedell 1976; Gessner and Chauvet 1994). During early stages of breakdown, labile materials are leached out or processed, leaving the more recalcitrant C compounds which require specialized enzymes to be broken down. This agrees with our previous work showing that cellulose and lignin on leaves can limit microbial respiration on leaves (Ardón et al. 2006).

What are the relative contributions of fungi, bacteria and insects to leaf breakdown?

Our results support previous claims that fungi and bacteria play a key role in leaf breakdown, as indicated by the strong and positive correlation of both fungal and bacterial biomass with breakdown rate (Table 5). Levels of fungal and bacterial biomass reported here are some of the highest reported in the literature. Fungal biomasses measured on *Trema* and *Ficus* litterbags (*Trema* mean 106 mg g AFDM^{-1}; *Ficus* mean 116 mg g AFDM^{-1}) are at the high end of values reported for temperate streams (mean value 88 mg g^{-1}; Gessner et al. 1997). Observed biomass concentrations were similar to those reported in another tropical stream in Colombia (Mathuriau and Chauvet 2002) and to those previously measured on decomposing leaves at La Selva (Rosemond et al. 2002). Bacterial biomass was high and similar to values reported in a nutrient-enriched stream in North Carolina (*Trema* biomass range 0.4–0.9 mg C g^{-1} AFDM; red maple biomass range 0.1–1 mg C g^{-1} AFDM; Gulis and Suberkropp 2003).

Two lines of evidence support the hypothesis that insects did not play a significant role in leaf breakdown (Irons et al. 1994). First, collector−gatherers, not shredders, were the most abundant functional feeding group on litterbags. This agrees with several studies of insect assemblages in tropical streams (Dudgeon 1982; Ramírez and Pringle 1998; Dobson et al. 2002; Mathuriau and Chauvet 2002). Second, we found a negative correlation between insect biomass and leaf breakdown rate (Table 5), which suggests that insects are using recalcitrant leaves as substrata for attachment and feeding on particles deposited on the leaves.

The mesh size of the litter bags we used (5 mm) could have excluded larger macroconsumers like crabs and shrimp, suggesting that our estimates of insect biomass on the leaves might be higher than if we had used a larger mesh. Despite the higher insect biomass, we did not see a positive correlation between insect biomass and breakdown rate, suggesting that insects play a minor role in the breakdown process. Our results agree with a previous study in this same stream, which used electricity to exclude macroconsumers from leaf packs (Rosemond et al. 1998). Despite increased insect biomass in the absence of macroconsumers, this did not result in faster breakdown rates (Rosemond et al. 1998).

What are the functional roles of leaves of different quality in a tropical rainforest stream?

Leaves of different quality, which are processed at varying rates, provide different types of resources for microbial and insect consumers. Rapidly decomposing species are quickly processed by fungi and bacteria without supporting much insect biomass. Some of the C provided by these leaves is lost as microbial respiration while some becomes fine particulate organic matter for consumers downstream. The rapid processing of *Trema* leaves, which had almost completely disappeared in 15 days, makes it an unlikely permanent substrate for chironomid larvae with a larval stage of 26 days (Ramírez and Pringle 2006). On the other hand, slow-decomposing species might be more important as substrata for attachment of collector−gatherers, and eventually also become sources of fine particles as they are slowly broken down. For example, *Carapa* and *Zygia* leaves could provide a permanent substratum for the development of 5–8 cohorts of Chironomidae larvae, given the long residence time of leaves in the stream.

The functional role of leaf litter in stream food webs depends upon the rate and timing at which leaves enter the stream and the residence time in the stream. Both the rate and timing of leaf litter inputs differ between temperate and tropical streams. In temperate streams, where leaf fall occurs once a year, the allochthonous resource base is composed of leaf litter that is processed at varying rates, thus providing food resources for insects throughout the year (Grubbs and Cummins 1996; Schofield et al. 2001). In contrast, leaf litter inputs into tropical rainforest streams are constant throughout the year; however, the rate of litter production differs among species (Hartshorn 1983). Even though we did not directly measure rate of leaf litter production for the eight species used in this study, we observed that those tree species which produced the most leaf litter (*Trema* and *Ficus*) were characterized by rapidly decomposing leaves, while tree species which produced lower amounts of leaf litter (*Zygia* and *Carapa*) produced slow-decomposing leaves. This pattern agrees with the hypothesis that plants which are constantly producing new leaves tend to produce them with lower concentrations of structural compounds (Coley and Aide 1991).

In conclusion, our results did not support our initial hypothesis that secondary compounds inhibit leaf breakdown in tropical streams. We found that concentrations of structural compounds (i.e., cellulose) were more important than secondary compounds in slowing leaf breakdown through limitation of bacterial and fungal biomass. Results suggest that fast-decomposing species are important carbon sources for microbial consumers while slow-decomposing species are important for invertebrates as substrata for attachment and eventually as a source of particulate organic matter. Changes to riparian plant communities can potentially alter the quality and availability of basal leaf litter resources in tropical stream food webs. These findings are important given that lowland tropical riparian forests are becoming severely degraded, often suffering decreases in tree species diversity and abundance (Pringle et al. 2000; Neill et al. 2001; Benstead et al. 2003).

Acknowledgments We thank M. Hidalgo for help in the field, D. Vázquez for helping with insect identification and S. Dye for assisting with bacteria enumeration. We are grateful to M. D. Hunter, J. L. Meyer and A. D. Rosemond for allowing us to conduct analyses in their laboratories. We thank M. M. Bustamante, A. Covich, D. Batzer, E. Lindquist, Pringle lab, and two anonymous reviewers for helpful suggestions about earlier versions of this manuscript. This research was supported by NSF LTREB grants DEB-0075339, 0545463 to C. M. Pringle, F. J. Triska and A. Ramirez; and NSF OISE-0434503, and a UGA Dissertation Completion Award to M. Ardón. Experiments complied with current Costa Rica law.

References

APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington, DC

Appel HM, Governor HL, D'Ascenzo M, Siska E, Schultz JC (2001) Limitation of foliar phenolics in ecological studies. J Chem Ecol 27:761–778

Ardón M, Stallcup LA, Pringle CM (2006) Does leaf quality mediate the stimulation of leaf breakdown by phosphorus in Neotropical streams? Freshw Biol 51:618–633

Bärlocher F, Canhoto C, Graca MAS (1995) Fungal colonization of alder and eucalytpus leaves in two streams in Central Portugal. Arch Hydrobiol 133:457–470

Bate-Smith EC (1975) Phytochemistry of proanthocyanidins. Phytochemistry 14:1107–113

Bate-Smith EC (1977) Astringent tannins of *Acer* species. Phytochemistry 16:1421–1426

Benfield EF (2006) Decomposition of leaf material. In: Hauer FR, Lamberti GA (eds) Methods in stream ecology, 2nd edn. Academic, San Diego, CA, pp 711–720

Benke AC, Huryn AD, Smock LA, Wallace JB (1999) Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. J North Am Benthol Soc 18:308–343

Benstead JP (1996) Macroinvertebrates and the processing of leaf litter in a tropical stream. Biotropica 28:367–375

Benstead JP, Douglas MM, Pringle CM (2003) Relationships of stream invertebrates communities to deforestation in eastern Madagascar. Ecol Appl 13:1473–1490

Bratbak G (1985) Bacterial biovolume and biomass enumerations. Appl Environ Microbiol 49:1488–1493

Campbell IC, Fuchshuber L (1995) Polyphenols, condensed tannins, and processing rates of tropical and temperate leaves in an Australian stream. J North Am Benthol Soc 14:174–182

Canhoto C, Graça MAS (1999) Leaf barriers to fungal colonization and shredders (*Tipula lateralis*) consumption of decomposing Eucalyptus globules. Microb Ecol 37:163–172

Chauvet E (1987) Changes in the chemical composition of alder, poplar and willow leaves during decomposition. Hydrobiol 148:35–44

Coley PD, Aide TM (1991) Comparisons of herbivory and plant defenses in temperate and tropical broad-leaved forests. In: Price PW, Lewinsohn TM, Fernandez GW, Benson WW (eds) Plant–animal interactions: evolutionary ecology in tropical and temperate regions. Wiley, Chichester, UK, pp 25–49

Dobson M, Magana A, Mathooko JD, Ndegwa K (2002) Detritivores in Kenyan highland streams: more evidence for the paucity of shredders in the tropics? Freshw Biol 47:909–919

Driebe EM, Whitman TG (2000) Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia 123:99–107

Dudgeon D (1982) An investigation of physical and biological processing of two species of leaf litter in Tai Po Kau forest stream, New Territories Hong Kong. Arch Hydrobiol 96:1–32

Dudgeon D, Wu KKY (1999) Leaf litter in a tropical stream: food or substrate for macroinvertebrates? Arch Hydrobiol 146:65–82

Enriquez S, Duarte CM, Sand-Jansen K (1993) Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457–471

Gessner MO, Chauvet E (1993) Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. Appl Environ Microbiol 59:502–507

Gessner MO, Chauvet E (1994) Importance of stream microfungi in controlling breakdown rates of leaf litter. Ecology 75:1807–1817

Gessner MO, Suberkropp K, Chauvet E (1997) Decomposition of plant litter by fungi in marine and freshwater ecosystems. In: The mycota IV: Environmental and microbial relationships. Springer, Berlin, pp 303–322

Gonçalvez JF, França JS, Medeiros AO, Rosa CA, Callisto M (2006) Leaf breakdown in a tropical stream. Int Rev Hydrobiol 91:164–177

Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshw Biol 48:123–134

Grubbs SA, Cummins KW (1996) Linkages between riparian forest composition and shredder voltinism. Arch Hydrobiol 137:39–58

Hartshorn GS (1983) Plants. In: Janzen DH (ed) Costa Rican natural history. University of Chicago Press, Chicago, IL, pp 118–183

Hieber M, Gessner MO (2002) Contributions of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. Ecology 81:3445–3463

Irons JG, Oswood MW, Stout RJ, Pringle CM (1994) Latitudinal patterns in leaf litter breakdown: is temperature really important? Freshw Biol 32:401–411

Janzen DH (1975) Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. Biotropica 6:69–103

Kaushik NK, Hynes HBN (1971) The fate of the dead leaves that fall into streams. Arch Hydrobiol 68:465–515

Kursar TA, Coley PD (2003) Convergence in defense syndromes of young leaves in tropical rainforests. Biochem Syst Ecol 31:929–949

Lecerf A, Dobson M, Dang CK, Chauvet E (2005) Riparian plant species loss alters trophic dynamics in detritus-based stream ecosystems. Oecologia 146:432–442

Martin JS, Martin MM (1982) Tannin assays 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. J North Am Benthol Soc 21:384–396
Melillo JM, Naiman RJ, Aber JD, Eshleman KN (1983) The influence of substrate quality and stream size on wood decomposition dynamics. Oecologia 58:281–285
Neill C, Deegan LA, Thomas SM, Cerri CC (2001) Deforestation for pasture alters nitrogen and phosphorus in small Amazonian streams. Ecol Appl 11:1817–1828
Ostrofsky ML (1997) Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. J North Am Benthol Soc 16:750–759
O'Connor PJ, Covich AP, Scatena FN, Loope LL (2000) Non-indigenous bamboo along headwater streams of the Luquillo Mountains, Puerto Rico: leaf fall, aquatic leaf decay and patterns of invasion. J Trop Ecol 16:499–516
Paul RW, Benfield EF, Cairns JJ (1983) Dynamics of leaf processing in a medium-sized river. In: Fontaine TDI, Bartell SM (eds) Dynamics of lotic ecosystems. Ann Arbor Press, Ann Arbor, MI, pp 25–35
Petersen RC, Cummins K W (1974) Leaf processing in a woodland stream. Freshw Biol 4:345–368
Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
Pringle CM, Hamazaki T (1997) Effects of fishes on algal response to storms in a tropical stream. Ecology 78:2432–2442
Pringle CM, Rowe GL, Triska FJ, Fernandez JF, West J (1993) Landscape linkages between geothermal activity, solute composition and ecological response in streams draining Costa Rica's Atlantic slope. Limnol Oceanogr 38:753–774
Pringle CM, Scatena F, Paaby-Haansen P, Nuñez-Ferrera M (2000) River conservation in Latin America and the Caribbean. In: Boon PJ, Davies BR, Petts GE (eds) Global perspectives on river conservation: science, policy and practice. Wiley, Chichester, UK, pp 41–77
Ramírez A, Pringle CM (1998) Structure and production of a benthic insect assemblage in a neotropical stream. J North Am Benthol Soc 17:443–463
Ramírez A, Pringle CM (2006) Fast growth and turnover of chironomid assemblages in response to stream phosphorus levels in a tropical lowland landscape. Limnol Oceanogr 51:189–196
Rier ST, Tuchman NC, Wetzel RG (2005) Chemical changes to leaf litter from trees grown under elevated CO_2 and the implications for microbial utilization in a stream ecosystem. Can J Fish Aquat Sci 62:185–194
Rincón J, Martínez I (2006) Food quality and feeding preferences of *Phylloicus* sp. (Trichoptera: Calamoceratidae). J North Am Benthol Soc 25:209–215
Roldán G (1996) Guía para el estudio de los macroinvertebrados acuaticos del departamento de antioquia, 2nd edn. Fondo Fen Colombia, Colciencias, Universidad de Antioquia, Bogotá
Rosemond AD, Pringle CM, Ramírez A (1998) Macroconsumer effects on insect detritivores and detritus processing in a tropical stream. Freshw Biol 39:515–523
Rosemond AD, Pringle CM, Ramírez A, Paul MJ, Meyer JL (2002) Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. Limnol Oceanogr 47:278–289
Rosset J, Bärlocher F, Oertli JJ (1982) Decomposition of conifer needles and deciduous leaves in two Black Forest and Swiss Jura streams. Int Rev Ges Hydrobiol 67:695–711
Sanford RL, Paaby P, Luvall JC, Phillips E (1994) Climate, geomorphology, and aquatic systems. In: McDade LA, Bawa KS, Hespenhedie HA, Hartshorn GS (eds) La Selva: ecology and natural history of a Neotropical rainforest. University of Chicago Press, Chicago, IL, pp 19–33
SAS (1999) Statistical analysis systems version 8. SAS, Cary, NC
Schofield KA, Pringle CM, Meyer JL, Sutherland AB (2001) The importance of crayfish in the breakdown of rhododendron leaf litter. Freshw Biol 46:1191–1204
Schultz JC, Baldwin IT (1982) Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217:149–151
Short RA, Ward JV (1980) Leaf litter processing in a regulated rocky-mountain stream. Can J Fish Aquat Sci 37:123–127
Stallcup LA, Ardón M, Pringle CM (2006) Does nitrogen become limiting under high-P conditions in detritus-based tropical streams? Freshw Biol 51:1515–1526
Stout RJ (1989) Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach. Can J Fish Aquat Sci 46:1097–1106
Suberkropp K, Weyers H (1996) Application of fungal and bacterial production methodologies to decomposing leaves in streams. App Env Microbiol 62:1610–1615
Suberkropp K, Godshalk GL, Klug MJ (1976) Changes in chemical composition of leaves during processing in a woodland stream. Ecology 57:720–727
Swain T (1979) The importance of flavonoids and related compounds in fern taxonomy and ecology. Bull Torrey Bot Club 107:113–153
Triska FJ, Sedell JR (1976) Decomposition of 4 species of leaf litter in response to nitrate manipulation. Ecology 57:783–792
Triska FJ, Sedell JR, Buckley B (1975) The processing of conifer and hardwood leaves in two coniferous forest streams. II. Biochemical and nutrient changes. Verh Int Ver Theor Angew Limnol 19:1625–1639
Tuchman NC, Wetzel RG, Rier ST, Wahtera KA, Teeri JA (2002) Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Glob Chang Biol 8:163–170
Wantzen KM, Wagner R, Suetfeld R, Junk WJ (2002) How do plant-herbivore interactions of trees influence coarse detritus processing by shredders in aquatic ecosystems of different latitudes? Verh Int Ver Theor Angew Limnol 28:815–821
Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. Annu Rev Ecol Syst 17:567–594
Wetzel RG, Likens GE (2000) Limnological analyses, 3rd edn. Springer, Berlin
Wright M, Covich AP (2005) The effect of macroinvertebrate exclusion on leaf breakdown rates in a tropical headwater stream. Biotropica 37:403–408