EFFECTS OF LEAF LITTER QUALITY ON DECOMPOSITION DYNAMICS IN LOWLAND NEOTROPICAL STREAMS

by

MARCELO LUIS ARDÓN SAYÃO (Under the Direction of CATHERINE M. PRINGLE)

ABSTRACT

Lowland Neotropical streams have a chemically-diverse detrital resource base, where leaf quality has the potential to play a key role in mediating effects of eutrophication on stream food webs. I examined the hypothesis that the quality of leaf substrata (i.e. carbon quality and nutrient content) determines: (1) the rate at which microbes and invertebrates process leaf litter; and (2) the magnitude of the microbial and invertebrate response to high ambient nutrient levels in water and substratum. First, I studied the effects of leaf carbon-quality and nutrient content on leaf breakdown of eight common riparian species in a stream at La Selva Biological Station, Costa Rica. Results indicate that concentrations of structural compounds, not secondary compounds, are the most important predictors of breakdown. Results also suggest that microbes rapidly colonize and process high-quality leaves, while low-quality leaves serve primarily as substrata for attachment of invertebrates. Second, I examined if the same trends I observed with respect to carbon-quality and breakdown at La Selva also occurred at a temperate site. I used standardized analytical techniques to measure leaf litter chemistry from seven common riparian trees from Coweeta Hydrologic Laboratory, N.C. and compared results to leaf chemistry of species from La Selva. Structural compounds were strongly correlated with leaf breakdown rate at both sites. However, concentrations of condensed tannins were

significantly greater (2.6 - fold) in Coweeta than in La Selva species and were negatively correlated to breakdown rate among Coweeta species, but not among La Selva species. Third, I examined if leaf carbon-quality mediates stimulation of leaf breakdown by elevated ambient phosphorus. I examined leaf breakdown rates of three species differing in carbon-quality across a natural landscape gradient in stream water P, and a whole-stream P-enrichment. Results suggest that high-P levels stimulate fungal biomass and microbial respiration to a greater extent on high than on low carbon-quality species. And finally, to avoid confounding effects of other leaf quality parameters, I manipulated both carbon-quality and phosphorus levels using a modified artificial (agar-diffusing) substratum technique. Results provide the first experimental demonstration supporting the hypothesis that carbon-quality of organic matter can determine the response of microbial respiration to P-enrichment of both substratum and water. My results indicate that carbon-quality has the potential to mediate the effects of elevated water nutrients on detrital-based food webs.

INDEX WORDS: Decomposition, Tannins, Phenolics, Lignin, Nitrogen, Tropical streams, Ergosterol, Bacteria, La Selva Biological Station, Costa Rica

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DEDICATION

A Erin, Pa, Ma, Josefo, y Sufa por todo su apoyo.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In their classic paper, Hairston, Smith and Slobodkin (1960) concluded that the "world is green" primarily because predators exert "top-down" control to maintain herbivore populations at low densities. On the other hand, Ehrlich and Raven (1964) provided an alternative "bottom-up" view by pointing out that the "world is green" primarily because plants vary in their quality as a resource to herbivores, and not all are edible. In the evolutionary arms race between plants and herbivores, plants have evolved a variety of mechanical and chemical adaptations to "defend" themselves against herbivores. Variation in plant defenses increase heterogeneity in their quality as a resource, which can determine energy flow within food webs (Hunter and Price 1992, Polis 1999, Hunter 2001). Partly because of this variation in quality, 70-90% of primary production goes unconsumed and enters detrital food webs (Pomeroy 1991, Wetzel and Ward 1992). While the importance of resource quality has been theoretically and empirically recognized in food webs based on living plants, we still lack a clear understanding of how the quality of detritus might affect ecosystem structure and function (Moore et al. 2004).

There are advantages of examining how detritus quality can determine ecosystem structure and function in tropical stream ecosystems. First, because of the closed forest canopy and continuous leaf litter inputs, tropical streams are primarily heterotrophic (Covich 1988, Pringle 2000). Second, due to the high diversity of plant species and their tendency to be better defended against herbivores (Coley and Aide 1991), a wide range of initial leaf litter chemistry naturally occur within the same habitat. And finally, the relatively high and stable temperatures in tropical regions alleviate temperature constraints over decomposition. This environment allows litter quality to play a more important role in decomposition dynamics than in temperate

systems (Lavelle et al. 1993, Aerts 1997). This study was aimed at quantifying how leaf litter quality (carbon-quality and nutrient content) potentially interacts with ambient nutrient levels in water to affect the process of decomposition, and both microbial and invertebrate response to high ambient nutrient levels.

Which chemical constituents are known to determine leaf litter quality?

Most studies on the chemical constituents that determine leaf litter quality have been conducted in temperate streams (Webster and Benfield 1986). Leaf litter quality is defined as the ease of decomposition (Heal et al. 1997), and is determined by the concentration and forms of carbon (C) present, and leaf nutrient content (Aber and Melillo 2001). Small, labile C molecules with high energy content (e.g. simple sugars) are easily broken down. In contrast, recalcitrant C compounds (e.g. cellulose, hemicellulose, lignin and tannins) have large three-dimensional, complex structures that can be broken down only by specialized enzymes, making them metabolically more costly for microbes to access (Sinsabaugh et al. 1993). Leaves from species with a high concentration of recalcitrant forms of C are broken down at a slow rate (Webster and Benfield 1986, Aerts 1997). Leaf nutrient content also affects its quality. Leaves with high concentrations of nitrogen (N) and phosphorus (P) tend to be broken down faster than leaves with low nutrient content (Enriquez et al. 1993).

Do breakdown dynamics differ in tropical versus temperate streams?

Given the differences between tropical and temperate ecosystems there is good reason to believe that leaf breakdown may differ between these two biomes. First, tropical streams have warmer and more consistent year-round water temperature, suggesting microbial activity is less seasonal (Suberkropp 1984). Second, lowland tropical streams tend to receive continuous leaf litter inputs throughout the year, in contrast to seasonal inputs in temperate streams. Third, invertebrate shredders, which play a vital role in leaf breakdown in temperate

streams (Cuffney et al. 1990), have been reported to be rare in lowland tropical streams (Ramírez and Pringle 1998, Rosemond et al. 1998, Dudgeon and Wu 1999, Dobson et al. 2002). Finally, it is generally accepted that leaf chemistry differs between temperate and tropical systems, with leaves from tropical species having higher concentrations of tannins and phenolics (Coley and Aide 1991, Dyer and Coley 2001). The high diversity and concentrations of leaf secondary compounds has been suggested to inhibit leaf breakdown rate and invertebrate feeding in tropical streams (Stout 1989, Wantzen et al. 2002).

Can leaf litter quality determine the effects of ambient nutrient enrichment on leaf breakdown?

In addition to leaf litter quality, ambient nutrient levels have been shown to be important in determining leaf breakdown in both tropical and temperate streams. Faster breakdown rates have been reported in response to increased nitrogen (Meyer and Johnson 1983, Suberkropp and Chauvet 1995), phosphorus (Elwood et al. 1981, Rosemond et al. 2002), and both nutrients combined (Robinson and Gessner 2000, Grattan and Suberkropp 2001, Gulis and Suberkropp 2003, Benstead et al. 2005). In contrast, some studies have reported no change in leaf breakdown rate in response to nutrient enrichment (Triska and Sedell 1976, Newbold et al. 1983, Royer and Minshall 2001).

Possible interactions between leaf litter quality and dissolved nutrients in streams have not been examined thoroughly. The few studies that have examined these interactions have focused on how nutrient enrichment affects organic matter with different nutrient content (Peterson et al. 1993, Royer and Minshall 2001, Stelzer et al. 2003, Gulis et al. 2004). These studies found a stronger effect of high ambient nutrients on the breakdown of substrata with lower intrinsic nutrient content. However, it is not clear how the concentration of recalcitrant C compounds in leaves can potentially mediate the effect of high ambient nutrients on leaf breakdown in streams.

Dissertation overview

The majority of the work for this dissertation was conducted at La Selva Biological Station, in the Caribbean lowlands of Costa Rica. La Selva is part of the last protected unbroken ecological corridor (spanning altitudinal extremes from 35m to 2906m) on the Caribbean coast of Central America. Due to solute-rich groundwater inputs, streams at La Selva have a wide range of variation in water chemistry (Pringle et al. 1993). The solute-rich inputs contain high concentrations of cations, anions, and phosphorus (Mg, Na, Si, Cl, SO₄, PO₄) and enter receiving streams at ambient temperature (Pringle et al. 1993). This research expands on previous studies at La Selva which examined the extent to which ecosystem processes are affected by variation in phosphorus levels (Rosemond et al. 2001, Rosemond et al. 2002, Ramírez et al. 2003).

I examined the hypothesis that the quality of leaf substrata (i.e. carbon quality and nutrient content) determines: (1) the rate at which microbes and invertebrates process leaf litter; and (2) the magnitude of the microbial and invertebrate response to high ambient nutrient levels in water and substratum. I focus on effects of leaf litter quality and dissolved phosphorus (P) on leaf breakdown in tropical streams because: (1) both factors have shown to be very important in temperate systems; (2) we lack a clear understanding of how these two factors control leaf breakdown in tropical streams independently; and (3) their interaction might play an important role in determining how tropical ecosystems will respond to anthropogenic nutrient loading. Projections are that by the year 2050, two-thirds of all fertilizer use worldwide will be applied in tropical ecosystems (Matson et al. 1999), making these ecosystems particularly vulnerable to eutrophication from non-point sources (Carpenter et al. 1998, Caraco and Cole 1999).

In Chapter 2, I examine the hypothesis that chemical changes in tropical leaf litter during breakdown affect both microbial (fungal and bacterial) and invertebrate processing of leaves. Based on existing paradigms (Stout 1989, Irons et al. 1994, Wantzen et al. 2002), I predicted that secondary compounds would play a more important role than structural compounds in

inhibiting leaf breakdown rate. I measured leaf breakdown rates, and concomitant changes in leaf litter chemistry, bacterial, fungal, and invertebrate biomass on leaves of eight species of common riparian trees in La Selva, representing a wide range in leaf quality. This comprehensive dataset enabled an evaluation of long-standing assumptions about leaf litter breakdown in tropical streams. Furthermore this chapter sets the stage for subsequent chapters by elucidating which chemical constituents determine leaf litter quality in lowland tropical streams.

In Chapter 3, I examine if the same trends observed with respect to carbon (C) quality and decomposition dynamics at La Selva also occurred at a temperate site, Coweeta Hydrologic Laboratory, N.C. U.S.A. Comparisons of the effects of leaf litter chemistry on breakdown rate in tropical versus temperate streams are hindered by the lack of comparability of analytical methods used to measure leaf chemistry between studies. As a first step to overcome this obstacle, I measured leaf chemical constituents from common riparian tree species from La Selva and Coweeta using standardized analytical techniques. I compared the initial leaf chemistry and breakdown rates from the eight species used in Chapter 2 to the initial chemistry and breakdown rate of seven common riparian species at Coweeta.

Chapter 4 examines the hypothesis that leaf C-quality (as determined by concentrations of cellulose, lignin and tannins) mediates the stimulation of high ambient P concentration on leaf breakdown in streams at La Selva. I predicted that high dissolved P would have a stronger stimulatory effect on microbial and insect processing of high- than of low- quality leaves. From the eight species used in Chapters 2 and 3, I selected three species that spanned the range of quality. I incubated single species leaf packs in five streams that had natural differences in ambient P due to solute-rich groundwater inputs, and in a stream that has been experimentally enriched with P. I measured fungal biomass, microbial respiration, invertebrate abundance and nutrient content of the leaves during breakdown.

In Chapter 5, I expand on results from Chapter 4 by experimentally examining the interactive effects of organic matter quality and ambient P concentration on heterotrophic biofilm respiration and breakdown of organic matter. I tested the hypothesis that C quality of organic matter determines the response of heterotrophic biofilms to P enrichment. To avoid confounding effects of other leaf quality parameters I manipulated both carbon-quality and phosphorus levels using an artificial (agar-diffusing) substratum technique modified from Tank and Winterbourn (1996) and Tank and Dodds (2003). I conducted two experiments placing substrata in low- and high-P sites of an on-going whole-stream P-enrichment experiment. I first used cellulose cloth as an artificial low-quality substratum and enhanced its carbon-quality and nutrient content by amending underlying agar with maltose and P, respectively. I then conducted a second experiment amending two natural leaf substrata (*Trema integerrima* a high-quality C source and *Zygia longifolia* a low-quality C source) with maltose.

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CHAPTER 2

CHEMICAL AND BIOTIC CHANGES DURING LEAF LITTER PROCESSING IN A TROPICAL STREAM: CONTRADICTING EXISTING PARADIGMS ¹

¹Ardón, M. and C.M. Pringle. To be submitted to *Oecologia*

Abstract

Here we present the first dataset of changes in the chemistry of decomposing leaves and concomitant changes in associated microbial and invertebrate biota for a tropical stream ecosystem. We examined the hypothesis that chemical changes in tropical leaf litter during breakdown affect both microbial (fungal and bacterial) and insect processing of leaves. We predicted that secondary compounds play a more important role than structural compounds in inhibiting leaf breakdown rate. We measured breakdown rates, leaf litter chemical changes, bacterial, fungal, and insect biomass on leaves of eight species of common riparian trees in lowland Costa Rica. The eight species represented a wide range in initial nutrient content, structural, and secondary compounds. Leaf breakdown rates were fast (i.e. relative to temperate studies) for all species, and ranged from 0.198 d⁻¹ (*Trema integerrima*) to 0.011 d⁻¹ (Zygia longifolia). Processing of individual chemical constituents was also very fast: cellulose was processed 6-fold faster, while lignin was processed 10-fold faster compared to similar studies in temperate streams. Leaf toughness (r = -0.86, p < 0.001) and structural compounds were important in determining breakdown rate (cellulose r = -0.78, p < 0.001; lignin r = -0.67, p < -0.001; lignin r = -0.001; ligni 0.001). Accordingly, fungal and bacterial biomass on leaves were correlated to leaf toughness (r = -0.84, p = 0.01) and chemical changes in structural compounds (lignin r = -0.55, p = 0.01). As in temperate studies, fungi and bacteria were important in driving breakdown rate (fungi r = 0.65, p = 0.04; bacteria r = 0.86, p = 0.01). However, in contrast to temperate studies where invertebrate shredders have been found to be the dominant group, invertebrate assemblages in leaf packs were dominated by collector-gatherers. Furthermore, invertebrate biomass was negatively correlated with breakdown rate (r = -0.70, p = 0.01) and microbial biomass (fungi + bacteria, r = -0.67, p = 0.04), suggesting that insects were not directly using leaves as a food resource even after microbial conditioning. Contrary to a prevailing paradigm that high secondary compounds in tropical leaves inhibit breakdown rate, we found that secondary compounds were rapidly leached (2-fold faster than rates reported for temperate leaves), and

structural compounds were more important than secondary compounds in inhibiting leaf breakdown rate. Overall our results suggest that fast-decomposing species are an important carbon source for fungi and bacteria, while slow-decomposing species primarily serve as substrata for attachment of insects.

Introduction

Leaf litter chemistry affects its quality, persistence and availability as a resource to consumers in stream ecosystems (Melillo et al. 1984, Webster and Benfield 1986, Tank et al. 2000). Leaves of different species are known to lose mass at different rates, and much effort has been devoted to determine which chemical constituents affect breakdown rate (Petersen and Cummins 1974, Webster and Benfield 1986). Leaf litter chemical constituents found 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, Hutchens and Benfield 2000); (2) low nutrient content such as nitrogen (Melillo et al. 1983); and (3) high ratios of constituents such as lignin to nitrogen ratio and carbon to nitrogen ratio (Enriquez et al. 1993).

Classic 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). However, to our knowledge no study has examined changes in leaf litter chemistry and associated biota on decomposing leaves in a tropical stream. Given the differences between tropical and temperate ecosystems there is good reason to believe that leaf breakdown and its controlling factors may differ between these two biomes.

Leaf inputs into tropical streams represent a wide range of chemical parameters, due to the high diversity of tropical plants and their tendency to be better chemically defended against

herbivores than temperate species (Levin 1976, Coley and Aide 1991, Dyer and Coley 2001). A prevailing paradigm in the literature states that high concentrations of secondary compounds in leaves of tropical species inhibit stream invertebrates (Janzen 1975, Wantzen et al. 2002) and retard leaf litter breakdown (Stout 1989). However, no study in a tropical stream has substantiated this. In fact, no study to date has even measured secondary compounds in tropical leaves through the breakdown process, as we do here.

In addition to differences in leaf litter chemistry, the relative role of detritivores has also been hypothesized to differ between temperate and tropical streams (Irons et al. 1994). Based on a cross-latitudinal comparison of leaf breakdown and a literature review, Irons et al. (1994) hypothesized that due to the lack of invertebrate 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 invertebrates using leaf bags of different mesh size (Rosemond et al. 2002, O'Connor et al. 2000, Wright and Covich 2005a). However, data on microbial (fungi and bacteria) biomass on decomposing tropical leaves are notably scarce (see reports of fungal biomass on tropical leaves: Mathurai and Chauvet 2002, Rosemond et al. 2002, Ardón et al. 2006). We know of no previous study that has reported fungal and bacterial biomass on decomposing leaves representing a wide range of chemical quality in a tropical stream.

We examined the hypothesis that chemical changes of leaf litter during breakdown affect microbial (fungal and bacterial) and invertebrate processing of leaves. We predicted that secondary compounds will play a more important role than structural compounds in inhibiting leaf breakdown rate. Objectives of this study were to: (1) compare leaf litter breakdown rates among eight tropical riparian tree species spanning a relatively wide range of initial chemistry; (2) document changes in leaf litter nutrient content (carbon, nitrogen, and phosphorus), structural (lignin, cellulose, and hemicellulose), and secondary compounds (condensed tannins,

total phenolics, and hydrolysable tannins) of these species during breakdown; and (3) examine whether fungal, bacterial and invertebrate biomass on leaves track these chemical changes.

Methods

Study site

This study was conducted at La Selva Biological Station, Costa Rica (10°26´ N, 84°01´W). The 1536 ha reserve is the lowland terminus of the last protected unbroken biological corridor, spanning altitudinal extremes from 35 to 2906 meters above sea level, on the Caribbean slope of Central America. La Selva receives 4000 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. 2001). This study was conducted 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 of small cobbles and gravel in riffles, and gravel and sediment in runs. Stream water temperature is relatively constant throughout the year (24-27 °C), with mean annual pH values ranging from 5.5 - 6 (Ramírez 2001). Twenty-six species of fish have been recorded in the Sabalo (Burcham 1988), and 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 3 month study period, 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 Millipore filters) 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 bi-weekly using handheld meters (Hanna Instruments, RI, USA). Daily maximum and minimum water temperatures were also recorded. Flow velocity over the leaf packs was measured bi-weekly using a Marsh-McBirney velocity meter (Frederick MD, USA). Gauge height was recorded monthly and used to calculate discharge based on staging equations (C.M. 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 of how many leaf bags one researcher could process immediately), we ran two separate experiments. In spring 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). In 2004 we repeated the experiment using leaves from two new species [Carapa nicaraguensis C. DC. (Meliaceae) and Simira maxonii (Standl.) Steverm. (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 two new species were selected to expand the range in initial chemistry. We repeated the use of two species (one fast-decaying species and one slow-decaying species) 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 10 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-gram leaf packs using plastic mesh bags (22 cm x 40 cm), with a coarse mesh (5 mm) to allow access by stream

fauna (Benfield 1996). We mixed leaves collected from different individual trees growing on different soil types before placing them into bags. Leaf packs were anchored to the streambed using metal stakes (Boulton and Boon 1991).

We collected one leaf pack per site on day '0' and six pre-determined 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 pre-select leaf collection retrieval dates for each species in order to cover most of the breakdown process (i.e., up 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 to determine initial concentrations of chemical constituents. On each collection date we removed one leaf pack 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 hours at 40 °C, weighed, and ground. A sub-sample (1 gram) was ashed at 500 °C for one hour and re-weighed 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, Bas 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 leaf pack were examined. On day '0' and three collection dates (selected to represent early, middle and late stages of breakdown), sub-samples were taken for chemical analysis. Sub-samples 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, New York). Three

separate analyses were conducted for phenolics: condensed tannins, hydrolysable tannins and total phenolics. For tannin analysis, samples were extracted in 70% acetone with 1mM ascorbic acid and evaporated under reduced pressure to provide aqueous extracts. Condensed tannins were estimated as proanthocyanidins (Rossiter et al. 1988). Hydrolysable tannins were estimated using a potassium-iodate technique (Hunter et al. 2003). Total phenolics were estimated with the Folin-Denis assay (Swain 1979). To avoid problems associated with using commercial standards, all samples were compared to standards prepared from pooled litter samples (Appel et al. 2001). 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 (again 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 were punched from randomly selected leaves from each leaf pack immediately after collection on three collection 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 hours at 40 °C, weighed, ignited at 500 °C for 1 hour, and re-weighed to determine AFDM. Ergosterol was extracted from leaf disks in alkaline methanol by refluxing for 30 minutes, partitioning into pentane, drying and re-dissolving 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 conversion factors (Gessner and Chauvet 1993).

Bacterial biomass was calculated by staining bacteria with 4',6-diamidino-2-phenylindole [DAPI; (Porter and Feig 1980)]. To dislodge bacterial cells from leaf disks, samples were sonicated in an ice bath for 10 minutes (VWR HT 150 Sonicator). Following sonication, 2 mL sub-samples were placed in a 12-port Millipore vacuum filter manifold and stained with DAPI (final concentration 10 μ g L⁻¹) for 10 minutes in the dark. Samples were filtered through black polycarbonate membrane filters (0.22 μ m, Poretics) backed with a 0.45 μ m Millipore cellulose nitrate filters and rinsed with 2 ml of 2% buffered formalin. Filters were mounted on glass slides with Cargille type FF non-fluorescent immersion oil. Bacteria were counted using 1000x epifluorescent microscopy (Olympus BH-2). At least 10 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 μ m⁻³ (Bratbak 1985).

Statistical analyses

Analysis of covariance (ANCOVA) was used to test for differences in breakdown rates among leaves of the eight tree species. We used analysis of variance (ANOVA) followed by *post-hoc* Tukey tests to examine differences in initial litter chemistry and in the cumulative (sum of biomass on each sampling date) fungal, bacterial and insects biomass among species. Logarithmic or arcsine square-root transformations were used to meet assumptions of normality. Pearson correlation analyses were performed to examine relationships between breakdown rates, invertebrate, bacterial and fungal biomass, and initial leaf litter chemistry (Gessner and Chauvet 1994). We also examined correlations between chemical constituents through the breakdown process and invertebrate, fungal, and bacterial biomass. All statistical analyses were performed on Statistical Analysis Systems (SAS 1999).

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 ($F_{1, 266} = 6.17$, 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 ($F_{1, 59} = 14.03$, p< 0.005). In 2004, the leaf packs were subject to higher flow velocity than in 2003 (Table 2.1; $F_{1, 54} = 30.52$, p < 0.001).

Mass loss

There was an order of magnitude variation in breakdown rate among the eight species (Fig. 2.1, Table 2.2). Breakdown rates were fastest for *Trema* ($k = 0.198 \text{ d}^{-1}$, 15 days to 95% mass loss), and slowest for *Zygia* ($k = 0.014 \text{ d}^{-1}$, 214 days to 95% mass loss; Table 2.2). We observed a faster breakdown rate for *Ficus* in 2004 than in 2003 (k in 2003 = 0.079 d⁻¹, k in 2004 = 0.11d⁻¹; F = 5.61, 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; F = 2.11, p = 0.15).

Chemical changes in leaves during breakdown

There was significant variation in initial leaf litter chemistry among the eight species (Table 2.3). 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 strongly correlated with leaf toughness and initial concentrations of structural compounds (leaf toughness r = -0.86, p< 0.001; cellulose r = -0.78, p < 0.01, carbon r = -0.77, p < 0.01, lignin r = -0.67, p < 0.01; Table 2.4). For ease of presentation of the chemical data, we grouped the eight leaf species into three categories which were processed at similar rates and followed similar chemical trajectories. The categories are:

(a) "Fast": *Ficus* (2003 and 2004) and *Trema*; (b) "Intermediate": *Castilla, Luehea, Terminalia,* and *Simira*; and (c) "Slow": *Zygia* (2003 and 2004) and *Carapa*.

Nutrient content: Initial carbon concentration was highest in *Zygia* leaf litter (47.5 % DM) and lowest in *Trema* (34.1 % DM, Table 2.3). Concentrations of carbon decreased in all species through time except for *Terminalia* which had a slight increase in the last sampling date (Fig. 2.2). Initial leaf litter nitrogen concentration was highest in *Simira* (2.05 % DM; Table 2.3) and lowest in *Carapa* (0.91 % DM; Table 2.3). Nitrogen concentrations increased until halfway through the decomposition process and then declined in *Ficus, Trema, Castilla,* and *Luehea* (Fig. 2.2 a, b). On the other hand *Terminalia* and *Simira* exhibited strong increases in % N (Fig. 2.2 b), while *Carapa* and *Zygia* had slight increases throughout the breakdown process (Fig. 2.2 c). *Castilla* had the highest initial phosphorus concentration (0.11 % DM), whereas *Zygia* had the lowest (0.06 % DM; Table 2.3). Phosphorus concentration increased rapidly in *Trema* and *Ficus* leaves, followed by a decline on the last sampling date (Fig. 2.2 a). In the other six species, P concentration declined on the first sampling date and then increased (Fig. 2.2 b, c).

Structural compounds: Lignin concentrations were highest in *Zygia* (30.2 % DM) and lowest in *Trema* (5.5 % DM; Table 2.3). Lignin concentrations tended to increase in *Ficus*, *Trema*, and *Simira* (Fig. 2.3 a, b), while the other species showed an initial increase followed by a decline (Fig. 2.3 b, c). Initial concentrations of cellulose were lowest in *Trema* (12.2 % DM), and highest in *Zygia* (24.5 % DM; Table 2.3). Concentrations of cellulose declined in leaf litter of all eight species during the breakdown process (Fig. 2.3). Hemicellulose was highest in *Castilla* (21.9 % DM) and lowest in *Carapa* (7.5 % DM; Table 2.3). Concentrations of hemicellulose increased in *Ficus* and *Trema* (Fig. 2.3 a) during the breakdown process, while they tended to decline in the other six species (Fig. 2.3 b, c).

Secondary compounds: Concentrations of condensed tannins were lowest in *Ficus* (0.48 % DM) and highest in *Carapa* (23.2 % DM; Table 2.3). Condensed tannin concentration declined rapidly in all eight species, with the decline being slowest in *Carapa* (Fig. 2.4). Total

phenolics were highest in *Carapa* (32.3 % DM) and lowest in *Trema* (0.97 % DM; Table 2.3). Similar to condensed tannins, concentrations of total phenolics declined rapidly in all 8 species (Fig. 2.4). Hydrolysable tannins were highest in *Carapa* (34.8 %DM) and lowest in *Ficus* (2.6 %DM; Table 2.3). Similar to the other secondary compounds, hydrolysable tannins were lost rapidly from all eight species (Fig. 2.4).

Fungal, bacterial, and invertebrate biomass on the leaves

Fungi: Cumulative fungal biomass was higher in the fast-decomposing than in the slowdecomposing species ($F_{9,39}$ =16, p < 0.001). Fungal biomass increased rapidly and then declined on *Trema* and *Ficus* leaves (Fig. 2.5 a, b). In contrast, fungal biomass increased steadily through out the breakdown process for all other species. *Trema* and *Ficus* leaves supported the most fungal biomass (Fig. 2.5 a, b). Fungal biomass accumulated on leaves was strongly positively correlated with leaf litter breakdown rate (r = 0.65, p < 0.05; Table 2.5). Fungal biomass on leaves was related to initial leaf toughness (r = -0.78, p < 0.01) and concentrations of carbon (r = -0.74, p = 0.01, Table 2.6 A). Fungal biomass through the breakdown process was related to changes in lignin concentration, lignin:N and lignin:P ratios during breakdown (lignin r = -0.55, p < 0.01; lignin:N r = -0.55, p < 0.01, lignin:P r = 0.45, p = 0.05; Table 2.6 B).

Bacteria: Biomass was significantly higher in fast-decomposing species than in the slow decomposing species ($F_{9,39}$ = 125, p <0.001). Bacterial biomass increased steadily in all leaf species (Fig. 2.5). Similar to fungal biomass, *Trema* and *Ficus* supported the highest bacterial biomass (Fig. 2.5 a, b). Bacterial biomass was strongly correlated to leaf litter breakdown rate (r = 0.86, p <0.01; Table 2.5). Both initial concentrations in cellulose (r = -0.73, p = 0.01) and carbon (r = -0.67, p < 0.05) affected bacterial biomass (Table 2.6 a). Changes in carbon (r = -0.73, p < 0.01), C:P (r = -0.49, p < 0.05), and lignin:P (r = -0.55, p = 0.01) ratios also affected bacterial biomass (Table 2.6 b). Since these are the first estimates of bacterial biomass on

decomposing leaves in a tropical stream we re-counted half of the samples. Out of 15 comparisons, we only found two dates in 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 (Table 2.7). There were significant differences in cumulative amount of insect biomass colonizing the leaves ($F_{9,39} = 3.81$, p < 0.05). *Carapa* had the highest cumulative biomass (biomass = 17.9 mg leaf pack⁻¹) and *Trema* the lowest (biomass = 2.2 mg leaf pack⁻¹; Fig. 2.6). In *Trema, Castilla, Luehea,* and *Zygia* we observed a peak in insect abundance followed by a decline (Fig. 2.6 a, e, f, h). Mean insect biomass was negatively correlated to the sum of mean fungal and bacterial biomass (r = - 0.67, p < 0.05, Fig. 2.7).

Discussion

The observed changes in the chemistry of decomposing leaves and concomitant changes in associated microbial and insect biota provide no support for the prediction that secondary compounds play a more important role than structural compounds in inhibiting leaf breakdown rate. Contrary to a prevailing paradigm that high secondary compounds in tropical leaves inhibit breakdown rate, secondary compounds were rapidly leached (2-fold faster than rates reported for temperate leaves), and structural compounds were more important than secondary compounds in retarding leaf breakdown rate. The initial hypothesis, that changes in leaf litter chemistry during breakdown affect both microbial and insect processing of leaf litter, was only partially supported. Leaf litter chemistry affected microbial biomass (Table 2.6) which, in turn, affected leaf litter breakdown (Table 2.5). On the other hand, insect assemblages on leaf packs, which lacked shredders and were dominated by collector-gatherers, appeared to play an insignificant role in the overall breakdown process (Table 2.5) and did not seem to rely on microbial conditioning of leaves (Fig. 2.7).

Changes in leaf litter chemistry during breakdown

Our results indicate that among these eight species, percent carbon (% C) is a good predictor of breakdown rate (Table 2.4). We did not expect this result because, while % C provides an overall index of carbon content, it does not provide information on the quality (i.e. availability) of carbon (Aerts 1997). Unlike other studies which have found carbon to nutrient ratios to be good predictors of breakdown rate (Enriquez et al. 1993), we did not find significant correlations between breakdown rate and initial C:N or C:P ratios.

Nitrogen is the element believed to have the strongest effect on breakdown rate in temperate streams (Webster and Benfield 1986). However, we did not find a relationship between initial nitrogen content of the eight tree species and breakdown rate (Table 2.4), which could be due to the relatively high initial nitrogen content of these leaves. Initial nitrogen concentrations of the eight species used in this study (mean = 1.60 % N) were higher than concentrations observed in temperate leaf litter [mean = 0.94 % N; (Aerts 1997)]. Our results support previous reports of high nitrogen concentration in forest leaf litter in La Selva [mean = 1.73 % N; (Wood et al. in press)]. Another line of evidence that indicates nitrogen was not limiting is that we only observed increases in % N in Simira and Terminalia leaves, and slight increases in Zygia and Carapa leaves (Fig. 2.2). Previously reported increases in % N of leaf litter during processing in temperate streams have been attributed to microbial colonization of leaves and immobilization of dissolved N from the overlying water (Kaushik and Hynes 1971, Triska et al. 1975, Meyer and Johnson 1983, Robinson and Gessner 2000). We hypothesize that the high initial leaf litter nitrogen content was sufficient for microbes colonizing the leaves to meet their nutrient requirements, without needing to immobilize N from overlying water (Suberkropp 1998, Chadwick and Huryn 2003). Our results agree with Stallcup (2004), who reported no increase in leaf breakdown of Trema and Ficus leaves in response to N-enrichment in La Selva streams.

Phosphorus (P) concentration increased during breakdown for all eight species (Fig. 2.2). We observed some initial leaching of leaf P-content in the intermediate and slow decomposing species, followed by an increase in % P (Fig. 2.2). We interpret this increase in % P to indicate that the microbial community colonizing the leaves was P-limited, which led to immobilization of P from the overlying water. Recent reviews on the C:N:P ratios of leaf litter across broad geographical scales suggest that terrestrial decomposition might be limited by low P-content of leaves in tropical systems (McGroddy et al. 2004, Reich and Oleksyn 2004). Although initial concentrations of P were not correlated to breakdown rate in our study (Table 2.4), increases in % P during breakdown provide evidence that leaf litter P-content might also be important in determining processing in tropical streams.

While it has long been recognized that overall mass loss occurs rapidly in tropical streams (Irons et al. 1994, Benstead 1996, Dudgeon and Wu 1999, Mathurai and Chauvet 2002), our work illustrates that processing of individual structural compounds is also much faster. It has been suggested that lignin degradation is faster and more complete in tropical than temperate terrestrial ecosystems (Lavelle et al. 1993, Coûteaux et al. 1995). Our results suggest that fast degradation of lignin also occurs in tropical stream ecosystems. In order to compare the processing of individual chemical constituents we calculated the absolute amount of each constituent (concentration x leaf pack weight; Suberkropp et al. 1976) and compared their disappearance rate to studies done in temperate streams (Table 2.8). Processing of cellulose was six-fold faster in this study than in similar studies done in temperate streams (2.87% per day in this study compared to 0.46% average of temperate studies; Table 2.8), and lignin was processed an order of magnitude (10-fold) faster in our tropical study stream when compared to studies carried out in temperate streams (2.4 % per day in this study, compared to 0.22% per day average in temperate studies, Table 2.8).

We suggest that high microbial enzymatic activity is responsible for the rapid processing of chemical constituents in this tropical stream. Under laboratory conditions, tropical aquatic

fungi have exhibited similar basal enzymatic production to temperate aquatic fungi (Bucher et al. 2004). However, since enzymatic activity has been shown to increase with higher water temperature (Jenkins and Suberkropp 1995), we believe the relatively high and stable (24 - 26 °C) water temperature in the study stream might provide an ideal environment for high enzymatic activity. High microbial respiration rates measured on leaves in streams in La Selva support this hypothesis (Ramírez et al. 2003, Ardón et al. 2006).

We found a strong correlation between leaf toughness and structural compounds (lignin r = 0.78, p < 0.01, cellulose r = 0.69, p < 0.05), which suggests that leaf toughness is a good indicator of the concentrations of structural compounds and a good predictor of breakdown rate, as has been suggested for terrestrial systems (Gallardo and Merino 1993). The importance of leaf toughness in determining leaf breakdown rates has also been observed for temperate streams. For example, thick cuticles in conifer needles (Bärlocher et al. 1978) and rhododendron (Webster and Waide 1982) have been reported to inhibit leaf breakdown. Leaf toughness also reduces physical abrasion, which might explain why the higher flow velocity in 2004 affected leaf breakdown rate of *Ficus* (physically softer leaves) but not of *Zygia* (physically tougher leaves).

We observed rapid loss of secondary compounds, similar to results reported from temperate streams (Triska et al. 1975, Suberkropp et al. 1976, Rosset et al. 1982, Paul et al. 1983). However, loss of these compounds was 2-fold greater than rates reported in temperate studies (Table 2.8). The fast disappearance of these compounds is due, in part, to the relatively high water temperature since leaching of soluble compounds can be enhanced by increased water temperature (Short and Ward 1980, Paul et al. 1983). The fast leaching of secondary compounds contradicts a prevailing paradigm 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 with

few species from each site [i.e two or less, (Stout 1989)], or incubated three or more tropical species in temperate streams (Campbell and Fuchshuber 1995, Wantzen et al. 2002). Neither of these approaches has provided solid evidence that would indicate an important role of secondary compounds in determining leaf breakdown of tropical species. The fact that these compounds are rapidly leached from the leaves and their concentrations are not correlated with breakdown rate, strongly suggests that leaf secondary compounds are unlikely to play an important direct role in leaf breakdown among tropical species.

It is possible that secondary compounds, which we assumed were leached from the leaves, actually reacted with leaf nitrogen to form recalcitrant lignin-like compounds, which could indirectly inhibit litter processing (Suberkropp et al. 1976). For example, Suberkropp et al. (1976) attributed increases in the absolute amount of lignin, during the first eight weeks of decomposition of oak and hickory, to reactions between leaf nitrogen and secondary compounds. We only observed increases in the absolute amount of lignin during the first week for *Castilla, Luehea, Terminalia,* and *Zygia,* followed by declines in the second week (data not shown). These increases suggest that for these four species, secondary compounds could play an indirect role by reacting with nitrogen to form new lignin-like compounds, although the increases in the absolute amount of lignin we observed were not as strong or prolonged as those observed by Suberkropp et al. (1976). Future studies should consider measuring the nitrogen content of the lignin fraction, as suggested by Suberkropp et al. (1976), to examine if reactions between litter N and secondary compounds might be occurring in tropical streams.

Changes in fungal, bacterial and insect biomass

Changes in fungal and bacterial biomass through time provide three lines of evidence for the important role of microbes in leaf breakdown. First, fungal and bacterial biomass reported here are some of the highest reported in the literature. Fungal biomass measured on *Trema* and *Ficus* leaf packs (*Trema* mean 106 mg g AFDM⁻¹; *Ficus* mean 116 mg g AFDM⁻¹) are on the
high-end of values reported for temperate streams [mean value 88 mg g^{-1} ; (Gessner et al. 1997)]. Yet, our values were similar to those reported in another tropical stream in Colombia (Mathurai 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). Second, both fungal and bacterial biomass were strongly positively correlated with breakdown rate, suggesting that they played an important role in the breakdown process (Table 2.5). Finally, concentrations of cellulose, carbon and lignin were important in determining both breakdown rate and microbial biomass, suggesting that carbon availability inhibited microbial processing of leaves.

We observed changes in the relative abundance of fungi and bacteria that are similar to reports from temperate streams. For example, in *Trema* and *Ficus* we observed a decline in fungal biomass, followed by an increase in bacterial biomass, suggesting that bacteria might play a more important role in later stages of breakdown of these two species, as has been observed in temperate streams (Webster and Benfield 1986). Bacteria are limited by the surface to volume ratio of the material they colonize and tend to dominate smaller fractions of organic matter, whereas fungi dominate larger fractions like leaves (Findlay et al. 2002). *Trema* and *Ficus* leaves were severely fractured in later stages of breakdown, which probably explains why bacteria became more predominant. Several studies have also shown antagonistic interactions between fungi and bacteria (Mille-Lindblom and Tranvik 2003, Wright and Covich 2005b). Whether observed changes in fungal and bacterial biomass were due to fragmentation, or antagonistic interactions between fungi and bacteria might and bacteria, merits further research.

Three 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 leaf packs (Table 2.7). This agrees with some previous

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studies of insect assemblages in tropical streams (Dudgeon 1982, Ramírez and Pringle 1998, Dobson et al. 2002, Mathurai and Chauvet 2002). Second, we found a negative correlation between insect biomass and leaf breakdown rate (Table 2.5), which we interpret to indicate that insects are using recalcitrant leaves as a substrate for attachment and feeding on particles deposited on the leaves. And third, insects did not track bacterial and fungal colonization of leaves, as evidenced by the negative correlation between microbial (fungal + bacterial) biomass and insect biomass (Fig. 2.7). This negative correlation suggests that the important role of microbial conditioning for insect colonization of leaves, which has been described thoroughly in temperate systems (Anderson and Sedell 1979), may not be as important in this tropical stream.

Emerging patterns in leaf breakdown in tropical streams

Overall, our results suggest significant differences in the leaf breakdown process between this tropical stream and what is known from temperate streams. First, the processing rate of individual chemical constituents seems to be faster in the study stream than previously reported in temperate streams, particularly for lignin. Second, we did not find any evidence for a direct inhibitory effect of leaf secondary compounds on leaf breakdown, although our results suggest the possibility for an indirect role of secondary compounds in some of the species. Third, insect assemblages were dominated by collector-gatherers and did not play an important role in driving leaf breakdown, and furthermore did not track fungal and bacterial biomass on leaves, as they do in temperate streams.

Together these results suggest that leaves of different chemical quality, which are processed at varying rates, potentially serve different functional roles (i.e. relative to temperate streams) in this tropical stream. The functional role that leaf litter inputs play in supporting stream food-webs depends upon the rate at which leaves enter the stream and the residence time of this litter once it is in the stream. In temperate streams, where leaf fall occurs once a year, an allochthonous resource base composed of leaf litter that is processed at varying rates

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will provide food at different times of the year for long-lived insects (Grubbs and Cummins 1996, Schofield et al. 2001). In contrast, in lowland tropical streams, overall leaf litter inputs are constant throughout the year; however the rate of leaf 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 did notice that trees which produced leaves that were rapidly processed (i.e. *Trema* and *Ficus*) produced more leaf litter than those species whose leaves were processed more slowly (i.e. *Zygia* and *Carapa*). This agrees with Coley and Aide (1991), who suggested that plants which produce more leaves, tend to produce them with lower concentrations of structural compounds. The rate of leaf litter production might be an important factor determining leaf litter chemistry, and thus breakdown rate in tropical streams.

Once in the stream, leaves that are processed at different 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 carbon provided by these leaves is lost as microbial respiration, while some becomes an important source of fine particulate organic matter for consumers downstream. The rapid processing of *Trema* leaves, which has almost completely disappeared in 15 days, would make 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 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 during the time the leaves are in the stream. Future studies into leaf breakdown in tropical streams are needed to provide further insights into these patterns.

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		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
рН	5.89	5.71 - 6.13	5.90	5.26 - 7.23
Conductivity (µS)	65.13	47.40 - 80.10	65.61	47.20 - 85.00
PO₄-P (µg L⁻¹)	4	1 - 12	6	1 - 27
NO₃-N (µg L⁻¹)	102	33 - 247	134	96 - 197
NH₄-N (µg L⁻¹)	20	0 - 36	3	0 - 29

Table 2.1. Chemical and physical characteristics of the Sabalo study stream, La Selva Biological Station during experiments.

* significantly different between years (p < 0.05)

Table 2.2. Breakdown rate coefficients for eight common riparian tree species incubated in the Sabalo study stream. Different letters denote significant differences using ANCOVAs.

	<u> </u>				
Species	Breakdown rate k (day ⁻¹)	Standard	r ²	Days to 95% mass loss	
2003	n (ddy)	01101	•		
Trema integerrima Ficus insipida	0.198 0.079	0.0170 0.0039	0.81 0.93	15 38	a b
Terminalia oblonga	0.039	0.0029	0.93	77	С
Castilla elastica	0.038	0.0018	0.94	79	С
Luehea seemannii	0.033	0.0045	0.83	91	С
Zygia longifolia	0.011	0.0014	0.86	272	d
2004					
Ficus insipida	0.111	0.0120	0.86	27	е
Simira maxonii	0.048	0.0044	0.90	62	bc
Carapa nicaraguensis	0.023	0.0020	0.92	130	cd
Zygia longifolia	0.014	0.0017	0.86	214	d

Species	Nitrogen	Carbon	Phosphorus	Lignin	Cellulose	Hemicellulose
2003						
Trema integerrima	1.61 (0.11)	34.08 (0.55)	0.10 ^a (0.010)	5.57 ^a (1.36)	12.26 (0.70)	10.10ª (1.22)
rienia nicegorinia	1.27	35.03	0.08 ^b	8.33ª	(011°) 19.07ª	(14.67 ^b
Ficus insipida	(0.03) 1.36	(0.64) 39 22ª	(0.006) 0.12ª	(1.55) 6.96ª	(1.34) 17 50ª	(1.29) 13 89 ⁶
Terminalia oblonga	(0.14)	(0.46)	(0.014)	(1.61)	(0.41)	(1.27)
elastica	(0.20)	40.44 ⁻ (1.05)	0.16 (0.025)	13.40 [×] (1.44)	19.47 ⁻ (1.67)	(2.73)
Luehea seemannii	1.27 (0.15)	44.18 ^{ab} (0.10)	0.11 ^a (0.010)	16.33 [⊾] (0.81)	19.85ª (0.70)	15.46 ^b (1.59)
Zygia	1.87 ^a	46.45 ^b	0.06 ^b	28.36 ^c	24.58 ^b	17.52
longitolia	(0.09)	(0.54)	(0.002)	(1.09)	(1.61)	(0.82)
2004 Ficus insipida	1.40 (0.06)	39.81ª (1.06)	0.08 ^b (0.004)	8.20ª (1.55)	19.54ª (1.20)	15.05 [⊾] (1.22)
Simira maxonii	2.05 ^a (0.14)	44.91 ^{ab} (0.83)	0.11ª (0.010)	15.33 [♭] (3.12)	22.93 ^b (0.60)	14.62 ^b (0.40)
Carapa nicaraguensis	0.91 (0.26)	41.71 ^a (0.43)	0.08 ^b (0.002)	18.34 ^b (4.71)	17.98 ^a (2.82)	7.57 ^a (2.17)
Zygia longifolia	1.87ª (0.06)	47.48 ^b (0.69)	0.06 ^b (0.009)	30.28 ^c (3.37)	24.50 ^b (2.21)	17.30 ^a (1.90)

Table 2.3. Mean initial concentration (percent dry mass \pm 1 standard deviation) of chemical constituents and leaf toughness (g) in eight species of common riparian trees at La Selva Biological Station. Means with same letters are not significantly different.

Species	Total phenolics	Hydrolisable Tannins	Condensed Tannins	Leaf toughness
2003				
	0.97	2.96 ^a	0.80 ^a	12
Trema integerrima	(0.28)	(0.24)	(0.11)	(2)
Ficus	11.78 ^a	2.66ª	0.48 ^a	120 ^a
insipida	(5.23)	(0.29)	(0.22)	(26)
	11.46 ^a	11.37	12.85 ^b	138ª
Terminalia oblonga	(2.69)	(2.01)	(7.52)	(9)
Castilla	12.29 ^a	9.44	11.29 ^b	152
elastica	(7.45)	(3.02)	(4.68)	(16)
Luehea	7.42	13.99	13.65 ^b	124 ^a
seemannii	(2.81)	(4.61)	(3.51)	(19)
Zygia	10.04 ^a	7.82 ^c	8.12 ^c	232 ^b
longifolia	(1.29)	(0.85)	(0.50)	(15)
2004				
Ficus	13.86ª	2.45 ^b	0.49 ^a	118
insipida	(5.23)	(4.46)	(0.18)	(8)
Simira	4.26	6.64 ⁶	2.80	134 ^{°a}
maxonii	(0.68)	(1.38)	(0.54)	(10)
	32.33	34.88	23.20	204 ⁶
Carapa nicaraguensis	(11.80)	(10.60)	(5.64)	(4)
Zygia	10.43 ^a	7.75°	8.10 ^c	230 ^b
longifolia	(0.87)	(0.65)	(0.49)	(10)

Parameter	r	р
Inicial		
% Carbon	-0.77	<0.01
% Nitrogen	-0.06	0.79
% Phosphorus	0.09	0.86
Lignin	-0.67	<0.05
Hemicellulose	-0.34	0.28
Cellulose	-0.78	<0.05
Condensed Tannins	-0.58	0.06
Hydrolysable Tannins	-0.42	0.20
Total Phenolics	-0.40	0.31
Leaf toughness	-0.86	<0.01

Table 2.4. Pearson correlation coefficients between exponential breakdown rate (k) and initial concentrations of leaf litter chemical constituents (N = 10).

Table 2.5. Pearson correlation coefficients between breakdown rate (k) and cumulative biomass of fungi, bacteria and insects (N = 10).

Parameter	r	р
Cumulative fungal biomass	0.65	0.04
Cumulative bacterial biomass	0.86	<0.01
Cumulative invertebrate biomass	-0.70	0.02

Table 2.6. (a) Significant Pearson correlations between decomposer biomass and mean initial leaf chemical constituents (N = 10); and (b) Pearson correlations between decomposer biomass and concentrations of chemical constituents during breakdown sampled on same date (N = 18).

	Chemical		
Parameter	constituent	r	р
(a) Initial chemistry			
Cumulative fungal biomass	Leaf toughness	-0.78	<0.01
	Carbon	-0.74	0.01
Cumulative bacterial biomass	Cellulose	-0.73	0.01
	Carbon	-0.67	<0.05
Cumulative invertebrate biomass	Condensed tannins	0.82	<0.01
(b) Chemical changes during breakdown			
Fungal biomass	Lignin	-0.55	<0.01
	Lignin:N	-0.55	<0.01
	Lignin:P	-0.45	0.05
Bacterial biomass	Carbon	-0.73	<0.01
	Carbon:P	-0.49	0.03
	Lignin:P	-0.55	0.01
Invertebrate biomass	No variables		

Table 2.7. Abundance of most common taxa (> 1%) of insects in leaf packs of eight common riparian species incubated in the Sabalo stream. Letters in parentheses refer to functional feeding group: CG= collector-gatherer, Sc = Scraper, F = filterer, P = predator.

Таха	Trema	Ficus	Simira	Terminalia	Castilla	Luehea	Zygia	Carapa
Ephemeroptera								
Thricorythodes (CG)	43	53	13	109	65	89	142	2
Baetis (Sc)	5	29	1	6	23	35	52	
Thraulodes (CG)	2	5	12	5	6	4	20	14
Leptohyphes (CG)	12	12	1	3	8	3	2	
Trichoptera								
Leptonema (F)	5	3	1	5	9	24	16	10
Hydroptila (Sc)	4	9	5	27	33	12	18	3
Neotrichia (Sc)	2	18	1	10	9	7	12	2
Odonata								
Argia (P)	2	2	2	6	3	2	8	1
Haeterina (P)	4	1	1	1	1	4	8	3
Libellulidae (P)				3	5	2	1	1
Coleoptera								
Microcylloepus (CG)	5	18	26	56	20	24	66	140
Elmidae (CG)	2	2	16	14	5	12	4	17
Diptera								
Ceratopogonidae (P)	3	12	7	31	34	6	12	6
Hemerodromia (P)	3	3	12	10	13	5	18	51
Chironomidae								
Tanypodinae (P)	11	44	4	73	77	62	95	19
Others (CG)	464	554	497	878	792	398	700	739
Hydracarina	15	17	5	9	10	9	6	5
Total	582	782	604	1246	1113	698	1180	1013

Study	Species	Cell	HC	Lig.	Ν	Phen.
Triska et al. 1975	Alnus rubra			0.32		
	Acer circinatum			0.30		
	Acer macrophyllum			0.28		
	Pseudotsuga menziesii			0.20		
Suberkropp et al. 1976	Quercus alba	0.44	0.39	0.23	0.18	6.25
	Carya glabra	0.59	0.47	0.27	0.65	5.36
Rosset et al.1982	Quercus petraea	0.42		-0.37	0.79	0.61
	Larix decidua	0.27		-0.24	0.28	0.63
	Picea abies	0.22		-0.20	0.00	0.47
Paul et al. 1983	Acer negundo	0.79	1.19	0.16		
	Platanus occidentalis	0.64	0.16	-0.46		
Chauvet 1987	Alnus glutinosa	0.60		0.13		
	Populus nigra	0.50		0.10		
	Salix alba	0.49		0.00		
Campbell et al. 1992	Eucalyptus viminalis				-0.05	0.86
	Acacia melanoxylon				-0.03	0.83
	Pomaderris aspera				0.00	0.59
Bärlocher et al. 1995	Alnus glutinosa					3.35
	Eucalyptus globulus					3.00
This study	Carapa nicaraguensis	1.03	1.03	0.95	0.95	1.08
	Castilla elastica	1.70	1.75	1.40	1.68	2.06
	Ficus insipida 03	4.40	3.79	3.30	4.23	4.70
	Ficus insipida 04	5.00	4.05	3.50	4.96	5.54
	Luehea seemannii	1.10	1.11	1.10	1.10	4.60
	Simira maxonii	4.00	3.79	3.00	3.34	4.11
	Terminalia oblonga	2.50	2.07	2.40	1.24	14.25
	Trema integerrima	7.50	6.36	5.80	7.13	19.30
	Zygia longifolia 03	0.85	0.88	0.70	0.58	4.60
	Zygia longifolia 04	0.59	0.40	0.20	0.48	0.80
Temperate average		0.46	0.51	0.22 / 0.05*	0.23	1.95
This study average		2.87	2.52	2.24	2.57	6.10
Difference between tro	pical and temperate	6x >	4x >	10 / 44x >*	11x >	2x >

Table 2.8. Rate of change (percent per day) for chemical constituents in studies conducted in temperate streams, compared to rate of changes in decomposing leaf litter in this study. Cell = cellulose, HC = hemicellulose, Lig = lignin, N = nitrogen, Phen = phenolics.

* First value does not include studies where absolute amount of lignin content increased during breakdown; second value includes studies where absolute amount of lignin increase.

Figure 2.1. Natural logarithm of ash free dry mass (AFDM) remaining in leaf packs over time in the Sabalo study stream. Line corresponds to linear regression used to determine breakdown rate (*k*). *Ficus* and *Zygia* were used both years. Solid line represents regression from the experiment in 2003; dashed line represents regression from the experiment in 2004.



Figure 2.2. Changes in nutrient concentration (carbon, nitrogen and phosphorus) in decomposing leaves of eight species of common riparian trees in the Sabalo study stream. Species are grouped into: (a) *"Fast*", (b) *"Intermediate"* and (c) *"Slow"* decomposing species based on their overall breakdown rate. Symbol legend for individual species appears above each group of graphs. Note different scales on y-axes.



(b) Intermediate ---- Castilla -∆-- Luehea -▲ Terminalia ---- Simira





Figure 2.3. Chemical changes in structural compounds (cellulose, hemicellulose and lignin) in decomposing leaves of eight species of common riparian trees in the Sabalo study stream. Species are grouped as: (a) "*Fast*", (c) "*Intermediate*" and (c) "*Slow*" decomposing species based on their overall breakdown rate. Symbol legend for individual species appears above each group of graphs. Note differences on y-axis on (c) "Slow".



Figure 2.4. Chemical changes in secondary compounds (total phenolics, condensed tannins and hydrolysable tannins) in eight species of common riparian trees. Species are grouped into: (a) "*Fast*", (b) "*Intermediate*" and (c) "*Slow*" decomposing species based on their overall breakdown rate. Symbol legend for individual species appears above each group of graphs. Note different scales on y-axes.



Day

Figure 2.5. Fungal and bacterial biomass on leaf packs of eight common riparian tree species over time in the Sabalo study stream. Mean biomass \pm 1 standard error.



Figure 2.6. Insect biomass on leaf packs of eight common riparian tree species over time in the Sabalo study stream. Mean biomass \pm 1 standard error. Note different scale of y-axis on (a) *Trema* graph.



Figure 2.7. Relationship between mean microbial (fungal plus bacterial) biomass and a) insect biomass per leaf pack (r^2 = 0.67, p= 0.04); and b) insect biomass per gram of AFDM (r^2 = 0.47, p = 0.07) in leaf packs of eight common riparian trees incubated in the Sabalo study stream.



CHAPTER 3

EFFECTS OF STRUCTURAL COMPOUNDS ON LEAF BREAKDOWN RATES IN A TROPICAL AND A TEMPERATE STREAM: IMPORTANCE OF USING STANDARDIZED ANALYTICAL TECHNIQUES TO MEASURE LEAF LITTER CHEMISTRY ²

² Ardón, M., C.M. Pringle, and S.L. Eggert. To be submitted to: Journal of the North American Benthological Society
Abstract

Comparisons of the effects of leaf litter chemistry on breakdown rate in tropical versus temperate streams are hindered by the lack of comparability of analytical methods used to measure leaf chemistry between studies and the paucity of comprehensive studies on tropical streams. Here, we used standardized analytical techniques to measure leaf litter chemistry from common riparian tree species at two sites where there is a relatively large amount of information on leaf litter chemistry and leaf breakdown: a tropical site (La Selva Biological Station, Costa Rica) and a temperate site (Coweeta Hydrologic Laboratory, N.C., USA). We selected eight common riparian tree species from La Selva and seven common riparian species from Coweeta to represent the range of leaf litter chemistry naturally entering streams at each site. Based on existing paradigms, we predicted that concentrations of secondary compounds (total phenolics, condensed, and hydrolysable tannins) would be higher and more strongly related to leaf breakdown rate among tropical species at La Selva than among temperate species at Coweeta. Previous studies that have examined differences in leaf breakdown in temperate and tropical streams have relied on literature values for leaf chemistry, conducted cross-site transplants of only a few (2 - 3) species, or incubated leaf litter from tropical species in temperate streams. Contrary to our initial hypothesis, mean concentration of condensed tannins was significantly (p < 0.001) greater (2.6 - fold) in Coweeta than in La Selva species. Also in contrast to our predictions, concentrations of condensed tannins were negatively correlated to breakdown rate among Coweeta species (r = -0.77), but not among La Selva species. Concentrations of structural compounds were strongly related to breakdown rate at both sites (Coweeta species: lignin r = -0.94, cellulose r = -0.76; La Selva species: cellulose r = -0.77, carbon r = -0.73, and lignin r = -0.65). Our results indicate that leaf litter from common riparian tree species at Coweeta is of lower quality than litter from common riparian trees at La Selva. Based on previous studies conducted in streams and data presented here, we recommend using the

forage fiber method to determine structural compounds, the acid-butanol assay with "selfstandards" to determine condensed tannins, and standard colorimetric assays after digestion to determine nutrient content. Use of standardized analytical techniques to measure leaf litter chemistry will better enable cross-site comparisons and synthesis of results from a broad range of studies.

Introduction

It is generally accepted that leaf chemistry differs between temperate and tropical ecosystems, with leaves from tropical species having higher concentrations and more recalcitrant tannins and phenolics (Levin 1976, Levin and York 1978 Coley and Aide 1991, Dyer and Coley 2001). Various researchers have hypothesized that the range of variation in leaf litter chemistry entering tropical streams is wider than that entering temperate streams, which also leads to a wider range in leaf breakdown rate (Covich 1988, Dobson et al. 2002, Wantzen et al. 2002). However, direct comparisons of effects of leaf litter chemistry on breakdown rate in tropical versus temperate streams are hindered by the lack of comparability in methodologies and the paucity of comprehensive studies from tropical streams. The lack of comparability in methodologies is particularly problematic for secondary compounds, because different analytical techniques provide relative measures that are not directly comparable.

An existing paradigm states that high concentrations of secondary compounds in tropical species play an important role in determining leaf breakdown rates (Stout 1989, Irons et al. 1994, Wantzen et al. 2002, Wantzen and Wagner 2006). But to our knowledge, the effects of leaf litter chemistry on breakdown rate of native species decomposing in their natural habitats at both a tropical and temperate site has not been determined. Stout (1989) examined literature values for condensed tannins (measured using a variety of techniques) and correlated them with leaf breakdown rates to suggest that high concentrations of condensed tannins in tropical species inhibit leaf breakdown. Other studies that have examined this hypothesis have been

conducted with tropical leaves in temperate streams. For example, working in a temperate Australian stream, Campbell and Fuchshuber (1995) showed that condensed tannins were important in determining breakdown rates of six species of *Eucalyptus*, but were not important for twelve other tropical and temperate species. Working in a stream in Germany, Wantzen et al. (2002) reported that leaf litter from tropical species, with higher concentrations of tannins, were processed more slowly than temperate species that were lower in tannins. These results might not be a true reflection of what occurs in tropical streams for two reasons: (1) chemical changes in leaves in tropical streams occur faster than in temperate streams and; (2) the microbial and invertebrate fauna of those temperate streams (i.e. where tropical leaves were incubated) may not have been adapted to deal with the chemical quality of tropical leaves.

Specific objectives of this study were to: (1) directly compare the initial leaf litter chemistry from common native riparian tree species from a tropical site (La Selva Biological Station, Costa Rica) and a temperate site (Coweeta Hydrologic Laboratory, N.C., USA) using standardized analytical techniques; (2) examine the importance of leaf litter chemistry on leaf breakdown at these two sites where there is a relatively large amount of information on leaf litter chemistry and leaf breakdown (Hartshorn 1983, Rosemond et al. 1998, Wood et al. in press, Swank and Crossley 1988, Webster et al. 1999); (3) make recommendations on analytical techniques to facilitate cross-site comparisons.

Methods

Site descriptions

La Selva Biological Station (10°26´ N, 84°01´W, 1536 ha) is located in the Caribbean lowlands of Costa Rica. It is the lowland terminus of the last protected unbroken biological corridor spanning altitudinal extremes in Central America. La Selva receives 4000 mm of rain a year, with more than 400 mm a month from May to December (Sanford et al. 1994). Mean annual stream water temperature ranges from 24 - 26° C. Due to geothermally-modified

groundwater inputs, streams at La Selva display a wide range of variation in solute concentrations (Pringle and Triska 1991), and pH averages ca. 4.5 - 6.5 (Ramírez 2001). Algal assemblages in all of these streams are light-limited due to dense canopy cover (>90%), resulting in detritus-based food webs (Pringle et al. 1993). Most trees in La Selva are non-deciduous, resulting in continuous leaf fall throughout the year (Hartshorn 1983).

Coweeta Hydrologic Laboratory (1625 ha drainage basin) is a U.S. Department of Agriculture Forest Service research facility and Long Term Ecological Research site in the Blue Ridge Province of the Southern Appalachian Mountains (North Carolina, USA). Coweeta Hydrologic Laboratory has a 50+ year history of hydrologic and climatological monitoring data. Coweeta receives approximately 1700 mm a year of rainfall and stream temperatures range from 4 to 17° C (Swank and Crossley 1988). Dissolved concentrations of most ions are usually low (<1mg/L), and pH averages ca 6.6 - 6.8 (Wallace et al. 1986). Streams in Coweeta drain mixed hardwood forests dominated by oaks and hickories, and are heavily shaded by riparian rhododendron (Wallace et al. 1982) resulting in detritus-based food-webs (Wallace et al. 1999).

Leaf litter chemical analyses

Based on the literature and on-going research at La Selva (Hartshorn and Hammel 1994, O. Vargas personal communication) we selected eight common riparian species that represented a wide range in leaf litter chemistry and quality. Freshly fallen leaves were collected in January and February of 2003 and 2004. Species selected were: *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), *Luehea seemannii* Triana & Planch (Tiliaceae), *Carapa nicaraguensis* C. DC. (Meliaceae) and *Simira maxonii* (Standl.) Steyerm. (Rubiaceae). Recently senesced leaves were collected from the ground (avoiding those that had begun to decompose or had signs of herbivory), brought to the

laboratory, and stored in an air conditioned room until used. For each species, leaves were collected from at least ten different individual trees growing in different soil types within La Selva.

Based on the literature, we selected seven common riparian species from Coweeta to represent a range of leaf litter quality (Wallace et al. 1982, Webster et al. 1999). Leaves from Coweeta were collected in October of 2003 and 2004. Species collected included: *Acer rubrum* (Aceraceae, red maple), *Cornus florida* (Cornaceae, dogwood), *Liquidambar styraciflua* (Hamamelidaceae, sweetgum), *Liriodendron tulipifera* (Magnoliaceae, tulip poplar), *Pinus strobus* (Pinaceae, white pine), *Quercus rubra* (Fagaceae, northern red oak), and *Rhododendron maximum* (Ericaceae, rhododendron). Freshly fallen leaves were collected and maintained in an air conditioned laboratory until used for leaf breakdown experiments.

We used standardized analytical methods to measure the chemistry of leaves from La Selva and Coweeta. We estimated structural compounds (i.e. cellulose, hemicellulose, and lignin) using the forage fiber technique which involves sequential neutral detergent/acid detergent digestion on an Ankom 2000 fiber analyzer (ANKOM Fiber Technologies, Fairport, New York; Goering and Van Soest 1970). Three separate analyses were conducted for secondary compounds: condensed tannins, hydrolysable tannins and total phenolics. For tannin analysis, samples were extracted in 70% acetone with 1mM ascorbic acid and evaporated under reduced pressure to provide aqueous extracts. Condensed tannins were estimated as proanthocyanidins using the acid-butanol assay (Rossiter et al. 1988). Hydrolysable tannins were estimated using a potassium-iodate technique (Hunter et al. 2003). Total phenolics were estimated with the Folin-Denis assay (Swain 1979). To avoid problems associated with using commercial standards, all samples were compared to standards prepared from pooled litter samples from each site (Appel et al. 2001). Leaf nutrient content was measured using the following methods: 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)].

Measurements of leaf breakdown rate

Breakdown rates at La Selva were measured in the Sabalo, a third-order stream on the east border of the reserve. We created 5-gram leaf packs using plastic mesh bags (20 cm x 36 cm), with a coarse mesh size (5 mm) in order to allow access to stream fauna (Benfield 1996). Before placing leaves into bags, we mixed leaf litter collected from different individual trees growing on different soil types. Litterbags were anchored to the streambed using metal stakes (Boulton and Boon 1991). Daily maximum and minimum temperatures were recorded. Litterbags were washed to remove invertebrates and sediments, oven dried (40 ° C for 24 – 48 hours), weighed, ashed (500° C for 1 hour), and re-weighed to obtain ash free dry mass (AFDM) remaining at each date.

Breakdown rates at Coweeta were measured in a first-order stream (catchment 53), using 15-gram leaf packs placed in plastic mesh bags [20 cm x 36 cm, mesh 5mm (Eggert and Wallace 2003)]. Daily mean, maximum and minimum temperatures were recorded using Ryan temp-mentors. Litterbags were placed in the stream on the first days of December each year and 3 - 7 replicates collected on nine to eleven collection dates (Eggert and Wallace 2003). Collected litterbags were placed on ice and transported back to the laboratory for processing. Litterbags were washed to remove invertebrates and sediments, oven dried (60° C for seven days), weighed, ashed (500° C for 12 hours), and re-weighed to obtain AFDM remaining for each date.

Data analyses

Breakdown rate, *k*, was estimated by linear regression of natural logarithm transformed percent ash-free dry mass remaining versus day (negative exponential model; Benfield 1996). Using the average of the maximum and minimum daily temperatures for the Sabalo study stream, we estimated breakdown rates in degree days. Water temperature in this stream is fairly constant with a diel range between 24 - 26 ° C and an annual range 24 - 27 ° C (Rosemond et al. 1998). For Coweeta species we divided breakdown rate, *k*, by average daily temperature to provide an estimate of breakdown per degree day (Irons et al. 1994). We examined relationships between breakdown rate (per day and per degree day) and concentrations of leaf litter chemical constituents in species from both sites using Pearson correlation coefficients (Proc Corr). To examine overall variation in leaf chemistry between the two sites we conducted principal component analysis on untransformed leaf chemistry data. We compared mean concentrations of chemical constituents using analysis of variance on natural logarithm (x+1) transformed data to meet assumptions of normality. All analyses were conducted using Statistical Analyses Software (SAS 1999).

Results

Leaf litter chemistry

The ranges of individual chemical constituents were different between the two sites (Table 3.1). Mean concentration of condensed tannins was higher for Coweeta species than for La Selva species ($F_{1,58} = 24.3$, p < 0.001, Fig. 3.1a). Both total phenolics and hydrolysable tannins were slightly higher for Coweeta than for La Selva species, although the difference was not significant (Fig. 3.1b, c). Lignin was higher for Coweeta species than for La Selva species ($F_{1,58} = 4.27$, p < 0.05; Fig. 3.1 d). Cellulose concentrations were similar between both sites, while hemicellulose was higher for La Selva species ($F_{1,58} = 4.27$, p < 0.05; Fig. 3.1 d). Phosphorus and nitrogen concentrations were higher in La Selva species than in Coweeta

species ($F_{1,58}$ = 13.5, p<0.001; $F_{1,58}$ = 102.5, p < 0.0001; respectively Fig. 3.1g, h). Carbon concentration was higher for Coweeta than for La Selva species ($F_{1,58}$ = 45.1, p < 0.0001; Fig. 3.1i).

Principal component analysis of leaf chemistry data did not show a clear separation of species from the two sites (Fig. 3.2). Principal component axis 1 explained 40% of the variation and was positively correlated to condensed tannins (r = 0.41) and negatively correlated to phosphorus (r = -0.40) and nitrogen (r = -0.39). Principal component axis 2 explained 27% of the variation and was positively correlated to cellulose (r = 0.52), hemicellulose (r = 0.50) and lignin (r = 0.37).

Leaf breakdown rate

Mean breakdown rates were 3 to 7-fold faster at La Selva (mean k = 0.058 day⁻¹, 0.0025 degree day⁻¹) than at Coweeta (mean k = 0.008 day⁻¹, 0.0008 degree day⁻¹; Table 3.2). The variance in breakdown rate between the slowest and the fastest species was much higher at La Selva (15-fold based on breakdown rate per day⁻¹; and 17-fold based on breakdown rate per degree day⁻¹) than at Coweeta (3-fold based on breakdown rate per day⁻¹; and 17-fold based on breakdown rate per degree day⁻¹) than at Coweeta (3-fold based on breakdown rate per day⁻¹ and 4-fold based on breakdown rate per degree day⁻¹; Table 3.2). Breakdown rates of slow-decomposing species calculated using degree day (i.e. *Castilla elastica, Luehea seemanii, Zygia longifolia* and *Carapa nicaraguensis* in La Selva; *Acer rubrum, Quercus rubra, Pinus strobes* and *Rhododendron maximum* in Coweeta) were not significantly different between the two sites ($F_{1,6} = 4.08$; p > 0.05; Fig. 3.3). While breakdown rates of the faster decomposing species were significantly different between the two sites ($F_{1,6} = 6.48$; p = 0.05).

Overall, there were stronger relationships between leaf chemical parameters and breakdown rate among Coweeta species than among La Selva species (Table 3.3.) Breakdown rate of Coweeta leaves was strongly correlated to: lignin (r = -0.94, p < 0.01), cellulose (r = -0.76, p < 0.05), phosphorus (r = 0.89, p < 0.01) and condensed tannins (r = -0.77, p < 0.05; Table 3).

Breakdown rate of La Selva leaves was strongly correlated to structural compounds: cellulose (r = -0.77, p < 0.05), carbon concentration (r = -0.73, p < 0.01), and lignin (r = -0.65, p < 0.05; Table 3.3). Concentration of condensed tannins was not significantly correlated to breakdown rates in La Selva species (Table 3.3). Breakdown rate was significantly correlated to %C and lignin when all 15 species were analyzed together using breakdown rates in degree days (Table 3.3).

Discussion

We examined the importance of carbon-based leaf litter chemistry on leaf breakdown at a tropical and a temperate stream using standardized analytical techniques for leaf chemical analyses. Previous studies that have examined differences in leaf breakdown between temperate and tropical streams have either relied on literature values for leaf chemistry [measured in a non-standardized manner among studies (Stout 1989)], conducted cross-site transplants of only a few (2 - 3) species (Stout 1989, Irons et al. 1994), or incubated leaf litter from tropical species in temperate streams (Campbell and Fuchshuber 1995, Wantzen et al. 2002). Direct comparison of initial concentrations, facilitated by the use of standardized analytical techniques, indicated that secondary compounds were higher and more strongly correlated to leaf breakdown in Coweeta than in La Selva species.

Contrary to our predictions based on the literature (Levin 1976, Coley and Aide 1991, Dyer and Coley 2001), concentrations of condensed tannins were significantly (p<0.001) greater (2.6-fold) in temperate Coweeta species than in tropical La Selva species (Fig. 3.1). Of the species analyzed, temperate white pine (*Pinus strobus*), which is considered a poor resource for aquatic invertebrates (Whiles and Wallace 1997), had the highest concentration of condensed tannins. Conifers are known to have high concentrations of tannins and phenolics which can inhibit decomposition and nutrient cycling (Northup et al. 1995). Campbell and Fuchshuber (1995) did not find differences in concentrations of condensed tannins and phenolics in litter of

eight tropical and ten temperate species in Australia. These results combined with the findings reported here bring into question the generalization that concentrations of secondary compounds are greater in tropical than in temperate species.

Our results indicate that leaf litter from common riparian tree species at Coweeta is of lower quality than litter from common riparian trees at La Selva. Despite significant differences in various chemical constituents (Fig. 3.1), PCA analyses of leaf litter chemistry did not provide a clear separation of the species (Fig. 3.2). Axes scores for *Carapa nicaraguensis* (which had low N and high condensed tannins) were similar to Coweeta species, and axes scores for *Liriodendron tulipifera* (which had high N and low condensed tannins) were similar to La Selva species. Future studies should examine the chemistry of the entire litterfall input, rather than just that of common species, in tropical versus temperate streams.

Functional differences in leaf breakdown at a tropical and a temperate site

Mean breakdown rates were faster at the La Selva site than at the Coweeta site. Variance in breakdown rate between the slowest and the fastest decomposing species at La Selva was much greater (15 to 17-fold) than the variance observed at Coweeta (3 to 4-fold). This variance is driven mostly by the extremely fast breakdown rate of *Trema integerrima*. However, when we compared the second fastest (*Ficus insipida*) decomposing species to the slowest (*Zygia longifolia*), the variance at La Selva was still greater (6-fold) than the variance at Coweeta. This agrees with a previous reciprocal transplant experiment of ten species (collected from five sites), where the variance between the slowest and the fastest decomposing species was greater in a Costa Rican stream (27-fold), than in a Michigan (17-fold) or an Alaskan (20fold) stream (Irons et al. 1994).

While average breakdown rate of all species was faster at La Selva, when we compared breakdown rates per degree day (to account for temperature) of slow-decomposing species (i.e. *Castilla elastica, Luehea seemanii, Zygia longifolia* and *Carapa nicaraguensis* in La Selva; *Acer*

rubrum, *Quercus rubra*, *Pinus strobes* and *Rhododendron maximum* in Coweeta), rates were not significantly different between the two sites ($F_{1,6} = 4.08$; p > 0.05; Fig. 3.3). Our results suggest that slow-decaying species in tropical streams can break down at similar rates to slowdecaying species in temperate streams. Previously we reported that, on average, leaves from *Zygia longifolia* lost 95% of their mass in 686 days in headwaters streams at La Selva (Ardón et al. 2006), which is similar to the 656 days for 95% mass loss reported for *Rhododendron maximum* at Coweeta headwater streams (Greenwood 2004).

We hypothesize that increased microbial activity (stimulated by higher temperatures) in tropical streams might accelerate the breakdown rate of higher quality species, increasing the variance between slow- and fast-decomposing species. This large variance might be important in structuring tropical stream food-webs. We previously suggested that, in a tropical stream, fast-decomposing species might be 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 (Ardón 2006, Chapter 2). In temperate systems it has been suggested that leaf litter inputs that decompose at varying rates, can support a higher diversity of organisms by serving as resources to consumers at different times of the year (Cummins et al. 1989, Whiles and Wallace 1997, Schofield et al. 2001, Moore et al. 2004).

Differences in breakdown rate between Coweeta and La Selva could have been confounded by the fact that breakdown rates were measured in a third-order stream at La Selva and a first-order stream at Coweeta. While breakdown rates in the study stream in La Selva were faster than if we had measured them in a smaller stream, probably due to increased physical abrasion related to greater discharge fluctuations, two lines of evidence suggest that breakdown rates observed in the Sabalo study stream are representative of overall patterns in breakdown in smaller streams. First, although breakdown rates of four species measured in the Sabalo stream were 2-3 times faster than rates previously measured in a first order stream at La

Selva (Table 3.4), the relative differences in breakdown rates among species were similar in both streams. For example the difference in breakdown rate between *Trema integerrima* and *Zygia longifolia* was 15-fold in the Sabalo study stream (Table 3.4), and 11-fold in a first-order stream (Ardón et al. 2006). Our results agree with previous reports of relatively constant differences in breakdown rates among species under different stream conditions (Webster and Benfield 1986). Secondly, cellulose and lignin were important chemical constituents inhibiting leaf breakdown both in this study and a previous experiment which incubated three species (*Trema integerrima, Castilla elastica,* and *Zygia longifolia*) in six headwater streams at La Selva (Ardón et al. 2006), suggesting that cellulose and lignin can inhibit breakdown rates in both first-and third-order streams.

Effects of litter chemistry on leaf breakdown rates at a tropical and a temperate site

Contrary to our initial predictions, concentrations of condensed tannins were more strongly related to leaf breakdown rate for temperate Coweeta species than for tropical La Selva species (Fig. 3.1, Table 3.3). There is actually more evidence for an inhibitory role of condensed tannins and other phenolic compounds on leaf breakdown in temperate than in tropical streams. Ostrofsky (1997) found a significant, yet weak, negative relationship between condensed tannins and leaf breakdown rate for forty-eight temperate species. Similarly, Gessner and Chauvet (1994) reported a negative relationship between condensed tannins and leaf breakdown rate of five species in a French stream. More recently, Driebe and Whitham (2000) reported that hybridization in cottonwood plants increased condensed tannin concentrations, which led to slower leaf breakdown in temperate streams. Phenolic compounds in *Eucalyptus* leaves have also been found to affect leaf breakdown by inhibiting fungal colonization (Bärlocher et al. 1995, Canhoto and Graça 1999) and invertebrate feeding (Canhoto and Graça 1999). The few studies conducted with tropical species do not provide evidence for a strong role of secondary compounds in leaf breakdown (Campbell and

Fuchshuber 1995, Wantzen et al. 2002, Chapter 2). These studies do not support Stout's (1989) hypothesis that condensed tannins play a major role in inhibiting leaf breakdown of tropical species.

Our results indicate that studies examining the importance of leaf litter chemistry on leaf breakdown in tropical and temperate ecosystems, should consider the key effects of structural compounds and nutrient content. Concentrations of cellulose and lignin in leaves were strongly correlated to breakdown rate at both sites (Table 3.3). Cellulose and lignin are recalcitrant forms of carbon that require microbes to produce specialized enzymes to break them down, so they are commonly found to inhibit leaf breakdown (Melillo et al. 1983, Gessner and Chauvet 1994, Hutchens and Benfield 2000). In addition to structural compounds, leaf phosphorus content was strongly correlated to breakdown rate at Coweeta, but not at La Selva (Table 3.3). We believe that this is due to the low P-content of Coweeta species. A previous experiment reported severe stoichiometric imbalances with regards to phosphorus (suggesting P-limitation) between consumers and leaf detritus in two Coweeta streams (Cross et al. 2003). We attribute the lack of correlation between leaf P-content and breakdown rate in La Selva species, to the high leaf P-content of leaves at La Selva. It has been suggested that high P-content in La Selva soils due to their volcanic origin is responsible for high leaf P-content (Wood et al. *in press*).

Recommendations for the use of standardized analytical techniques to measure leaf chemistry

Direct comparisons of the effects of leaf litter chemistry on breakdown rate in tropical versus temperate streams are hindered by the lack of comparability of analytical methods used to measure leaf chemistry between studies and the paucity of comprehensive studies on tropical streams. We propose that adopting the use of standardized analytical methods, while in some cases costly and time consuming, will allow for cross-site comparisons and synthesis of results from a broad range of studies. Below, we discuss advantages and disadvantages of some of

the most common analytical methods used to measure leaf chemistry in studies conducted in streams and suggest methods that we believe should be used routinely.

Structural compounds: We suggest that studies should use the forage fiber method to determine structural compounds (Goering and Van Soest 1970), because the forage fiber technique is simpler and more precise than the Klason method to determine lignin and cellulose (Rowland and Roberts 1994, Ryan et al. 1990). Since most methods to determine structural compounds depend on sequential extractions, they tend to have many steps and are thus likely to be less precise and comparable between laboratories (Palm and Rowland 1997). Studies in streams have usually measured lignin and cellulose using the forage fiber technique (Suberkropp et al. 1976, Triska and Sedell 1976, Gessner and Chauvet 1994), which determines the residual weight of samples following successive removal of various tissue constituents (Goering and Van Soest 1970). The "forest products" technique, also known as Klason lignin (Ryan et al. 1990), has also been used in stream studies (Kennedy and Hobbie 2004).

Secondary compounds: We propose that future studies should measure condensed tannins with the acid-butanol technique using "self-standards", for an accurate measure of the concentrations of tannins and phenolics in the leaves. The structural diversity of tannins and phenolics provide challenges for quantitative analyses (Martin and Martin 1982). The Folin-Denis (Suberkropp et al. 1976, Ostrofsky 1993, Campbell and Fuchshuber 1995, Tuchman et al. 2002, this study), Folin-Ciocalteau (Rosset et al. 1982), acid-butanol (Driebe and Whitham 2000, this study), and radial diffusion assay (Gessner and Chauvet 1994, Mathurai and Chauvet 2002) have all been commonly used to measure phenolics and tannins in stream studies. Most of these techniques depend on a calibration curve based on a known standard to estimate concentrations of tannins and phenolics in samples being analyzed. A majority of studies in streams have used commercial tannic acid as the standard, despite the recognition in the terrestrial literature that using commercial tannic acid can over- or under-estimate

concentrations by up to a factor of two (Martin and Martin 1982, Wisdom et al. 1987, Hagerman and Butler 1989). To avoid this problem we used standards made from purified tannins from the samples being analyzed (i.e. "self-standards"), which have been reported to provide the most accurate results (Hagerman and Butler 1989, Appel et al. 2001).

Nutrient content: We suggest that colorimetric methods after acid-digestion of leaf material can provide accurate comparable results for both nitrogen and phosphorus. Leaf litter nitrogen concentrations have been measured with the total Kjeldahl-N method (Triska and Sedell 1976) and more recently using carbon-hydrogen-nitrogen (CHN) analyzers (Flindt and Lillebo 2005). Phosphorus can also be measured after Kjeldahl digestion, followed by colorimetric determination of phosphate (PO_4 -P) using the ascorbic acid method (Flindt and Lillebo 2005). Different analytical methods for leaf nutrient content generally provide similar results, allowing comparisons of literature values of leaf nitrogen and phosphorus content across different biomes and organisms (Enriquez et al. 1993, Elser et al. 2000, McGroddy et al. 2004).

It is important to consider, that even when using standardized analytical techniques, leaf litter chemistry is highly variable and can change due to biotic and abiotic factors. Soil characteristics at the collection site, and differences due to leaf age, nutritional state and presence of herbivores can alter leaf litter chemistry. For example, lignin concentration of *Rhododendron* leaves [21.1 % dry mass (DM)] reported here is higher than previous reports from Coweeta using the same methods [10 - 16 % DM (McGinty 1972, Hunter et al. 2003)]. This difference could be due to variation in precipitation, different soil characteristics, and/or altitude at which leaves were collected. Variation in leaf litter chemistry, either natural or due to different analytical methods, indicate that in order to examine the importance of leaf litter chemistry on leaf breakdown, it is important to analyze the same leaves that are used in decomposition experiments, since using literature values might be misleading.

Conclusions

Direct comparisons of initial leaf litter chemistry, facilitated by the use of standardized analytical techniques, indicated that contrary to our initial hypothesis, mean concentration of condensed tannins was significantly (p<0.001) greater (2.6-fold) in Coweeta than in La Selva species (p < 0.001). Concentrations of condensed tannins were negatively correlated to breakdown rate among Coweeta species (r = -0.77), but not among La Selva species. Our results indicate that leaf litter from common riparian tree species at Coweeta is of lower quality than litter from common riparian trees at La Selva. Structural compounds and nutrient content of leaves are key factors affecting leaf breakdown rate at both sites, while phosphorus was important only in Coweeta. Use of standardized analytical techniques will better enable cross-site comparisons and synthesis of results from a broad range of studies.

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	Condensed	Total	Hydrolizable			
Species	tannins	phenolics	tannins	Lignin	Cellulose	Hemicell.
Coweeta Hydrologic Lab.						
Acer rubrum	26.8 (1.10)	35.3 (15.20)	39.7 (1.68)	16.5 (0.46)	14.8 (0.24)	9.5 (0.21)
Cornus florida	9.3 (0.48)	12.7 (0.97)	14.7 (0.33)	8.4 (0.15)	12.4 (0.07)	9.0 (0.09)
Liquidambar styraciflua	22.3 (1.63)	6.8 (0.19)	9.6 (0.43)	14.9 (0.29)	13.0 (0.09)	9.3 (0.24)
Liriodendron tulipifera	5.5 (0.27)	4.4 (0.41)	3.9 (0.09)	15.9 (0.96)	18.6 (0.31)	15.8 (0.54)
Pinus strobus	57.7 (4.88)	5.6 (0.41)	10.2 (1.37)	19.2 (1.79)	22.3 (0.66)	15.6 (0.15)
Quercus rubra	35.9 (3.50)	7.7 (1.64)	13.1 (1.68)	16.9 (0.21)	21.3 (0.34)	16.0 (0.67)
Rhododendron maximum	24.8 (2.31)	25.3 (12.60)	17.2 (1.88)	21.1 (0.40)	23.6 (0.25)	9.8 (0.70)
Range	5.5 – 57.7	4.4 – 35.3	3.9 – 39.7	8.4 – 21.1	12.4 – 23.6	9.0 – 16
La Selva Biological Station						
Carapa nicaraguensis	23.1 (2.82)	32.3 (5.94)	34.8 (5.32)	18.3 (2.36)	18.0 (1.41)	7.5 (1.09)
Castilla elastica	11.2 (2.34)	12.2 (3.72)	9.4 (1.51)	13.4 (0.72)	19.5 (0.83)	22.0 (1.37)
Ficus insipida	0.4 (0.11)	11.7 (2.62)	2.7 (0.15)	8.3 (0.78)	19.1 (0.67)	14.7 (0.65)
Luehea seemannii	13.6 (1.75)	7.4 (1.41)	14.0 (2.30)	16.3 (0.41)	19.9 (0.35)	15.5 (0.65)
Simira maxonii	2.8 (0.27)	4.3 (0.34)	6.6 (0.55)	15.3 (1.56)	23.0 (0.30)	14.6 (0.20)
Terminalia oblonga	12.8 (3.76)	11.5 (1.35)	11.4 (1.00)	6.9 (0.81)	17.5 (0.20)	13.9 (0.64)
Trema integerrima	0.8 (0.06)	0.9 (0.14)	2.9 (0.12)	5.5 (0.68)	12.2 (0.35)	10.1 (0.61)
Zygia longifolia	8.1 (0.25)	10.0 (0.65)	7.8 (0.42)	28.3 (0.55)	24.6 (0.80)	17.5 (0.41)
Range	0.4 – 23.1	0.9 – 32.3	2.7 – 34.8	5.5 – 21.1	12.2 – 24. 6	7.5 – 22.0

Table 3.1. Mean concentrations (percent dry mass <u>+</u> 1 standard error) of chemical parameters of seven common species in Coweeta Hydrologic Laboratory, N.C. and eight species from La Selva Biological Station, Costa Rica.

Table 3.1 Continued

Species	Р	N	С
Coweeta Hydrologic Lab.			
Acer rubrum	0.032 (1 x 10 ⁻⁵⁾	0.41 (0.002)	47.6 (0.06)
Cornus florida	0.161 (2 x 10 ⁻³⁾	0.80 (0.010)	44.9 (0.44)
Liquidambar styraciflua	0.069 (8 x 10 ⁻⁴)	0.58 (0.002)	44.4 (0.06)
Liriodendron tulipifera	0.095 (8 x 10 ⁻⁴)	1.24 (0.010)	45.5 (0.09)
Pinus strobus	0.041 (2 x 10 ⁻⁴)	0.40 (0.002)	50.3 (0.09)
Quercus rubra	0.036 (1 x 10 ⁻⁴)	0.68 (0.003)	47.3 (0.01)
Rhododendron maximum	0.032 (1 x 10 ⁻³)	0.32 (0.004)	47.3 (0.10)
Range	0.032 - 0.161	0.32 – 1.24	44.4 – 50.3
La Selva Biological Station			
Carapa nicaraguensis	0.082 (2 x 10 ⁻⁴)	0.91 (0.13)	41.7 (0.23)
Castilla elastica	0.150 (0.01)	2.02 (0.10)	40.4 (0.52)
Ficus insipida	0.076 (2 x 10 ⁻⁴)	1.27 (0.02)	35.0 (0.32)
Luehea seemannii	0.111 (0.01)	1.27 (0.07)	44.2 (0.05)
Simira maxonii	0.114 (0.01)	2.05 (0.07)	44.9 (0.41)
Terminalia oblonga	0.117 (0.01)	1.36 (0.07)	39.2 (0.23)
Trema integerrima	0.100 (0.01)	1.61 (0.06)	34.1 (0.27)
Zygia longifolia	0.058 (1 x 10 ⁻³)	1.87 (0.04)	46.4 (0.27)
Range	0.058 - 0.117	0.91 - 2.04	34.1 - 46.4

Table 3.2. Breakdown rates calculated on a per day and on a per degree day basis for seven common riparian species from Coweeta Hydrologic Laboratory, N.C. and eight species from La Selva Biological Station, Costa Rica. Breakdown rates are classified as slow (S), medium (M), or fast (F) based on days (Webster and Benfield 1986), and on degree days (Cummins et al. 1989).

Breakdown rate						
Species	Source					
Coweeta Hydrological						
Laboratory						
Acer rubrum	0.008 M	0.0008 S	Eggert and Wallace unpublished data			
Cornus florida	0.017 F	0.0017 F	Wallace et al. 1982			
Liquidambar styraciflua	0.008 M	0.0009 S	Eggert and Wallace unpublished data			
Liriodendron tulipifera	0.010 F	0.0011 M	Eggert and Wallace unpublished data			
Pinus strobus	0.003 S	0.0002 S	Eggert and Wallace unpublished data			
Quercus rubra	0.007 M	0.0007 S	Eggert and Wallace unpublished data			
Rhododendron maximum	0.004 S	0.0004 S	Eggert and Wallace unpublished data			
La Selva Biological Station						
Carapa nicaraguensis	0.023 F	0.0009 S	Ardón 2006			
Castilla elastica	0.038 F	0.0014 M	Ardón 2006			
Ficus insipida	0.079 F	0.0031 F	Ardón 2006			
Luehea seemannii	0.033 F	0.0013 M	Ardón 2006			
Simira maxonii	0.048 F	0.0018 F	Ardón 2006			
Terminalia oblonga	0.039 F	0.0015 M	Ardón 2006			
Trema integerrima	0.198 F	0.0076 F	Ardón 2006			
Zygia longifolia	0.013 F	0.0005 S	Ardón 2006			

Table 3.3. Pearson correlation coefficients between *in situ* breakdown rate and concentrations of leaf litter chemical constituents of seven species from Coweeta Hydrologic Laboratory, N.C. and eight species from La Selva Biological Station, Costa Rica.

	•		Both sites
	Coweeta	La Selva	Both sites
Chemical constituent	r	r	r
Carbon	-0.74	-0.73*	-0.78*
Nitrogen	0.58	0.03	0.44
Phosphorus	0.89**	0.01	0.26
Lignin	-0.94**	-0.65*	-0.60*
Cellulose	-0.76*	-0.77*	-0.37
Hemicellulose	-0.36	-0.36	-0.07
Condensed tannins	-0.77*	-0.61	-0.50
Total phenolics	-0.05	-0.50	-0.37
Hydrolizable tannins	-0.01	-0.45	-0.39
C:N	-0.71	-0.39	-0.47
N:P	-0.38	-0.10	-0.10

* p < 0.05, ** p < 0.01

Table 3.4. Breakdown rates of four common riparian species in the Sabalo (third-order stream) and Saltito (first-order stream) in La Selva Biological Station. F-values are from analysis of covariance (ANCOVA).

	Sabalo	Saltito-100	F value	Source
Trema integerrima	0.198	0.067	37.74***	Ardón et al. 2006
Ficus insipida	0.079	0.053	22.53***	Stallcup et al. in press
Castilla elastica	0.038	0.010	117.40***	Ardón et al. 2006
Zygia longifolia	0.013	0.006	8.79**	Ardón et al. 2006

* p < 0.05, ** p < 0.01, ***p < 0.001

Figure 3.1. Mean concentrations (percent dry mass <u>+</u> 1 standard error) of chemical constituents of seven species from Coweeta Hydrologic Laboratory, N.C. and eight species from La Selva Biological Station, Costa Rica. Significant differences are from analysis of variance and *posthoc* Tukey comparison. * p < 0.05, ** p < 0.01, *** p < 0.001



Figure 3.2. Principal component analysis (PCA) of leaf chemistry of seven species from Coweeta Hydrologic Laboratory, NC and eight species from La Selva Biological Station. PCA Axis 1 explained 40% of the variation and PCA Axis 2 explained 27% more of the variation. Coweeta species are denoted by black symbols, La Selva species are denoted by white symbols. P = phosphorus, N = Nitrogen, CT = condensed tannins, Hemicell. = hemicellulose.



Figure 3.3. Breakdown rates (degree day ⁻¹) of common riparian species from (a) La Selva Biological Station, and (b) Coweeta Hydrologic Laboratory.



CHAPTER 4

DOES LEAF QUALITY MEDIATE THE STIMULATION OF LEAF BREAKDOWN BY PHOSPHORUS IN NEOTROPICAL STREAMS? ³

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Summary

1. Lowland tropical streams have a chemically-diverse detrital resource base, where leaf quality could potentially alter the effect of high nutrient concentrations on leaf breakdown. This has important implications given the extent and magnitude of anthropogenic nutrient loading to the environment.

2. Here, we examine if leaf quality (as determined by concentrations of cellulose, lignin and tannins) mediates the effects of high ambient phosphorus (P) concentration on leaf breakdown in streams of lowland Costa Rica. We hypothesized that P would have a stronger effect on microbial and insect processing of high- than of low- quality leaves.

3. We selected three species that represented extremes of quality as measured in leaves of eight common riparian species. Species selected were, from high- to low- quality: *Trema integerrima* > *Castilla elastica* > *Zygia longifolia*. We incubated single-species leaf packs in five streams that had natural differences in ambient P concentration (10-140 μ g SRP L⁻¹), due to variable inputs of solute-rich groundwater, and also in a stream that was experimentally enriched with P (~200 μ g SRP L⁻¹).

4. The breakdown rate of all three species varied among the six streams: *Trema integerrima* (*k* values range: 0.0451 - 0.129 day⁻¹); *Castilla elastica* (*k* values range: 0.0064 - 0.021 day⁻¹); and *Zygia longifolia* (*k* values range: 0.002 - 0.008 day⁻¹). Both ambient P concentration and flow velocity had significant effects on the breakdown rate of the three species.

5. Results supported our initial hypothesis that litter quality mediates the effect of high ambient P concentrations on leaf processing by microbes and insects. The response of microbial respiration, fungal biomass and invertebrate density to high ambient P concentration was greater in *Trema* (high quality) than in *Castilla* or *Zygia* (low quality). Variation in flow velocity, however, confounded our ability to determine the magnitude of stimulation of breakdown by P.
6. Cellulose and lignin appeared to be the most important factors in determining the magnitude of P-stimulation. Surprisingly, leaf secondary compounds did not have an effect. This contradicts predictions made by other researchers, regarding the key role of plant secondary compounds in affecting leaf breakdown in tropical streams.

Introduction

Leaf breakdown is a vital process in headwater streams, linked to such ecosystem processes as carbon cycling, nutrient spiraling and energy transfer (Webster & Benfield, 1986; Tank & Webster, 1998; Wallace *et al.,* 1999). While factors, such as leaf quality and ambient nutrient concentrations, are known to affect leaf breakdown rate independently (see reviews by Webster & Benfield, 1986; Boulton & Boon, 1991; Abelho, 2001), their potential interactions have received much less attention.

Leaf *quality* is determined by the concentration and forms of organic carbon (C) present, and leaf nutrient content (Aber & Melillo, 2001). Small, labile C molecules with a high energy content (e.g. simple sugars) are easily broken down. Whereas recalcitrant C compounds (e.g. cellulose, hemicellulose, lignin and tannins) have large three-dimensional, complex structures that can be broken down only by specialized enzymes, making them metabolically more costly for microbes (Sinsabaugh *et al.*, 1993). Plant species with a high concentration of recalcitrant forms of C are broken down at a slow rate (Webster & Benfield, 1986; Aerts, 1997). Leaf nutrient content also affects breakdown rate. Leaves with high concentrations of nitrogen (N) and phosphorus (P) tend to be broken down faster than leaves from species with low nutrient content (Enríquez, Duarte & Sand-Jensen, 1993).

In addition to leaf quality, various studies have demonstrated that ambient nutrient concentration can affect the breakdown process. Faster breakdown has been reported in response to high N (Meyer & Johnson, 1983; Suberkropp & Chauvet, 1995), P (Elwood *et al.*, 1981; Rosemond *et al.*, 2002), and both nutrients together (Howarth & Fisher, 1976; Robinson &

Gessner, 2000; Grattan & Suberkropp, 2001; Gulis & Suberkropp, 2003). Increases in ambient nutrient concentrations can alter microbial activity, leading to faster leaf breakdown rates and increased leaf nutritional quality for invertebrate consumers (Elwood *et al.*, 1981, Suberkropp & Chauvet, 1995; Pearson & Connolly, 2000; Robinson & Gessner, 2000; Gulis & Suberkropp, 2003; Ramírez, Pringle & Molina, 2003). In contrast, some studies have reported no change in leaf breakdown rate in response to ambient nutrient enrichment (Triska & Sedell, 1976; Newbold *et al.*, 1983; Royer & Minshall, 2001).

The interaction between leaf quality and ambient nutrients in stream water has not been examined thoroughly; most studies have focussed on the nutrient content of the organic matter itself (Peterson *et al.*, 1993; Royer & Minshall, 2001; Stelzer, Heffernan & Likens, 2003; Gulis *et al.*, 2004). These studies found a stronger effect of high ambient nutrients on the breakdown of substrates with lower intrinsic nutrient content (Peterson *et al.*, 1993; Stelzer *et al.*, 2003; Gulis *et al.*, 2004). However, no study has examined how the concentration of recalcitrant C compounds in leaves can potentially mediate the effect of high ambient nutrients on leaf breakdown in streams.

Based on the breakdown dynamics of wood, Melillo *et al.* (1983, 1984) proposed that C availability in organic matter can determine microbial nutrient demand. Microbes growing rapidly on high quality substrates with small, labile C molecules would have high nutrient requirements. Consequently, such microbial communities (which are not carbon-limited) might become nutrient-limited in oligotrophic stream water (Melillo *et al.*, 1984). In contrast, microbes on low-quality substrates with a high concentration of recalcitrant C compounds, are primarily carbon- (not nutrient-) limited, and are thus unable to respond to increases in ambient nutrients. Therefore an increase in nutrients should accelerate activity of microbes on leaf litter with high, more than low, quality.

Tropical rainforest streams are ideal systems to examine interactions between ambient nutrient concentrations and leaf quality. They are heterotrophic and rely on leaf litter inputs as

the carbon base of the food-web (Covich, 1988; Pringle, 2000). Leaves that fall into tropical streams are chemically heterogeneous, due to the high diversity of tropical plants, and their tendency to be highly chemically defended against herbivores (Coley & Aide, 1991). In a literature review, Aerts (1997) demonstrated that initial leaf chemistry explained more of the variation in decomposition rates in tropical terrestrial systems than in temperate or Mediterranean systems. This suggests that, in the tropics, leaves are more chemically diverse, and their chemistry exerts a stronger control over decomposition (Lavelle *et al.*, 1993; Aerts, 1997).

Here, we examine how initial leaf quality (as determined by concentrations of cellulose, lignin and tannins) of riparian tree species interacts with ambient P concentration to determine leaf breakdown in lowland streams of Costa Rica. We measured both structural (lignin, cellulose and hemicellulose) and secondary (condensed tannins, hydrolysable tannins and total phenolics) compounds in leaves. Secondary compounds were measured because they have been reported to affect breakdown rate in tropical streams (Stout, 1989; Campbell & Fuchshuber, 1995). We hypothesized that P would stimulate microbial and invertebrate processing on high-quality leaves (i.e., low concentrations of structural and secondary compounds) more than on low-quality leaves.

Methods

Site description

La Selva Biological Station (10°26´ N, 84°01´W), is a 1536 ha reserve that is the lowland terminus of the last intact forested biological corridor on the Caribbean slope of Central America, spanning altitudinal extremes from 35 to 2906 m above sea level. La Selva receives 4000 mm of rain a year, with more than 400 mm a month from May to December (Sanford *et al.*, 1994). Stream temperature is relatively constant throughout the year (24-27 °C), with mean annual pH values ranging from 4.5 - 6 (Ramírez, 2001).

Due to geothermally-modified groundwater inputs, streams at La Selva display a wide range of variation in solute concentrations (Pringle, 1991; Pringle & Triska, 1991). Streams receiving geothermally-modified groundwater inputs contain high concentrations of cations (up to 18 mg Ca L⁻¹, 44 mg Na L⁻¹, 25 mg Mg L⁻¹), anions (up to 28 mg Cl L⁻¹, 13 mg SO₄ L⁻¹), and phosphorus (up to 350 μ g SRP L⁻¹; Pringle *et al.*, 1993). Nearby streams that do not receive geothermally-modified inputs have low solute concentrations (<2 mg Ca L⁻¹, <2 mg Na L⁻¹, <1 mg Mg L⁻¹, <3 mg Cl L⁻¹, <2 mg SO₄ L⁻¹, <10 μ g SRP L⁻¹; Pringle *et al.*, 1993). Inorganic nitrogen concentrations are relatively high (typically >70 μ g NO₂ + NO₃-N L⁻¹; Pringle, 1991) in both geothermal and non-geothermal streams. In all of these streams, algal assemblages are light-limited due to dense canopy cover (>90%), resulting in detritus-based food webs (Pringle *et al.*, 1993; Rosemond *et al.*, 2001).

Streams with varying ambient P concentrations and experimental P-enrichment

Based on ongoing research at La Selva, we selected six streams for a leaf breakdown experiment. Due to varying geothermal inputs, five streams represent a natural gradient in P concentrations (Pringle *et al.*, 1993; Ramírez, 2001). Three of the streams (Piper, Taconazo and Saltito-100) have relatively low concentrations of dissolved phosphorus ($P \le 15 \ \mu g \ SRP \ L^{-1}$), while the other two (Sura and Arboleda) have naturally high concentrations ($P \ge 50 \ \mu g \ SRP \ L^{-1}$). To isolate the effect of phosphorus from other solutes, we also incubated leaves in a solute-poor headwater stream (Carapa) enriched with P, to a target concentration of 200 $\ \mu g \ SRP \ L^{-1}$.

We have been experimentally enriching the Carapa (which does not receive geothermal inputs) with P since July 1998, as part of a larger study examining the effects of geothermally-modified groundwater inputs on ecosystem processes (Ramirez *et al.*, 2003; Ramírez & Pringle *in press*; C.M. Pringle unpublished data). Phosphoric acid has been added to increase P concentrations from <10 μ g to 200 μ g SRP L⁻¹, which is at the high end of P concentrations exhibited by solute-rich streams (Pringle, 1991). A Mariotte bottle is used to add phosphoric

acid continuously, adjusting the concentration of acid and drip rate according to stream discharge, the experimental method having been described in more detail elsewhere (Ramírez *et al.*, 2003).

During the three month study period, water samples from each site were taken every ten days to be analysed for NO₃-N, NH₄-N and SRP. Samples were filtered (0.45 µm Millipore filters) and kept frozen until analysis at the University of Georgia. We recognise that freezing water samples might be problematic for NH₄-N, but logistical constraints limit our ability to analyse samples immediately at the field site. Phosphorus was measured as soluble reactive phosphorus (SRP) using the molybdenum-blue technique. Nitrate and NH₄-N were measured using the cadmium reduction and phenate methods respectively (APHA 1998). Temperature, pH (both with a meter: Hanna instruments, RI, USA) and flow velocity (Marsh-McBirney meter, MA, USA) were also measured above leaf packs when water samples were collected.

Initial leaf quality

In order to select three species representing a range of leaf quality, we collected leaves from the eight most common riparian species at La Selva during December 2001 and conducted chemical analyses. We collected freshly fallen leaves of from three different individual trees growing on different soil types within La Selva. Leaves were air-dried, ground and refrigerated until analysis. We estimated cellulose, hemicellulose and lignin by sequential neutral detergent/acid detergent digestion on an Ankom A200 fibre analyser (ANKOM Technologies, NY, USA: Madritch & Hunter, 2002). Three separate analyses were conducted for phenolics: condensed tannins, hydrolysable tannins and total phenolics. For tannin analysis, samples were extracted in 70% acetone with 1mM ascorbic acid and evaporated under reduced pressure to provide aqueous extracts. Condensed tannins were estimated as proanthocyanidins (Rossiter, Schultz & Baldwin, 1988). Hydrolysable tannins were estimated using a potassium-iodate technique (Hunter *et al.*, 2003). Total phenolics were estimated with the Folin-Denis

assay (Swain, 1979). To avoid problems associated with using commercial standards, all samples were compared to standards prepared from pooled litter samples (Appel *et al.*, 2001). Leaf total carbon and nitrogen content were determined using a Carlo Erba NA 1500 CHN Analyser (Carlo Erba, Milan, Italy).

Based on Principal Component Analysis (PCA) of secondary (tannins and phenolics) and structural (hemicellulose, cellulose and lignin) compounds, we selected three species that differed in the forms and concentrations of organic C, but had similar nitrogen content. The species selected were: *Trema integerrima* (Beurl) Standl (family Ulmaceae; from now on referred to as *Trema*), *Castilla elastica* Sessé ex Cerv. (family Moraceae; from now on referred to as *Castilla*) and *Zygia longifolia* (Humb. & Bonpl. Ex Willd.) Britton & Rose (family Fabaceae; from now on referred to as *Zygia*).

Leaf breakdown rate

We collected freshly fallen leaves from at least 10 different individual trees of each species; leaves were air-dried for 3 days, and stored in an air conditioned room until use. We created 5-g leaf packs using plastic mesh bags (22 cm x 40 cm), with a coarse mesh (5 x 5 mm) in order to allow access to stream fauna (Benfield, 1996). Before placing leaves into bags, we mixed leaves from different individual trees growing on different soil types. We placed 21 leaf packs of each species in each of six streams on 20 June 2002. Leaf packs were anchored to the streambed using metal stakes (Boulton & Boon, 1991).

We randomly collected three replicate leaf packs of each species at each site on predetermined days (0, 1, 4, 7, 11, 16 and 21 for *Trema*; and 0, 4, 7, 18, 29, 44 and 80 for *Castilla* and *Zygia*). Day 0 samples were taken to the sites and immediately returned to the laboratory to control for handling losses (Benfield, 1996). On each collection date we removed leaf packs from the stream with a fine mesh net and placed them into separate plastic bags. In the laboratory, leaves were rinsed over a 300 µm mesh sieve (to remove sediments and

insects), dried for a minimum of 24 h at 40 °C and weighed. A 1-g sub-sample was ashed at 500 °C and re-weighed to determine ash free dry mass (AFDM). Day 0 leaf packs were used to estimate initial AFDM and leaf quality as described above.

We examined invertebrate density and fungal biomass on leaf packs approximately halfway through the experiment (day 11 for *Trema* and day 44 for *Castilla* and *Zygia*). Invertebrates were preserved in 10% formalin and later identified to the lowest possible taxonomic level (genus in most orders, except Diptera which were identified to family or subfamily). Fungal biomass was estimated using ergosterol (Suberkropp & Weyers, 1996). Immediately after collection, 40 leaf disks were punched from randomly selected leaves from each leaf pack. Thirty-five disks were stored in methanol for ergosterol analysis, the five remaining disks were dried for at least 24 hours at 40 °C, weighed, ashed at 500 °C for 1 h, and re-weighed to determine AFDM. Ergosterol was extracted from leaf disks in alkaline methanol by refluxing for 30 minutes, partitioning into pentane, drying and re-dissolving in methanol. Ergosterol concentration was determined after separation from other lipids by high-performance liquid chromatography (HPLC; Suberkropp & Weyers, 1996).

Microbial respiration was measured on four leaf packs of each species at each of three sites: a low-P stream (Saltito), a geothermally-modified high-P stream (Sura), and the P-enriched stream (Carapa). Microbial respiration was measured in these three streams using *in situ* re-circulating metabolism chambers (Ramirez *et al.*, 2003) on days 10-11 for *Trema* and days 17-18 for *Castilla* and *Zygia*. Each chamber was 12 x 15 cm in cross-section and was equipped with a pump that produced a re-circulating flow velocity of 2.4 cm s⁻¹ throughout the chamber. Chambers were filled with stream water, anchored to the stream bottom to maintain water temperature and covered with black plastic to prevent photosynthesis.

Leaf nutrient content during breakdown

In order to follow nutrient uptake and release during breakdown, we measured leaf total carbon, nitrogen and phosphorus content on four sampling dates. We determined nutrient content of *Trema* leaves on days 0, 1, 7 and, 16 and of *Castilla* and *Zygia* leaves, on days 0, 7, 44 and 80. Total carbon and nitrogen content were measured as described above. For phosphorus analysis, ground leaf material was weighed into acid-washed and pre-ashed ceramic crucibles, ashed at 500 °C, acid-digested (Aqua regia double acid, Jones *et al.*, 1990), and analysed spectrophotometrically (ascorbic acid method: APHA, 1998).

Statistical analyses

Principal Component Analysis of initial litter chemistry of the eight species was conducted with untransformed data from three individual trees for each species. Chemical parameters on day 0 of the three species were compared using one way ANOVA and a *posthoc* Tukey's test on arcsine ($x^{0.5}$) transformed data to meet assumptions of normality. Breakdown rate, *k*, was estimated by linear regression of natural log-transformed percent AFDM remaining versus day (exponential model; Benfield 1996). Differences in *k* were determined with analysis of covariance (ANCOVA) followed by Tukey's test to compare slopes. We also used ANCOVA to test for possible effects of P and water flow velocity on breakdown rate of all three species. Differences in respiration, fungal biomass and macroinvertebrate density for each species were assessed among sites using ANOVA of natural log of (x+1) transformed data. Nitrogen and phosphorus immobilization in leaves was estimated by looking at the slopes of the regression of percent AFDM remaining versus percent nutrient concentration (Melillo *et al.*, 1984). All analyses were carried out in JMP statistical software 4.0.4 (SAS Institute).

Results

Landscape variation in P concentration and experimental P-enrichment

Stream chemical and physical characteristics varied among sites (Table 4.1). Streams varied greatly in SRP concentration, with Arboleda having the highest mean (186 μ g SRP L⁻¹) and Taconazo the lowest (10 μ g SRP L⁻¹; Table 4.1). The experimental P-enrichment of Carapa resulted in mean SRP concentration of ~186 μ g SRP L⁻¹. Flow velocity also varied among sites, with Piper having two to three times higher mean flow velocity than other sites (Table 4.1). Temperature was relatively constant among sites during the study period (range 24-26 °C). Average pH ranged from 4.46 in the P-enriched stream to 6.12 in a geothermally-modified stream (Table 4.1). Variation in stream pH during the period fell within the range observed in streams at La Selva (Ramírez, 2001).

Initial leaf quality

Leaves collected from the eight tree species represented a wide range of chemical parameters (Table 4.2), and Principal Component Analysis (PCA) provided good separation of species (Fig. 4.1). PCA Axis 1 explained 56% of the variation and was negatively correlated with condensed tannins, hydrolysable tannins and total phenolics. PCA Axis 2 explained 24% more of the variation and was positively correlated with lignin and cellulose (Fig. 4.1). We observed differences in lignin and nitrogen content between the initial leaf collection and the leaves used in the breakdown study (Table 4.2).

Day 0 (initial) leaf chemical parameters differed among the three species selected for the breakdown experiment (Table 4.2). *Castilla* and *Zygia* had higher concentrations of secondary compounds (condensed tannins, hydrolysable tannins and total phenolics) than *Trema*, but were not different from each other (condensed tannins $F_{2,36} = 202.4$, *P* < 0.0001; hydrolysable tannins $F_{2,36} = 231.32$, *P* < 0.0001; total phenolics $F_{2,36} = 47.67$, *P* < 0.0001; Table 4.2). *Zygia* had higher concentrations of lignin, cellulose and carbon than *Castilla* and *Trema* (lignin $F_{2,36} = 202.4$).

110.57, P < 0.0001; cellulose $F_{2,36} = 104.64$, P < 0.0001; carbon $F_{2,36} = 74.01$, P < 0.001; Table 4.2). *Trema* had lower concentrations of nitrogen than the other two species, and *Castilla* had higher concentrations of phosphorus than the other two species (nitrogen $F_{2,36} = 25.35$, P < 0.001; phosphorus $F_{2,36} = 13.05$, P < 0.001; Table 4.2).

Leaf breakdown rate

The three species decayed at different rates (Table 4.3; Fig. 4.2) being fastest for *Trema* $(k = 0.0401 - 0.1129 \text{ day}^{-1})$, intermediate for *Castilla* $(k = 0.0064 - 0.021 \text{ day}^{-1})$, and slowest for *Zygia* $(k = 0.0020 - 0.008 \text{ day}^{-1}; \text{ Table 4.3})$. Both phosphorus and flow velocity affected breakdown rate (ANCOVA phosphorus F_{1,9} = 17.43, *P* < 0.005; flow velocity F_{1,9} = 13.11, *P* < 0.05). However, the effects of flow and P were different among species (species*flow interaction, F_{2,9} = 9.70, *P* < 0.05; species*P interaction, F_{2,9} = 8.87, *P* < 0.05). Due to the high flow velocity at the Piper site, we ran the model without the Piper data. Without Piper, P was still a significant predictor of leaf breakdown rates but flow velocity was not (F_{1,7} = 26.83, *P* < 0.05; F_{1,7} = 1.13, *P* = 0.33; respectively).

Microbial respiration on *Trema* leaves was higher in the P-enriched stream, when compared both to a stream naturally high in P stream (Sura) and a low-P stream (Saltito; $F_{2,9} =$ 14.06, *P* < 0.05; Fig. 4.3). Microbial respiration on *Castilla* was significantly higher both in the P-enriched stream and in a naturally high P-stream compared to a low-P stream ($F_{2,9} = 9.12$, *P* < 0.05; Fig 4.3). There was no difference in microbial respiration across these three sites on *Zygia* leaves ($F_{2,9} = 0.30$, *P* = 0.3; Fig 4.3).

Ergosterol concentration on *Trema* leaves was higher in Arboleda (naturally high-P stream) and in the enriched stream than the other streams lower in P ($F_{5,12} = 7.99$, P < 0.05; Fig. 4.4). There was no difference in ergosterol among sites for *Castilla* or *Zygia* ($F_{5,12} = 0.66$, P = 0.65; $F_{5,12} = 1.28$, P = 0.33; respectively; Fig. 4.4). Invertebrate density on *Trema* on day 11 was higher at Arboleda and at the enriched stream, than at sites with lower P, for both

chironomids and non-chironomids ($F_{5,12} = 18.96$, P > 0.001; $F_{5,12} = 7.33$, P < 0.05; respectively; Fig. 4.5a & b). Chironomid density on *Castilla* was higher at the enriched and a naturally high-P stream (Arboleda) than at any of the other stream sites ($F_{5,12} = 11.12$, P < 0.005; Fig. 4.5a). Non-chironomids on *Castilla* were also higher in density in the experimentally enriched stream than at any of the other sites ($F_{5,12} = 5.44$, P < 0.05; Fig. 4.5b). Chironomid density on *Zygia* did not differ among sites (Fig. 5a). However, there was a higher density of non-chironomids on *Zygia* in Arboleda than at other stream sites ($F_{5,12} = 6.90$, P < 0.003; Fig. 4.5b).

Leaf nutrient content

The nitrogen content of *Trema* leaves increased slightly over the first three sampling dates, and then declined (Fig. 4.6a). *Castilla* showed an overall decline in N content over time (Fig. 4.6b). The N content of *Zygia* remained fairly constant throughout the incubation period (Fig. 4.6c). There was no clear trend in the relationship between percent AFDM remaining and leaf N content during breakdown for any of the three species (Table 4.4). Phosphorus content of *Trema* increased over time in two naturally high-P streams (Fig. 4.6d). In *Castilla* there was a period of initial leaching of leaf P, followed by an increase in leaf P content in three sites with relatively high P concentration (Fig 4.6e). *Zygia* P content declined slightly by day 7, but then increased only at the stream with the highest natural P concentration (Arboleda).

Discussion

This is the first study done *in situ* to support the hypothesis that high concentrations of recalcitrant C compounds in leaves, can mediate how microbes and invertebrates respond to nutrient loading. Several lines of evidence support our initial hypothesis that leaf quality determines how microbes and insects respond to high ambient P levels in stream water. The magnitude of the stimulation of fungal biomass and chironomid density in streams with higher ambient P levels was higher in *Trema* leaves, than in *Castilla* or *Zygia* (Table 4.5). Microbial

respiration was stimulated in the stream enriched with P on *Trema* and *Castilla*, but not on *Zygia* leaves (Fig. 4.3). *Trema* leaves also exhibited an increase in leaf P content throughout the breakdown process, suggesting P immobilisation (Table 4.4; Melillo *et al.,* 1984). In contrast, P immobilisation was not observed for *Castilla* or *Zygia* (Table 4.4). We believe that physical abrasion in a site with higher flow velocity (Piper) confounded our ability to determine P effects on breakdown rate. If this site is removed, however, our results do suggest that P had a stronger effect on the breakdown rate of *Trema* than of *Castilla* or *Zygia* (Table 4.5).

Our results are consistent with the hypothesis that leaf quality mediates the effects of high P on microbial processing. We are currently conducting experiments using artificial substrates of varying chemical quality as a direct test of this hypothesis (Ardón dissertation *in progress*). Since we selected our three species to represent extremes of quality from 8 common riparian species, we believe our results are an accurate representation of how leaves of different quality respond to high P.

Leaf breakdown rates observed in this study ranged from very fast (*Trema* leaves on average lost 95% of their mass in 39.1 days) to slow (*Zygia* leaves lost 95% of their mass in 686.3 days). The fast breakdown of *Trema* leaves agrees with previous reports of rapid decay in tropical streams (Stout, 1989; Irons *et al.*, 1994; Benstead, 1996; Rosemond, Pringle & Ramírez, 1998). Our results show that leaves from some tropical species are broken down at rates similar to those of low-quality leaves in temperate streams: average breakdown rate observed for *Zygia* (average $k = 0.005 \text{ day}^{-1}$) is comparable to the average breakdown rate reported for *Rhododendron* (low quality species) in North Carolina streams (average $k = 0.0046 \text{ day}^{-1}$, 656 days to 95% mass loss; Greenwood, 2004). In temperate systems it has been shown that a high diversity of detrital resources can increase diversity of consumers (Moore *et al.*, 2004). Accordingly, the availability of very fast and slow decomposing species in tropical streams might play a similarly important, though still unexplored, role in determining energy transfer and ultimately the evolution of invertebrate consumers.

Which aspects of leaf quality mediate the effect of nutrients on breakdown rate?

Our results suggest that initial concentrations of cellulose, lignin and total carbon were more important than secondary compounds (condensed tannins, hydrolysable tannins and total phenolics) in determining the response of leaf breakdown to high ambient P concentration. We observed significant effects of P on microbial respiration, fungal biomass, and Chironomidae density on *Trema* leaves. When we removed the site with high flow velocity (Piper), we observed a stronger P stimulation of breakdown rate on *Trema* (which had lower concentrations of lignin, cellulose and total carbon), than on *Castilla* or *Zygia* leaves (Tables 4.2 & 4.5). Despite *Zygia* having slightly lower concentrations of secondary compounds, *Zygia* decayed much more slowly than *Castilla*, and both species responded similarly to high ambient P. This suggests that leaf secondary compounds do not mediate the effect of P on breakdown rate.

Due to the longer evolutionary history between plants and herbivores, tropical species have a higher diversity and concentration of secondary compounds than many temperate species (Coley & Aide, 1991). Since secondary compounds affect the decomposition rate of leaves in terrestrial systems (Palm & Sanchez, 1990; Hattenschwiler & Vitousek 2000), we were surprised to find they did not affect decomposition rate in our study streams. Our results contrast with predictions made by other researchers. For example, based on a review of the literature, Stout (1989) proposed that a high concentration of secondary compounds (condensed tannins) slow down decomposition rates in tropical streams. More recently, it has been suggested that high concentrations of secondary compounds are crucial in determining decomposition in tropical streams (Wantzen *et al.*, 2002).

The importance of the concentration of recalcitrant C compounds, specifically lignin, in determining effects of nutrient enrichment has been shown in other systems. In a stream microcosm study, Melillo *et al.* (1984) reported that breakdown of alder wood (*Alnus rugosa*; low lignin concentration 13.1% DW) was stimulated by P enrichment, while breakdown of spruce

wood (*Picea mariana;* high lignin concentration 24.6% DW) was not. In terrestrial systems in Hawaii, Hobbie (2000) reported greater increases in decomposition of leaves with low lignin concentrations (\leq 12%) in response to N enrichment, than leaves with high lignin concentrations (\geq 18%). In a North Carolina wetland, Bridgham & Richardson (2003) hypothesized that low quality (lignin \geq 30%) was the main reason why they did not observe stimulation of decomposition rates in response to soil nutrient enrichment.

In contrast, two recent studies have reported that nutrient enrichment has a greater effect on microbial activity and decomposition rate of wood (high lignin) than leaves (low lignin; Stelzer *et al.*, 2003; Gulis *et al.*, 2004). Stelzer *et al.* (2003) found that nutrient enrichment increased microbial respiration on wood more than on leaves. Similarly, Gulis *et al.* (2004) reported that enhanced nutrients led to higher microbial activity and faster breakdown rates of wood veneers and sticks than of leaves. Differences in the magnitude of response to nutrient enrichment between leaves and wood might be related to the lower nutrient content of wood compared to leaves, and to differences in the physical structure of these two substrates. Further studies are needed in which C availability and nutrient content of substrates, as well as ambient nutrient concentration, are manipulated independently.

Our study shows that PCA can be a valuable statistical technique to evaluate simultaneously structural (cellulose, hemicellulose and lignin) and secondary compounds (condensed tannins, hydrolysable tannins and total phenolics) for the selection of species for breakdown experiments. The use of PCA provided a comprehensive measure of leaf quality for our initial selection of species. PCA has been used to examine factors affecting decomposition rates in terrestrial and wetland ecosystems (Vervaet *et al.*, 2002; Bridgham, Updegraff & Pastor, 1998), but it has not been used in decomposition studies in streams. We suggest that future studies which examine effects of leaf chemical parameters should use PCA to evaluate the importance of various chemical parameters.

Does leaf quality also affect the response of leaf nutrient content to high P in stream water?

We found evidence that initial concentrations of cellulose and lignin affect changes in the nutrient content of leaves during the breakdown process in response to high ambient P. The strong relationships between AFDM remaining and leaf P content observed for *Trema* leaves suggests that microbes on high quality leaves will take up more P at high-P sites (Table 4.4). *Trema* and *Zygia* had a similar initial concentration of P. Microbes on *Trema* leaves immobilised P at sites with relatively high P, while *Zygia* leaves increased in P content only at one high-P site (Fig. 4.6 d & e). Previous studies in terrestrial systems have reported that leaf quality can mediate changes in nutrient content in decomposing leaves in response to nutrient enrichment. For example, Hobbie (2000) found that leaves with lower concentrations of lignin had higher rates of nitrogen uptake in response to N addition. Similarly, Bridgham & Richardson (2003) found higher P immobilisation, in response to P-addition, in species of leaves with a lower initial concentration of lignin. Here we demonstrate that the same can occur for leaves in tropical streams.

In our study, C to nutrient ratios in leaves were not predictive of leaf breakdown rate or the response of breakdown rate to high P in water. Stoichiometric ratios (C:N, C:P and N:P) have been shown to predict breakdown rate and the response of microbes to nutrient enrichment in previous studies (Webster & Benfield 1986; Enríquez *et al.*, 1993; Qualls & Richardson 2000; Stelzer *et al.*, 2003). C:N ratios would have incorrectly predicted that *Trema* (C:N molar ratio 29.4) and *Zygia* (C:N molar ratio 26.3) would decay at similar rates. In contrast, *Trema* decayed much faster than *Zygia*. Similarly, C:P and N:P ratios would have incorrectly predicted that microbes on *Zygia* (C:P 1692.4, N:P 70.5), which had the highest C:P and N:P ratios, would have responded more strongly than *Trema* (C:P 1187.9, N:P 44.1) or *Castilla* (C:P 819.3, N:P 39), to high P. The poor predictive power of leaf stoichiometry supports our hypothesis that variation in the concentrations of recalcitrant forms of C among the three

species was the main factor determining the response of microbial and insect processing to high ambient P concentration.

Differences in leaf chemistry we observed between the initial collection and the leaves used in the breakdown experiment were most probably due to seasonal changes in leaf chemistry (Table 4.2). Leaves for the initial chemical analyses were collected during the rainy season, while those used in the breakdown study were collected in the dry season. Changes in leaf litter chemistry in La Selva forests have been linked to precipitation (Wood *et al.*, 2005).

What are the biotic mechanisms driving effects of high P concentration on leaf breakdown?

We examined microbial respiration, fungal biomass and invertebrate density to determine possible mechanisms driving differences in leaf breakdown. Our results must be interpreted conservatively, since we only sampled biotic components in each species once during the incubation period. However, results suggest that microbes and insects play different roles in leaf breakdown in the three species. Stimulation of breakdown of high-quality *Trema* in high-P streams was mostly reflected by changes increases in ergosterol and microbial respiration (Figs. 4.3 & 4.4). Ergosterol concentration in *Trema* was similar to that reported previously for *Ficus insipida* Willd. in La Selva streams (Rosemond *et al.*, 2002). The high microbial respiration on *Trema* (2.25 mg O₂ g AFDM⁻¹ h⁻¹) in the P-enriched stream illustrates that microbes play an important role in the breakdown of this species. Microbial respiration on *Trema* was also similar to respiration rates previously reported for *F. insipida*, while respiration rates on *Castilla* and *Zygia* were similar to the lower respiration rates on naturally accumulated mixed leaves reported for La Selva (Ramírez *et al.*, 2003).

For *Zygia*, we found an increase in leaf breakdown rate and in density of invertebrates other than chironomids with increasing P levels in the water (Table 4.2; Fig. 4.5b). This suggests that breakdown of low-quality leaves might be driven more by invertebrate consumers than by microbes. The importance of invertebrates in the breakdown of poor quality species

has been shown in temperate systems, where invertebrate removal affected the breakdown rate of *Rhododendron* (low-quality) more than red maple (high-quality) (Chung, Wallace & Grubaugh, 1993). Invertebrate densities reported here were 2-50 g⁻¹ AFDM of leaf, which is relatively low compared to those reported from some temperate streams (2-111 g⁻¹ DM Sedell, Triska & Triska, 1975; 6-30 g⁻¹ DM, Short, Canton & Ward, 1980). Our densities were similar to those reported in a previous study in La Selva (10-50 invertebrates g⁻¹ AFDM; Ramírez & Pringle, 2004). Like other studies in lowland tropical streams, we found very few insect shredders and collector-gatherers were the most abundant functional-feeding group (Walker, 1987; Pringle & Ramírez, 1998; Rosemond *et al.*, 1998). Chironomids, many of which are classified as collector-gatherers, may play an important role in the breakdown process because of their leaf-mining behaviour (Rosemond *et al.*, 1998)

Our overall results are consistent with the hypothesis that high a concentration of recalcitrant forms of C in leaves mediates the effect of high-P in stream water on fungal biomass, microbial respiration and invertebrate density. Future studies of the effect of ambient nutrient concentration on organic matter processing should consider C availability, in addition to nutrient content of the leaf substrate. In contrast to predictions made by other researchers regarding the key role of plant secondary compounds in mediating leaf breakdown rates in tropical streams, our study suggests that leaf secondary compounds did not affect breakdown. Moreover, the concentrations of cellulose and lignin were better predictors than leaf carbon to nutrient ratios of the effect of high ambient P concentration on microbial processing.

In conclusion, lowland tropical streams have a chemically-diverse detrital resource base, where leaf quality has the potential to play a key role in mediating effects of eutrophication on leaf breakdown. This has important implications, given the increasing extent and magnitude of anthropogenic nutrient loading in tropical ecosystems (Mattson *et al.*, 1999; Pringle, 2000).

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	Piper	Taconazo	Saltito 100	Sura	Arboleda	Carapa
Flow (m s⁻¹)	0.22	0.07	0.13	0.14	0.13	0.12
Range	(0.1-0.31)	(0.03-0.12)	(0.08-0.16)	(0.1-0.19)	(0.06-0.16)	(0.08-0.14)
T	00 5	00.7	00 F	00.0	00.0	00 5
Temperature (°C)	26.5	26.7	26.5	26.3	26.6	26.5
Range	(26.3-26.8)	(25.5-27.5)	(26.1-27)	(25.4-27)	(25.6-27.1)	(26.3-26.9)
рH	4.91	5.02	5.14	5.86	6.12	4,46
Range	(4.82-5.21)	(4.8-5.5)	(4.83-6.08)	(5.83-6.02)	(6.09-6.25)	(4.07-5.61)
	((110 010)	((0.00 0.01)	(0.00 0.20)	(
Conductivity (µS)	22.3	19.4	16.1	115.9	266.2	17.5
Range	(22-23)	(17-21)	(15-17)	(105-126)	(241-300)	(14-22)
SRP (µg P L ⁻ ')	11	12	12	49	137	187
Range	(9-14)	(10-21)	(9-19)	(35-74)	(127-159)	(10-289)
1						
NO₃-N (µg L⁻')	163	125	158	186	165	168
Range	(129-189)	(108-139)	(130-176)	(161-211)	(126-193)	(109-198)
NH₄-N (µg L ⁻¹)	1	2	3	2	11	3
Range	(0-3)	(0-3)	(0-7)	(0-8)	(2-26)	(0-16)
-						. ,
N:P molar ratio	34	23	29	9	3	2

Table 4.1. Physical and chemical characteristics of the study streams. Data presented for Carapa (the P-enriched stream) is from 20 m downstream of the P addition. Numbers in parentheses are the range observed during the study period.

Table 4.2. Chemical parameters (mean percent dry mass) of eight common riparian species from La Selva Biological Station. Leaves for the initial survey were collected in December of 2001. Leaves for the breakdown experiment were collected in April-June of 2002. Lower case letters denote significant differences after ANOVA and *post-hoc* Tukey (parameters with same letter are not significantly different between species).

	Condensed	Hydrolizable	Total						
	tannins	tannins	phenolics	Hemicellulose	Cellulose	Lignin	Ν	Р	С
Breakdown experiment									
Trema integerrima	0.97 a	2.92 a	1.15 a	8.88 a	11.51 a	1.25 a	1.35 a	0.074 a	34.09 a
Castilla elastica	16.45 b	16.02 b	16.05 b	23.16 b	16.57 b	7.59 b	2.07 b	0.128 b	40.67 b
Zygia longifolia	13.07 b	12.85 b	11.79 b	19.55 b	21.74 c	21.36 c	2.07 b	0.071 a	46.60 c
Initial survey									
Trema integerrima	1.48	8.09	1.31	16.53	12.45	10.98	2.42		37.46
Castilla elastica	18.66	20.88	21.58	15.06	18.93	9.63	2.31		42.82
Zygia longifolia	13.49	18.69	12.65	10.95	21.71	25.35	2.16		45.35
Ficus insipida	0.40	6.71	0.74	13.05	17.74	11.76	1.35		34.08
Terminalia									
oblonga	3.17	34.64	26.42	10.23	17.11	6.7	2.00		40.11
Luehea seemannii	14.48	33.22	22.31	13.09	20.95	19.37	1.74		45.36
Simira maxonii	0.59	9.86	3.35	13.91	23.40	14.31	2.31		42.77
Carapa									
nicaraguensis	23.58	36.84	34.75	9.88	19.2	22.95	1.375		41.94

Table 4.3. Breakdown rate ($k \text{ day}^{-1} \pm 1 \text{ SE}$) of three species incubated in six streams in La Selva Biological Station. Sites are in order of increasing P concentration. Letters denote significant differences using ANCOVA (Days to 95% mass loss = $-1(\ln(0.05)/k)$). All *P* values for the regression lines were < 0.05.

			Days to 95%	
	Decay rate (day ⁻¹)	r²	mass loss	ANCOVA
Trema				
Piper	0.129 <u>+</u> 0.0166	0.75	23.2	а
Taconazo	0.0451 <u>+</u> 0.0047	0.82	66.4	b
Saltito100	0.0665 <u>+</u> 0.0083	0.76	45.0	b
Sura	0.0638 <u>+</u> 0.0107	0.67	47.0	b
Arboleda	0.109 <u>+</u> 0.0103	0.86	27.5	а
Carapa	0.117 <u>+</u> 0.011	0.83	25.6	а
Castilla				
Piper	0.0119 <u>+</u> 0.0058	0.45	251.7	а
Taconazo	0.0064 <u>+</u> 0.0023	0.60	468.1	а
Saltito100	0.00991 <u>+</u> 0.0034	0.63	302.3	а
Sura	0.0087 <u>+</u> 0.0043	0.44	344.3	а
Arboleda	0.02144 <u>+</u> 0.0049	0.78	139.7	b
Carapa	0.01256 <u>+</u> 0.0053	0.55	238.5	а
Zygia				
Piper	0.003612 <u>+</u> 0.0014	0.62	829.4	а
Taconazo	0.002085 <u>+</u> 0.001	0.51	1436.8	b
Saltito100	0.005691 <u>+</u> 0.001	0.89	526.4	а
Sura	0.005901 <u>+</u> 0.0012	0.87	507.7	а
Arboleda	0.006755 <u>+</u> 0.0016	0.82	443.5	ac
Carapa	0.008003 <u>+</u> 0.0018	0.82	374.3	С

	%N)
_	Slope	r ²	Slope	r ²
Trema integerrima				
Piper	45.44	0.22	-2362	0.66
Taconazo	66.81	0.68	-651.44	0.84
Saltito	-28.29	0.25	-2333.86	0.82
Sura	27.19	0.16	-362.76	0.23
Arboleda	24.76	0.05	-418.52	0.94
Carapa	25.11	0.08	-730.05	0.81
Castilla elastica				
Piper	-29.3	0.15	-585.2	0.15
Taconazo	22.27	0.75	160.12	0.82
Saltito	27.32	0.34	149.89	0.06
Sura	9.94	0.08	-93.2	0.048
Arboleda	27.25	0.0013	-226.2	0.42
Carapa	27.25	0.42	404.39	0.3
Zygia longifolia				
Piper	-41.63	0.031	604.72	0.62
Taconazo	38.3	0.39	234.13	0.16
Saltito	-44.68	0.22	-371.35	0.064
Sura	-51.66	0.14	1117.19	0.51
Arboleda	90.1	0.44	-262.87	0.61
Carapa	1176.76	0.92	146.75	0.02

Table 4.4. Slopes and correlation coefficients of the inverse linear function relating mass remaining and percent nitrogen and phosphorus in the remaining material in three species of common riparian trees in six streams. Bold indicates P < 0.05

Table 4.5. Relative response (slope) of breakdown rates, ergosterol, chironomid and nonchironomid density to increasing stream water P concentration in leaves of *Trema*, *Castilla* and *Zygia*. The regressions are run with (w Piper) and without (w/o Piper) the Piper site, which had higher flow velocity than other sites. Numbers in parentheses are the r^2 values of the regressions. Bold numbers indicate $P \leq 0.05$.

Depedent variable	Depedent variable Trema		Cas	stilla	Zygia		
	w Piper	w/o Piper	w Piper	w/o Piper	w Piper	w/o Piper	
Breakdown rates	0.000295	0.000378	0.0000423	0.0000493	0.0000231	0.000022	
	(0.25)	(0.91)	(0.37)	(0.44)	(0.66)	(0.61)	
Ergosterol	2.29	2.27	-0.017	0.0049	-0.13	-0.18	
	(0.91)	(0.90)	(0.01)	(0.01)	(0.05)	(0.03)	
Chironomid density	0.21	0.21	0.15	0.15	0.0026	0.0033	
	(0.79)	(0.79)	(0.71)	(0.67)	(0.01)	(0.13)	
Non-chironomid density	0.11	0.12	0.074	0.08	0.035	0.0035	
	(0.59)	(0.59)	(0.60)	(0.61)	(0.26)	(0.25)	

Figure 4.1. Ordination of initial leaf chemical parameters for eight common riparian species in La Selva Biological Station using Principal component analysis. Species selected for the breakdown experiment are in solid black.



Trema Castilla Zygia Ficus Terminalia

Luehea

Simiira

Carapa

0

Δ

 ∇

 \diamond

Figure 4.2. Mean In of %AFDM remaining over time for three species: a) *Trema*, b) *Castilla* and c) *Zygia* in six streams in La Selva Biological Station. White symbols denote sites with low P (<15 μ g SRP L⁻¹) and black symbols indicate sites with high P (>50 μ g SRP L⁻¹).









Figure 4.3. Microbial respiration on three species of leaves in three sites in La Selva Biolgical Station. Saltito has low ambient P concentrations (>10 μ g SRP L⁻¹), Sura has high ambient P (49 μ g SRP L⁻¹), and Carapa is the P-enriched stream (188 μ g SRP L⁻¹). Capital letters denote significant differences within *Trema* and lower case letter denote significant differences within *Castilla*, there were no significant differences within *Zygia*.




Figure 4.4. Mean ergosterol concentration (<u>+</u> 1 S.E.) on leaves of three riparian trees incubated in six streams in La Selva Biological Station. Samples were collected on day 11 for *Trema* and day 44 for *Castilla* and *Zygia*. Sites are arranged in order of increasing SRP. Letters denote significant differences within species using ANOVA and *post hoc* Tukey. There were no significant differences in *Castilla* or *Zygia*.



Site

Figure 4.5. Mean density (<u>+</u> 1 S.E.) of: a) Chironomids and b) Non-chironomids on leaves of three riparian trees incubated in six streams in La Selva Biological Station. Invertebrates were collected on day 11 for *Trema* and day 44 for *Castilla* and *Zygia*.



Site

Figure 4.6. Leaf nutrient content during breakdown of three species of leaves incubated in six streams in La Selva Biological Station. Percent nitrogen in: a) *Trema*, b) *Castilla* and c) *Zygia* over time. Percent phosphorus in: d)*Trema*, e) *Castilla* and f) *Zygia* over time. Note the different scale on the x-axis for a) & d) *Trema*.



CHAPTER 5

SPATIAL HETEROGENEITY IN CARBON AND NUTRIENT RESOURCES AFFECT RESPIRATION OF HETEROTROPHIC BIOFILMS ON LEAVES IN A NEOTROPICAL STREAM⁴

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Abstract

Heterotrophic biofilms are important drivers of community respiration, nutrient cycling, and decomposition of organic matter in stream ecosystems. Both organic matter quality and ambient nutrient levels have been shown to individually affect heterotrophic biofilm biomass and activity, but both factors have not been manipulated simultaneously. To experimentally manipulate carbon (C) quality and nutrient content of organic matter, along with phosphorus (P) in overlying water, we first used an artificial low-quality substratum (cellulose cloth) and enhanced its C quality and nutrient content by amending underlying agar with maltose and P, respectively (Experiment I). Artificial substrata were incubated in low- and high-P sites of a whole-stream P-enrichment in lowland Costa Rica. Results from this experiment suggest that heterotrophic biofilm respiration on cellulose cloth is co-limited by C and P. Furthermore, biofilm respiration responded in an additive manner to combined effects of maltose and P-enrichment of overlying water and synergistically to maltose and high-P in substrata. Since decomposing organic matter that supports heterotrophic biofilms varies naturally in its C quality along with other physical and chemical properties, we conducted a second experiment amending two natural leaf substrata (Trema integerrima a high-quality C source and Zygia longifolia a lowquality C source) with maltose, and incubating them in low- and high-P sites of the P-enrichment stream. Results indicate that biofilm respiration on a naturally high-quality C source (Trema) is not C-limited, while biofilm respiration on a low-quality C source (Zygia) is C-limited. P stimulated biofilm respiration and breakdown rate on a high quality C-source (Trema), but not on a low-quality C-source (Zygia), supporting the hypothesis that the effect of P-enrichment is dependent on the quality of the C-source in decomposing leaves. These results demonstrate that C quality of organic matter can determine the response of microbial biofilms to Penrichment of both substratum and water.

Introduction

Heterotrophic biofilms, composed of fungi and bacteria, are important drivers of community respiration, nutrient cycling, and organic matter decomposition in stream ecosystems (Mulholland et al. 1984, Tank and Webster 1998, Battin et al. 2003, Tank and Dodds 2003). Because of their important role in transforming and transferring allochthonous carbon, increases in biofilm activity and biomass can stimulate the abundance and biomass of invertebrate consumers (Wilcox et al. 2005). Previous studies have shown that the biomass and activity of heterotrophic biofilms are influenced by spatial heterogeneity in: (1) quantity and quality of dissolved organic matter (McArthur et al. 1985, Hedin 1990); (2) quantity and quality of particulate organic matter (McArthur et al. 1985, Hedin 1990); and (3) forms and concentrations of ambient nutrients (Tank and Webster 1998, Grattan and Suberkropp 2001, Tank and Dodds 2003). Resource spatial heterogeneity is defined here as spatial variation in the source (substratum versus water) and quality of different resources.

Organic matter quality [defined here as the ease of microbial decomposition; (Strauss and Lamberti 2002)] is determined by carbon quality and nutrient content (Aber and Melillo 2001). Carbon (C) quality is determined by the concentrations and forms of carbon present. High concentrations of labile forms of C in particulate organic matter can lead to high microbial activity and fast breakdown (Melillo et al. 1984). Microbes growing rapidly on high-quality substrata with high concentrations of small, labile C molecules would be expected to have high nutrient requirements. Consequently, such microbial communities (which are not C-limited) could become nutrient-limited in nutrient-poor stream water (Melillo et al. 1984). In contrast, microbes on low-quality substrata with high concentrations of recalcitrant C compounds are primarily C- (not nutrient-) limited, and are thus unable to utilize increases in ambient nutrients. Therefore we predict that an increase in ambient nutrients should lead to greater microbial activity on high C, versus low C quality organic matter. Likewise, microbial biofilms on substrata

with high nutrient content will not be nutrient-limited (Suberkropp 1998) and will not respond strongly to nutrient enrichment of the overlying water.

Whereas many previous studies have addressed how resources provided from different spatial sources (i.e. substratum versus water) affect heterotrophic biofilms (McArthur et al. 1985, Tank and Webster 1998, Olapade and Leff 2005), few studies have examined their potential interactions. For example, research has shown that ambient nutrient enrichment can stimulate biofilm biomass and respiration on wood (high carbon : nitrogen ratio) more than on leaves [with low C:N; (Stelzer et al. 2003, Gulis et al. 2004)]. Greenwood (2004) showed that low quality *Rhododendron maximum* leaves (with high C:N) tended to respond more strongly to ambient nutrient enrichment than high-quality *Acer rubrum* leaves (low C:N), and Ardón et al. (2006) reported that microbial respiration and fungal biomass responded more strongly to increases in ambient phosphorus (P) on a high-quality leaf species (*Trema integerrima*) than on two low-quality leaf species (*Zygia longifolia*). However, none of these studies have experimentally manipulated the quality of the organic matter itself.

Here, we experimentally examine the interactive effects of organic matter quality and ambient P on heterotrophic biofilm respiration and breakdown of organic matter. We conducted two experiments: we first used an *artificial* low-quality substratum (cellulose cloth) and artificially enhanced its C quality and nutrient content by amending underlying agar with maltose and P, respectively. Artificial substrata were incubated in low- and high-P sites of a whole-stream P-enrichment. Since decomposing organic matter varies naturally in its C quality along with other physical and chemical properties, we conducted a second experiment, amending two *natural* leaf substrata (*Trema integerrima* a high-quality C source and *Zygia longifolia* a low-quality C source) with maltose and incubated them in low- and high-P sites of the P-enrichment stream.

Methods

Site description

This study was conducted at La Selva Biological Station, Costa Rica (10°26′ N, 84°01′W). The 1536 ha reserve is the lowland terminus of the last protected unbroken biological corridor spanning the full altitudinal range on the Caribbean slope of Central America. La Selva receives 4000 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. 2001). Streams at La Selva exhibit natural variation in ambient phosphorus concentration [5 - 350 µg soluble reactive phosphorus (SRP) L⁻¹] and other solutes (Mg, Fe, Na, Cl and SO₄) due to inputs of solute-rich groundwater (Pringle et al. 1993). This study was conducted in the Carapa, a firstorder stream that drains into the Sura River and does not receive solute-rich groundwater. We have been experimentally enriching a 100 m reach of the Carapa with phosphoric acid since July 1998 as part of a larger study examining the effects of solute-rich groundwater inputs on ecosystem processes (Ramírez et al. 2003, Ardón et al. 2006, Ramírez and Pringle 2006). Phosphoric acid has been added to increase P concentrations from <10 µg to a target concentration of 300 µg SRP L⁻¹, which is at the high end of P levels exhibited by streams receiving solute-rich groundwater (Pringle and Triska 1991). A Mariotte bottle (carboy) is used to add phosphoric acid continuously, with concentration and drip rate adjusted according to stream discharge. Discharge was measured bi-weekly, and water samples were taken weekly at one site upstream and three sites downstream from the site of P addition (10 m, 50 m, and 100 m). The experimental method was described in greater detail in a previous study (Ramírez et al. 2003).

Artificial substrata

Artificial substrata were constructed by modifying techniques developed to examine nutrient-limitation of heterotrophic biofilms (Winterbourn 1990, Corkum 1996, Tank and Webster 1998, Tank and Dodds 2003). They consisted of 30 ml plastic containers filled with a 2% (weight by volume) agar solution. We conducted two separate experiments placing the artificial substrata above and below the site of P-enrichment.

In Experiment I, conducted from 2nd to 18th of March 2005, we examined interactions between presence/absence of high-quality DOC and phosphorus both in water and substratum. We used an artificial standardized low-quality substratum (cellulose cloth, American Livestock Supply Inc., Madison, WI, USA) as an organic site for attachment of fungi and bacteria (J.L. Tank, personal communication). The cellulose cloths were placed to completely cover the agar and secured with a tight fitting snap-on cap exposing a 4 cm diameter circle on the top of the containers. We conducted a 2³ factorial experiment: carbon guality (presence or absence of maltose in the agar), substratum phosphorus (present or absent), and ambient phosphorus (low and high). For the substratum phosphorus treatment, we added 0.5 M of KH_2PO_4 to the agar (Tank and Dodds 2003). To manipulate carbon quality, we added maltose to the agar at a concentration of 4% (weight by volume). Four replicates of each treatment were placed in plastic L-beams and secured to the bottom of the stream above and 10m below the site of Penrichment (4 treatments x 2 sites x 4 replicates = 32 substrata). Samples were collected on day 16 and brought to the laboratory to measure biofilm respiration on the cellulose cloths. Previous experiments had shown that 16 days was enough time for microbial colonization of the cellulose cloths (M. Ardón unpublished data). We placed the entire cellulose cloth in respiration chambers (26 ml) filled with stream water from the site where the substrata had been collected. Changes in dissolved oxygen concentration were measured every 5 minutes for 30 minutes with a YSI Model 58 Dissolved Oxygen meter with a self-stirring probe (Gulis and Suberkropp 2003). Chambers containing only stream water were used as controls. Oxygen consumption is

expressed as mg O_2 per gram of ash free dry mass (AFDM) per hour. AFDM was determined by drying each disc at 70° C for 24 hours, weighing, and ashing at 500° C for 1 hour and reweighing.

In Experiment II, conducted from 3rd to 19th of July, 2005, we determined whether increasing the carbon quality (by providing maltose as a high-quality carbon source), of natural leaf substrata that differed in carbon quality, alters the response of biofilms to ambient phosphorus. We used leaf discs from two different tree species and placed them on top of agar-based substrata. Cellulose cloths on agar were used as controls. Two species of common riparian trees were selected: *Trema integerrima* [(Beurl) Standl (family Ulmaceae; hereafter referred to as *Trema*)], and *Zygia longifolia* [(Humb. & Bonpl. Ex Willd.) Britton & Rose (family Fabaceae; hereafter referred to as *Zygia*)]. Leaves of *Trema* have low concentrations of tannins, phenolics, lignin, and cellulose, and have fast breakdown rates, while leaves of *Zygia* have high concentrations of tannins, phenolics, lignin, and cellulose, lignin, and cellulose and have slow breakdown rates (Table 5.1). Both species have similar initial P content (Table 5.1). The leaf discs were held in place with a tight fitting snap-on cap exposing a 4 cm diameter circle on the top of the containers. For each of the three substrata types (*Trema, Zygia*, and cellulose cloths), half of the cups received agar amended with 4% (weight by volume) maltose as a source of high-quality carbon, while the other half received agar only.

Artificial substrata were secured to plastic L-beams in groups of six (one replicate per treatment), as follows: *Trema* without maltose, *Trema* with maltose, *Zygia* without maltose, cellulose without maltose, and cellulose with maltose. The placement of the treatments was randomly assigned within each plastic beam and secured to the bottom of the stream with aluminum stakes. Due to their rapid breakdown, we collected *Trema* leaf discs on days 2, 4 and 8 (3 collection dates x 2 maltose treatments x 2 P treatments x 4 replicates = 48 substrata). We collected substrata with *Zygia* and cellulose cloth discs on days 2, 4, 8, and 16 (4 dates x 2 maltose treatments x 4 replicates = 64 substrata each). A total of

176 artificial substrata were made. Samples were taken back to the laboratory to measure biofilm respiration and AFDM remaining as described above.

To examine the rate of maltose release from agar to the water, we used a semiquantitative colorimetric assay for dissolved sugars (Benedict's reagent). To examine if the agar itself could be a carbon source, we conducted a preliminary experiment where we compared respiration after 16 days on cellulose cloths secured on the top of plastic containers without agar to cellulose cloths on containers with agar.

Statistical analyses

We analysed data from Experiment I using three-way analysis of variance (ANOVA), with substratum phosphorus, maltose and ambient phosphorus as the main effects, followed by *post-hoc* comparison of least squares means (Students-t). Respiration data were logarithm-transformed to meet assumptions of normality. In Experiment II, we calculated breakdown rates as the negative slope of the linear regression of *natural log of percent AFDM remaining* versus *days in the stream* [negative exponential model, (Benfield 1996)]. Analysis of covariance (ANCOVA) was used to compare breakdown rates among treatments. Repeated measures multiple analysis of variance (MANOVA) was used to test for effects of maltose and phosphorus on biofilm respiration within each substratum type (*Trema, Zygia* and cellulose). Respiration data were logarithm transformed to meet assumptions of normality. For Experiment II we also tested effects of maltose and P among the three substratum types by conducting a three-way analyses of variance (ANOVA) with substratum type (*Trema, Zygia*, or cellulose cloth), maltose and ambient phosphorus as the main effects on respiration measured on the last sampling date, followed by *post-hoc* comparison of least squares means (Students-t). Analyses were conducted using JMP 5.0.1 Statistical Software (SAS Institute, Cary, NC, USA).

Results

Whole-stream phosphorus enrichment of the Carapa during the experiment was characterized by temporary shifts in P-concentration due to changes in discharge and variable rates of P-release from the Mariotte bottle (Table 5.2). In both time periods, P declined rapidly in the first 50 m of enrichment, primarily as a result of P-sorption to sediments (Triska et al. 2006; Table 5.2). Temperature, conductivity, and pH were similar between the two experiments (Table 5.2).

We found a constant rate of maltose release for up to 21 days, followed by a rapid decline afterwards. This suggests that biofilms on substrata with maltose amendments received a constant source of labile carbon throughout the duration of experiments. We found that there was significantly higher respiration on substrata with agar than those without (mean respiration with agar = 0.41 mg O₂ g AFDM⁻¹, mean respiration without agar = 0.13 mg O₂ g AFDM⁻¹; F = 7.96, p < 0.05), indicating that the agar itself could serve as a carbon source for biofilms.

In Experiment I, different treatments supported significantly different rates of biofilm respiration (F = 12.0, p < 0.001, Fig. 5.1). Phosphorus, from both water and substratum, and presence of maltose increased microbial respiration (Table 5.3). Maltose amendment significantly (p < 0.05) increased (1.5x) respiration when compared to the control treatment. Increases in water phosphorus doubled biofilm respiration compared to the control (p < 0.05, Fig. 5.1), while increases in substratum phosphorus did not significantly affect biofilm respiration. When maltose was added in combination with P-enrichment of the water, biofilm respiration was similar to the sum of the individual treatments, indicating an additive response (Table 5.3, Fig. 5.1). Biofilm respiration responded more strongly to maltose amendments and substratum-derived P, than to maltose and P-enrichment of overlying water (p<0.05). Respiration in response to both maltose and substratum-derived P was higher than the sum of the individual treatments, indicating a synergistic response.

In Experiment II, breakdown rates were fastest for *Trema*, and slowest for cellulose cloth (Table 5.4). P-enrichment in the water significantly accelerated breakdown rate of *Trema* with (F = 12.49, p < 0.005) and without maltose (F= 14.88, p < 0.005; Fig. 5.2, Table 5.4), but maltose had no significant effect on breakdown rate. Maltose and P-enrichment appeared to accelerate breakdown rate of *Zygia* discs and cellulose cloth, although treatment differences were not significant (Fig. 5.2, Table 5.4).

Biofilm respiration on *Trema* leaf discs increased with time in the stream, and was stimulated by P-enrichment in the water (date F = 26.88, p < 0.001; date*P F = 3.91, p < 0.05; Fig. 5.3a). The effect of P-enrichment was highest on day 4 (Fig. 5.3a). Maltose amendments did not change the response to ambient P (date*P*maltose F = 2.16, p = 0.16; Fig. 5.3a). Respiration rates on *Zygia* and cellulose cloth were much lower than on *Trema*. Respiration rates on *Zygia* increased over time, and were stimulated by the presence of maltose (date F = 4.72, p < 0.05, date*maltose F = 3.45, p < 0.05; Fig. 5.3b). However, presence of maltose did not alter the response of biofilm respiration to high-P in the water on *Zygia* (date*P*maltose F = 2.11, p = 0.16; Fig 5.3b). Respiration on cellulose cloth increased over time (date F = 13.28, p < 0.001), but was not significantly affected by high-P in the water, the presence of maltose in the agar, or their interaction (date*P F = 2.03, p = 0.17; date*maltose F = 1.15, p = 0.37; date*P*maltose F = 1.48, p = 0.27; Fig. 5.3). Maltose and substrate type determined microbial respiration on the last sampling date (Table 5.5), and substrate type determined the response to both P-enrichment in the water and maltose (Table 5.5).

Discussion

Our results provide the first experimental evidence to support the hypothesis that carbon quality of organic matter determines the response of microbial biofilms to phosphorus enrichment of both substratum and water. We found evidence for both carbon (C) and phosphorus (P) limitation of biofilm respiration and leaf breakdown rate. The combined effects

of high-quality C amendments and P-enrichment can lead to both additive and synergistic responses of biofilm respiration depending on the source of P (water versus substratum). Moreover, our results suggest that heterotrophic biofilms on leaves that are high-quality C sources can become P-limited, whereas biofilms on leaves that are poor-quality C sources are C-limited and therefore do not exhibit a significant response to P-enrichment of overlying water.

Evidence for carbon-limitation of heterotrophic biofilms

Positive response to maltose amendments in both experiments suggest that heterotrophic biofilms are C-limited in the Carapa stream, but the extent of limitation depends on the C quality of the substratum. Maltose amendments increased microbial respiration when compared to controls in both experiments. In Experiment II, the C quality of natural leaf substrata similarly appeared to determine biofilm response to maltose amendments (Fig. 5.3). Maltose increased biofilm respiration rate on *Zygia* leaf discs (low-quality substratum), but did not affect respiration or breakdown in *Trema* leaf discs (high-quality substratum; Fig 5.3). However, we did not find an increase in the breakdown rate of *Zygia*, suggesting that microbial biofilms increased respiration by taking advantage of the labile dissolved organic carbon (DOC) from maltose amendments, without increasing their use of leaf litter carbon. *Zygia* leaf discs have high concentrations of tannins, phenolics, lignin and cellulose which can limit microbial processing (Ardón 2006, Chapter 2).

We hypothesize that the delayed (i.e. day 16) response of microbial respiration to Penrichment on *Zygia* leaf discs was due in part to initial presence of secondary compounds in natural leaves. Secondary compounds extracted from red maple leaves have been shown to affect bacterial populations on artificial substrates (McNamara and Leff 2004). In a previous study, we showed that the majority of tannins and phenolics were leached out from *Zygia* leaves in approximately seven days (Ardón 2006, Chapter 2). The presence of these recalcitrant secondary compounds likely prevented biofilms on *Zygia* leaf discs from responding to maltose

amendments earlier during the incubation period. Once the secondary compounds were leached from the leaves (around day 8) biofilms were mostly carbon-limited due to high concentrations of cellulose and lignin in *Zygia* leaves. It is probable that if we had run the experiment for a longer period of time we would have seen a stronger response to maltose addition.

Even though we did not measure the concentration of dissolved organic carbon (DOC) in our study stream, mean concentration in a nearby similar stream (Taconazo) is approximately 1.7 mg L⁻¹ (M.D. McDowell, C.M. Pringle, and D. Genereux unpublished data), suggesting that DOC is relatively low in these sites. Thus, positive responses to maltose amendments would be predicted. Our findings of increased respiration in response to maltose amendments agree with previous reports of increased bacterial biomass due to glucose amendments using artificial substrata in a stream in Ohio (Olapade and Leff 2005). Similarly, a whole-stream addition of labile dissolved organic carbon (glucose) at Hubbard Brook Long-Term Ecological Research Site led to increases in microbial respiration without affecting mass loss of maple leaf packs (Bernhardt and Likens 2002). A similar study at Coweeta Hydrologic Laboratory Long-Term Ecological Research Site, likewise found increases in microbial respiration on red maple leaves in response to enrichment with labile DOC (dextrose), but the authors did not report whether there were differences in leaf mass loss (Wilcox et al. 2005).

Interactive effects between carbon-quality and phosphorus on heterotrophic biofilms

It has generally been hypothesized that heterotrophic biofilms rely primarily on nutrients from the overlying water when the substratum they colonize has a low nutrient content (Gessner and Chauvet 1994, Suberkropp 1998). Our results indicate that the carbon-quality of the substratum can determine the magnitude of stimulation of water- versus substratum-derived nutrient enrichment on microbial biofilms. Results from Experiment I indicate an additive response to combined influences of maltose amendments and high-P in the water, and a

synergistic response to maltose and substratum-derived P on biofilm respiration (Fig. 5.1). The presence of labile C can therefore increase biofilm demand for substratum-derived P.

We hypothesize that the greater increase in biofilm respiration to substratum Penrichment in the presence of maltose, than to P-enrichment of overlying water, is driven mostly by a fungal response. Because fungal hyphae are imbedded in the organic matter matrix, and thus in close contact with the substratum, it facilitates extra-cellular enzymatic secretion for the uptake of labile C and nutrients from the substratum (Suberkropp 1998). It has been similarly suggested that algal periphyton respond more strongly to substratum-derived nutrients than to enrichment of overlying water via similar mechanisms (Pringle 1990).

We did not observe a significant interaction between P-enrichment and maltose amendments in Experiment II. Instead, we found that P-enrichment only had a significant effect on microbial respiration and leaf breakdown of *Trema* leaf discs, whereas maltose had no effect. We interpret this result to mean that biofilms on *Trema* leaf discs were not C-limited and thus did not respond to increased labile DOC from maltose amendments. The increase in respiration and breakdown rate on *Trema*, in response to P-enrichment of the overlying water, supports our initial hypothesis (and previous research) that high C-quality of organic matter can increase demand for water-derived P (Ardón et al. 2006). Similarly, previous studies have shown that the presence of labile DOC (leaf leachate or glucose) can increase nitrogen uptake by microbial biofilms in sediments (Bernhardt and Likens 2002, Sobczak et al. 2003).

Effectiveness of artificial substrata as bioassays

Major considerations when using artificial substrata to examine heterotrophic biofilms include variability in the rate of nutrient and C supply, selection of type of substratum for microbial attachment, and experimental artifacts due to agar itself serving as a C source. Issues regarding the rate and duration of nutrient supply using agar substrates have been previously discussed (Pringle 1987, Tank and Winterbourn 1995, Pringle and Triska 1996). Our

results clearly indicate that the type of substratum for microbial attachment can have important effects on microbial response to C and nutrient manipulations (Fig. 5.2 and 5.3).

Cellulose cloths provided a standardized low-quality substratum, as evidenced by similar respiration rates on the control treatments between the two experiments (Fig. 3.1 and 3.3). The lack of treatment separation in Experiment II on cellulose cloths was partly due to lower discharge during Experiment II, which led to increased sediment accrual on the cellulose cloths and increased colonization by larval chironomids (Diptera: Chironomidae). Inorganic matter on cellulose cloths was lower in Experiment I (mean = 9 mg inorganic matter per disc) than in Experiment II (mean = 44 mg inorganic matter per disc). We also observed higher Chironomidae density on cellulose cloths in Experiment II (mean = 7 individuals per cellulose cloth) than in Experiment I (mean = 3.2 individuals per cellulose cloth), which could have decreased microbial biomass through their feeding activities.

Higher respiration on substrata with agar, indicate that agar can serve as a C source. To minimize possible experimental artifacts associated with variation in the C quality of the agar itself, we suggest using the same lot of a refined brand of commercial agar for experiments. Agar is a strongly gelling seaweed hydrocolloid that is derived from cell wall polysaccharides of Rhodophyta species, and is primarily composed of galactose (Guiseley 1968). The composition of this polysaccharide varies according to the nutritional states of the algae from which it was derived, along with different manufacturing and processing techniques. Previous work has suggested that using different lots of agar in the substratum may lead to different amounts of phosphorus leaching from the substrata (Pringle 1987).

What are the ecological implications of alterations to heterotrophic biofilm activity due to nutrient enrichment and increased C quality?

Land use change, agriculture, domestic and industrial wastewater, and food processing wastes all can increase organic C and nutrient loading to streams (Jones 2001). Anthropogenic

nutrient and organic C loading can stimulate biofilm biomass and activity, which can affect higher trophic levels and ecosystem processes. For example, nutrient enrichment of stream water has been shown to increase biomass and nutrient content of microbial biofilms, leading to higher secondary production and faster turnover ratios of invertebrate consumers (Cross et al. 2005, Ramírez and Pringle 2006). Similarly, increases in labile DOC have been shown to affect nutrient cycling by increasing microbial biofilm demand for water nutrients (Bernhardt and Likens 2002, Sobczak et al. 2003).

Heterotrophic biofilm respiration is influenced by resource spatial heterogeneity. Factors that might alter the C quality of organic matter inputs into streams, like changes in riparian vegetation (Whiles and Wallace 1997), or changes in riparian leaf litter chemistry due to increases in atmospheric CO₂ (Tuchman et al. 2002), could alter the response of heterotrophic biofilms to eutrophication. Future research should consider going beyond elemental ratios (C:N) and incorporating more detailed information about C quality of organic matter (e.g. Joffre et al. 2001), in order to accurately predict microbial biofilm processes in human-impacted aquatic ecosystems.

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Table 5.1.	Initial I	leaf litter	chemistry	(percent o	dry mass)) of two c	ommon	riparia	an spec	ies in La
Selva Biolo	ogical S	Station, C	Costa Rica.	Breakdo	wn rates	are from	Ardón e	et al. (2	2006).	Hemicell =
hemicellul	ose.									

Species	Lignin	Cell.	Hemicell.	С	Ν	Р	Breakdown
	%DM	%DM	%DM	%DM	%DM	%DM	<i>k</i> day⁻¹
Trema integerrima	1.25	11.51	8.88	34.09	1.35	0.074	0.066
Zygia longifolia	21.36	21.74	19.55	46.60	2.07	0.071	0.005

	Discharge	pH Conduct.	Temp.	PO₄-P		
	L s ⁻¹		μS	°C	mean (µg L⁻¹)	range
Experiment I						
Reference	1.81	6.48	14.6	24.5	79	42 - 89
10m	1.81	5.16	24.6	24.5	2781	516 - 4419
50m	1.81	6.11	14.2	24.6	87	53 - 150
Experiment II						
Reference	0.45	5.88	12.8	25.2	27	20 - 37
10m	0.45	5.47	19.8	25.3	561	63 - 2105
50m	0.45	5.61	17.7	25.3	30	14 - 52

Table 5.2. Physical and chemical characteristics of the Carapa study stream during the study periods. Reference reach is 10 m upstream of the site of P-enrichment, 10 m and 50 m denote distance downstream from site of P-enrichment. Conduct = conductivity, Temp = temperature

Factor	DF	F ratio
Substratum P	1	15.5***
Water P	1	11.7**
Maltose	1	43.3***
Substratum P * water P	1	4.1
Water P * maltose	1	0.2
Substratum P * maltose	1	6.8*
Subs P * water P *maltose	1	2.3

Table 5.3. F-ratios for three-way analysis of variance for Experiment I. P = phosphorus, subs = substratum. * p < 0.05, ** p < 0.01, *** p < 0.001.

Table 5.4. Breakdown rates (*k*) of different treatments. Treatment symbols: (0 0) no maltose, low P; (+ 0) maltose under low P; (0 +) no maltose under high P; (+ +) maltose under high P. Letters denote significant differences among breakdown rates based on ANCOVA.

Substrate	Treatment (maltose, P)	k (day ⁻¹)	St. Error
Cellulose	(0 0)	0.009	0.003 a
	(0 +)	0.013	0.002 a
	(+ 0)	0.012	0.003 a
	(+ +)	0.015	0.002 a
Trema	(0 0)	0.090	0.025 b
	(0 +)	0.170	0.021 c
	(+ 0)	0.119	0.014 b
	(+ +)	0.166	0.024 c
Zygia	(0 0)	0.017	0.008 a
	(0 +)	0.027	0.006 a
	(+ 0)	0.024	0.005 a
	(+ +)	0.028	0.005 a

Table 5.5. F-ratios for three-way analysis of variance for respiration data measured on the last collection date during Experiment II. Phosphorus (P) was added to the water. * p < 0.05, ** p < 0.01, ***p < 0.001.

Factor	DF	F ratio
Phosphorus	1	2.20
Maltose	1	3.63
Substrate	2	745.0***
P*maltose	1	1.19
P*substrate	2	3.46*
Maltose*substrate	2	5.03*
P*maltose*substrate	2	0.23

Figure 5.1 Mean (\pm 1 standard error) biofilm respiration rate on artificial substrates after 16 days in the Carapa stream. Different letters denote significant difference from *post-hoc* comparison of least-square means (Students-t). Subs P = substrate phosphorus.



Figure 5.2. Natural logarithm of percent ash free dry mass (AFDM) remaining through time in the stream for (a) *Trema* leaf discs; (b) *Zygia* leaf discs; and (c) cellulose cloth on artificial substrata. Error bars represent \pm 1 standard error. Symbols refer to the four different treatment combinations for each substratum type (a-c).





Figure 5.3. Microbial respiration on (a) *Trema* leaf discs; (b) *Zygia* leaf discs; and (c) cellulose cloth on artificial substrata. Factors on graph are from repeated measure multiple analysis of variance (MANOVA). * p< 0.05, ** p < 0.001. Note different scales on both x- and y-axes for *Trema*.


CHAPTER 6

GENERAL CONCLUSIONS

Dissertation Summary

I tested the hypothesis that the quality of leaves (i.e. carbon quality and nutrient content) can determine: (1) the rate at which microbes and invertebrates process leaf litter; and (2) the magnitude of the microbial and invertebrate response to high ambient nutrient levels in water and substratum. I combined several approaches: (1) measurement of chemical changes of leaves and breakdown rate of eight common riparian species in a stream at La Selva Biological Reserve (Chapter 2); (2) use of standardized analytical techniques to measure and compare initial chemistry and its effect on breakdown of common riparian species between a tropical site (La Selva) and a temperate site (Coweeta Hydrological Laboratory, N.C.; Chapter 3); (3) use of a natural landscape gradient in stream water phosphorus (P), coupled with a long-term whole-stream phosphorus enrichment experiment, to examine leaf breakdown rates of three species differing in leaf litter quality (Chapter 4); and (4) modification of an artificial agar-diffusing substratum technique to experimentally test the hypothesis that leaf carbon(C)-quality determines the magnitude of stimulation by P-enrichment on biofilm respiration and organic matter processing (Chapter 5).

Importance of carbon-quality in leaf breakdown in a tropical and a temperate site

The role of chemical constituents, in particular secondary compounds, in driving leaf litter breakdown in tropical streams has been debated in the literature, and is currently cited as a possible explanation for the paucity of invertebrate shredders in tropical streams (Dobson et al. 2002, Wantzen et al. 2002, Wantzen and Wagner 2006). However, there are few studies that have actually examined the role of secondary compounds on breakdown in tropical streams. In fact, no previous study that I am aware of has examined chemical changes in decomposing leaves in a tropical stream.

In Chapter 2 I found that concentrations of cellulose, and lignin were more important than secondary compounds in inhibiting leaf breakdown rates of eight common riparian species in La Selva. My results indicate that, contrary to an existing paradigm stating that high concentrations of secondary compounds in tropical leaves inhibit breakdown rate, secondary compounds were rapidly leached (2-fold faster than rates reported for temperate leaves) and thus were unlikely to be important in breakdown beyond early stages. As in temperate studies, fungal and bacterial biomass were strongly correlated with breakdown rate. Furthermore, I found that, in contrast to temperate studies where invertebrate shredders are the dominant group, invertebrate assemblages in leaf packs were dominated by collector-gatherers. Also, invertebrate biomass was negatively correlated with breakdown rate and microbial biomass, suggesting that invertebrates were not directly using leaves as a food resource even after microbial conditioning. Overall our results suggest that fast-decomposing species are an important carbon source for fungi and bacteria, while slow-decomposing species primarily serve as substrata for attachment of invertebrates.

In Chapter 3, I compared initial leaf chemistry and breakdown rates between a tropical site (La Selva) and a temperate site (Coweeta). Contrary to what I expected, mean concentration of condensed tannins was significantly greater (2.6-fold) in Coweeta than in La Selva species. Furthermore, concentrations of condensed tannins were negatively correlated to breakdown rate among Coweeta species, but not among La Selva species. Similar to results in Chapter 2, structural compounds of leaves were key factors affecting leaf breakdown rates, with concentrations of cellulose and lignin strongly correlated to breakdown rate at both sites. Our results suggest that leaf litter from common riparian tree species at Coweeta is of lower quality than litter from common riparian trees at La Selva. Based on previous studies conducted in streams and data presented here, recommendations are made for implementation of

standardized analytical techniques that will better enable cross-site comparisons and synthesis of results from a broad range of studies.

Interactive effects of phosphorus and leaf litter quality

Results from Chapter 4 support my overall hypothesis that litter quality mediates the effect of high ambient P concentration on leaf processing by fungi and invertebrates. I found a stronger effect on microbial respiration, fungal biomass and invertebrate density on *Trema integerrima* (a fast-decomposing, high C-quality species) than on *Castilla elastica* or *Zygia longifolia* (slower-decomposing, low C-quality species). High flow velocity in a low-P site confounded our ability to evaluate the effect of increased water P on breakdown rates. Similar to results from Chapters 2 and 3, cellulose and lignin appeared to be the most important chemical constituents determining leaf breakdown. Also in agreement with Chapter 2 and 3, we did not find a significant role for secondary compounds in leaf breakdown.

Results from Chapter 5 provide the first experimental demonstration supporting the hypothesis that C-quality of organic matter can determine the response of microbial biofilms to P-enrichment of both substratum and water. Results indicate that heterotrophic biofilm respiration rates on artificial substrata with cellulose cloth are co-limited by C and P. Furthermore, biofilm respiration responded in an additive manner to combined effects of maltose and P-enrichment of overlying water and synergistically to maltose and high-P in substrata. P stimulated biofilm respiration and breakdown rate on a high quality C-source (*Trema*), but not a low-quality C source (*Zygia*), supporting the hypothesis that the effect of P-enrichment is dependent on the quality of the C-source in decomposing leaves. My results indicate that artificial substrata can be useful tools to examine interactions between the quality of the substratum and the nutrient content of the water, similar to how they have been used for algal assemblages (Pringle 1990, Bernhardt and Likens 2004).

Functional differences between tropical and temperate streams

I found both similarities and differences between leaf litter breakdown in tropical streams to what is known from temperate streams. I found consistent evidence that structural compounds were important in determining leaf litter breakdown in tropical lowland streams, which has been observed in temperate streams (Melillo et al. 1984, Gessner and Chauvet 1994). I found that fungi and bacteria played similar important roles in determining leaf litter breakdown as they do in temperate streams, reaching biomass levels which were on the high end of those reported in temperate areas (Chapter 2). In contrast to the importance of invertebrate shredders that has been reported in temperate streams, I found invertebrate assemblages on litterbags to be dominated by collector-gatherers and their biomass was negatively correlated to leaf breakdown rate.

Results suggest that fungi and bacteria are the most important group contributing to the overall breakdown of leaf litter, and that due to the high water temperature their biomass and activity is responsible for the rapid breakdown of leaf litter. Currently there is much debate regarding the applicability of general theory of organic matter processing developed in temperate streams to tropical streams (Wantzen et al. 2006). Work in various tropical regions have indicated that insect shredders are extremely scarce and species-poor (Ramírez and Pringle 1998, Rosemond et al. 1998, Dudgeon and Wu 1999, Dobson et al. 2002). While some studies do indicate that macroconsumers (mostly shrimp and crabs) can be important shredders in tropical streams (Crowl et al. 2001, March et al. 2001, Moss 2004), there is also evidence that exclusion of macroconsumers does not affect leaf litter breakdown (Rosemond et al. 1998, Mantel and Dudgeon 2004). Throughout most of my dissertation I used litterbags that excluded macroconsumers because previous work in these streams (Rosemond et al. 1998, Ramírez and Pringle 2004) and our own work (M. Ardón and K. Maynard, unpublished data) indicated that macroconsumers were not very important in determining leaf litter breakdown in this system.

Eutrophication of tropical aquatic systems is becoming increasingly problematic with deforestation and agricultural use of fertilizers (Matson et al. 1999, Martinelli et al. 2006). As tropical riverine ecosystems are increasingly subject to eutrophication and modifications to the riparian plant community, it is important to understand how these alterations synergistically affect the temporal and spatial availability of carbon sources to aquatic consumers. My results indicate that the quality of leaf litter that comprise the resource base of stream food webs, could affect the response of stream ecosystems to nutrient loading. For example a stream, where natural riparian vegetation has been replaced by fast-colonizing pioneer species (which produce high-quality leaves), could be more susceptible to loss of carbon through increased microbial respiration. In the long-term, increased microbial respiration on high-quality leaf litter due to altered riparian vegetation and eutrophication could lead to carbon limitation of stream consumers (Cross et al. 2005). On the other hand, an intact riparian vegetation that provides litter with a wide range of leaf chemistry can guarantee the availability of resources to consumers.

As tropical stream ecosystems continue to be altered by changes in land use for agriculture and urban development (Matagi 1996, Neill et al. 2001, Biggs et al. 2004), overharvest of fish (Brashares et al. 2004), increased dam construction (March et al. 2003), and eutrophication (Caraco and Cole 1999, Matson et al. 1999), it is important to increase our understanding of their structure and function. Recent studies have shown that tropical riverine ecosystems can play an important role in the global carbon cycle (Richey et al. 2002, Mayorga et al. 2005). Increasing our understanding of basic ecosystem process like organic matter processing will aid in the sustainable management of these ecosystems, which could mediate negative impacts on global biogeochemical cycles (Mayorga et al. 2005).

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