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Does leaf quality mediate the stimulation of leaf breakdown by phosphorus in Neotropical streams?

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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 hypothesised 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 μg soluble reactive phosphorus (SRP) L^{-1}), because of variable inputs of solute-rich groundwater and also in a stream that was experimentally enriched with P (approximately 200 μg SRP L^{-1}).
4. The breakdown rate of all three species varied among the six streams: *T. integerrima* (*k*-values range: 0.0451–0.129 day^{-1}); *C. elastica* (*k*-values range: 0.0064–0.021 day^{-1}); and *Z. 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 concentration 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 rate 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.

Keywords: Costa Rica, decomposition, ergosterol, lignin, phenolics

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.

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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 specialised 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 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 of 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, because of 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 hypothesised that P would stimulate microbial and invertebrate processing on high-quality leaves (i.e. low concentrations of struc-

tural and secondary compounds) more than on low-quality leaves.

Methods

Site description

La Selva Biological Station (10°26′N, 84°01′W), in the lowlands of Costa Rica, 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 a.s.l. 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 to 6 (Ramírez, 2001).

Because of geothermally modified groundwater inputs, streams at La Selva display a wide range of variation in solute concentrations (Pringle & Triska, 1991; Pringle, 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 SO_4 L^{-1}) and phosphorus (up to 350 μg soluble reactive phosphorus (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 SO_4 L^{-1}, <10 μg SRP L^{-1}; Pringle *et al*., 1993). Inorganic nitrogen concentrations are relatively high (typically >70 μg NO_2 + NO_3-N L^{-1}; Pringle, 1991) in both geothermal and non-geothermal streams. In all of these streams, algal assemblages are light-limited because of 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. Because of 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 ($P \leq 15$ μg SRP L^{-1}), while the other two (Sura and Arboleda) have naturally high concentrations ($P \geq 50$ μg 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 μg 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 (Ramírez *et al*., 2003; Ramírez & Pringle, 2006; C.M. Pringle, unpublished data). Phosphoric acid has been added to increase P concentrations from <10 μg to 200 μg SRP L^{-1}, which is at the high end of P concentrations exhibited by solute-rich streams (Pringle, 1991). A Mariotte (Carboy) 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 3 month study period, water samples from each site were taken every 10 days to be analysed for NO_3-N, NH_4-N and SRP. Samples were filtered (0.45 μm Millipore filters, Billerica, MA, U.S.A.) and kept frozen until analysis at the University of Georgia. We recognise that freezing water samples might be problematic for NH_4-N, but logistical constraints limit our ability to analyse samples immediately at the field site. Phosphorus was measured as SRP using the molybdenum-blue technique. Nitrate and NH_4-N were measured using the cadmium reduction and phenate methods respectively [American Public Health Association (APHA), 1998]. Temperature, pH (both with a meter: Hanna Instruments, Woonsocket, RI, U.S.A.) and flow velocity (Marsh-McBirney meter; Marsh-McBirney, Frederick, MA, U.S.A.) 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 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, hemicel-

lulose and lignin by sequential neutral detergent/acid detergent digestion on an Ankom A200 fibre analyser (ANKOM Technologies, Macedon, NY, U.S.A.; 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 1 mM 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 with 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 (22×40 cm), with a coarse mesh (5×5 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 h 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 min, 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 (Ramírez *et al*., 2003) on days 10–11 for *Trema* and days 17–18 for *Castilla* and *Zygia*. Each chamber was 12×15 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, Wolf & Mills, 1991) 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 *post hoc* 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 immobilisation 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, Cary, NC, U.S.A.).

Results

Landscape variation in P concentration and experimental P-enrichment

Stream chemical and physical characteristics varied among sites (Table 1). Streams varied greatly in SRP concentration, with Arboleda having the highest mean (186 μg SRP L^{-1}) and Taconazo the lowest (10 μg SRP L^{-1}; Table 1). The experimental P-enrichment of Carapa resulted in a mean SRP concentration of 187 μg SRP L^{-1}. Flow velocity also varied among sites, with Piper having two to three times higher mean than other sites (Table 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 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 2) and PCA provided good separation of species (Fig. 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 posi-

Table 1 Physical and chemical characteristics of the study streams

	Piper	Taconazo	Saltito 100	Sura	Arboleda	Carapa
Flow (m s^{-1}) (range)	0.22 (0.1–0.31)	0.07 (0.03–0.12)	0.13 (0.08–0.16)	0.14 (0.1–0.19)	0.13 (0.06–0.16)	0.12 (0.08–0.14)
Temperature (°C) (range)	26.5 (26.3–26.8)	26.7 (25.5–27.5)	26.5 (26.1–27)	26.3 (25.4–27)	26.6 (25.6–27.1)	26.5 (26.3–26.9)
pH (range)	4.91 (4.82–5.21)	5.02 (4.8–5.5)	5.14 (4.83–6.08)	5.86 (5.83–6.02)	6.12 (6.09–6.25)	4.46 (4.07–5.61)
Conductivity (μS) (range)	22.3 (22–23)	19.4 (17–21)	16.1 (15–17)	115.9 (105–126)	266.2 (241–300)	17.5 (14–22)
SRP (μg P L^{-1}) (range)	11 (9–14)	12 (10–21)	12 (9–19)	49 (35–74)	137 (127–159)	187 (10–289)
NO_3-N (μg L^{-1}) (range)	163 (129–189)	125 (108–139)	158 (130–176)	186 (161–211)	165 (126–193)	168 (109–198)
NH_4-N (μg L^{-1}) (range)	1 (0–3)	2 (0–3)	3 (0–7)	2 (0–8)	11 (2–26)	3 (0–16)
N : P molar ratio	34	23	29	9	3	2

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 2 Chemical parameters of eight common riparian species from La Selva Biological Station

	Condensed tannins (%DM)	Hydrolysable tannins (%DM)	Total phenolics (%DM)	Hemicellulose (%DM)	Cellulose (%DM)	Lignin (%DM)	N (%DM)	P (%DM)	C (%DM)
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 seemanii	14.48	33.22	22.31	13.09	20.95	19.37	1.74		45.36
Simiira 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	1.37		41.94

Leaves for the initial survey were collected in December of 2001. Leaves for the breakdown experiment were collected in April to 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).

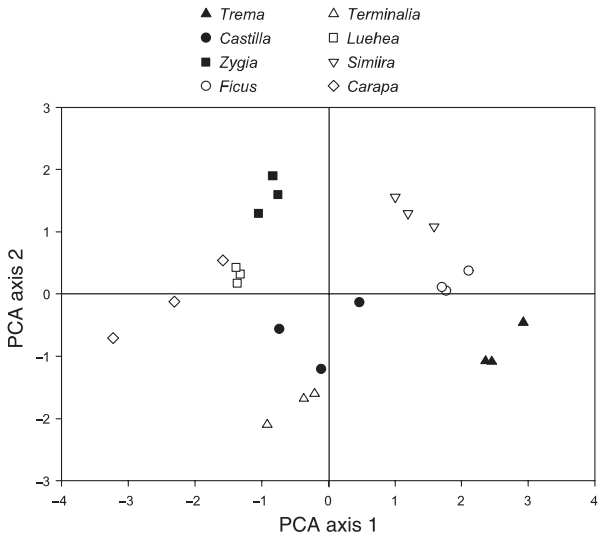

Fig. 1 Ordination of initial leaf chemical parameters for eight common riparian species in La Selva Biological Station using principal component analysis. Species selected for the breakdown experiment are in solid black.

tively correlated with lignin and cellulose (Fig. 1). We observed differences in lignin and nitrogen content between the initial leaf collection and the leaves used in the breakdown study (Table 2).

Day 0 (initial) leaf chemical parameters differed among the three species selected for the breakdown experiment (Table 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 2). *Zygia* had higher concentrations of lignin, cellulose and carbon than *Castilla* and *Trema* (lignin $F_{2,36} = 110.57$, $P < 0.0001$; cellulose $F_{2,36} = 104.64$, $P < 0.0001$; carbon $F_{2,36} = 74.01$, $P < 0.001$; Table 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 2).

Leaf breakdown rate

The three species decayed at different rates (Table 3; Fig. 2), being fastest for *Trema* ($k = 0.0401$–0.1129 day^{-1}), intermediate for *Castilla* ($k = 0.0064$–0.021 day^{-1}) and slowest for *Zygia* ($k = 0.0020$–0.008 day^{-1}; Table 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, $F_{2,9} = 8.87$, $P < 0.05$). Because of 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

Table 3 Breakdown rate (k day^{-1} ± 1 SE) of three species incubated in six streams in La Selva Biological Station

	Decay rate (day^{-1})	r^2	Days to 95% mass loss	ANCOVA
Trema				
Piper	0.129 ± 0.0166	0.75	23.2	a
Taconazo	0.0451 ± 0.0047	0.82	66.4	b
Saltito100	0.0665 ± 0.0083	0.76	45.0	b
Sura	0.0638 ± 0.0107	0.67	47.0	b
Arboleda	0.109 ± 0.0103	0.86	27.5	a
Carapa	0.117 ± 0.011	0.83	25.6	a
Castilla				
Piper	0.0119 ± 0.0058	0.45	251.7	a
Taconazo	0.0064 ± 0.0023	0.60	468.1	a
Saltito100	0.00991 ± 0.0034	0.63	302.3	a
Sura	0.0087 ± 0.0043	0.44	344.3	a
Arboleda	0.02144 ± 0.0049	0.78	139.7	b
Carapa	0.01256 ± 0.0053	0.55	238.5	a
Zygia				
Piper	0.003612 ± 0.0014	0.62	829.4	a
Taconazo	0.002085 ± 0.001	0.51	1436.8	b
Saltito100	0.005691 ± 0.001	0.89	526.4	a
Sura	0.005901 ± 0.0012	0.87	507.7	a
Arboleda	0.006755 ± 0.0016	0.82	443.5	ac
Carapa	0.008003 ± 0.0018	0.82	374.3	c

Sites are in order of increasing P concentration. Letters denote significant differences using ANCOVA. All P-values for the regression lines were <0.05.

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 (Sura) and a low-P stream (Saltito; $F_{2,9} = 14.06$, $P < 0.05$; Fig. 3). Microbial respiration on *Castilla* was significantly higher both in the P-enriched stream and in a naturally high P-stream compared with a low-P stream ($F_{2,9} = 9.12$, $P < 0.05$; Fig. 3). There was no difference in microbial respiration across these three sites on *Zygia* leaves ($F_{2,9} = 0.30$, $P = 0.3$; Fig. 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). 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). 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. 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. 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. 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. 5b).

Leaf nutrient content

The nitrogen content of *Trema* leaves increased slightly over the first three sampling dates and then declined (Fig. 6a). *Castilla* showed an overall decline in N content over time (Fig. 6b). The N content of *Zygia* remained fairly constant throughout the incubation period (Fig. 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). Phosphorus content of *Trema* increased over time in two naturally high-P streams (Fig. 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. 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 5). Microbial respiration was stimulated in the stream enriched with P on *Trema* and *Castilla*, but not on *Zygia* leaves (Fig. 3). *Trema* leaves also exhibited an increase in leaf P content throughout the breakdown process, suggesting P immobilisation (Table 4; Melillo *et al.*, 1984). In contrast, P immobili-

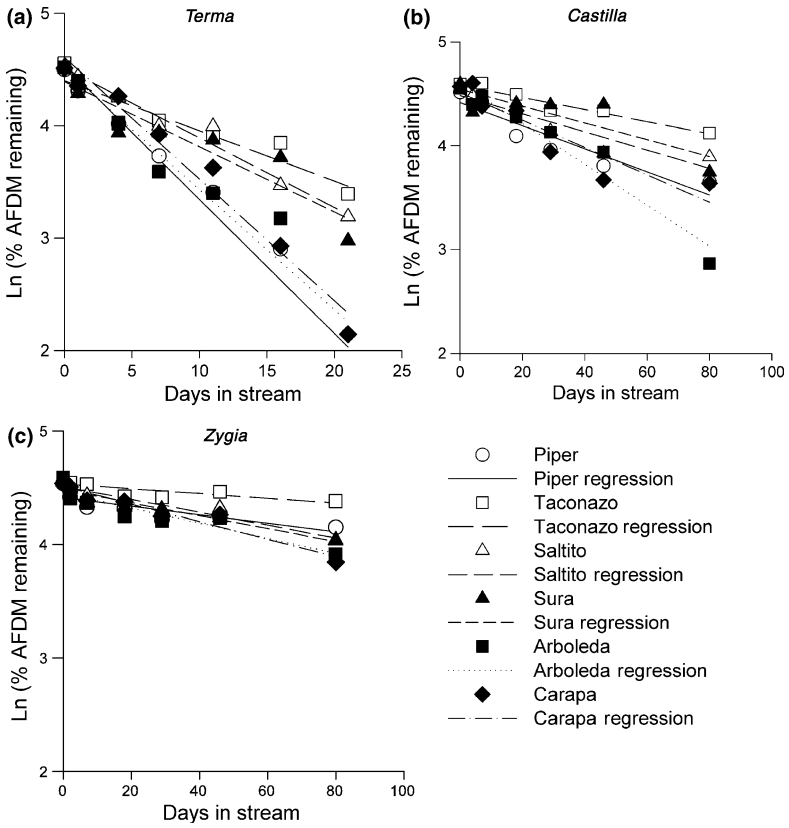

Fig. 2 Mean ln of %AFDM remaining over time for three species: (a) *Trema*, (b) *Castilla* and (c) *Zygia* in six streams in La Selva Biological Station. White symbols denote sites with low P (<15 μg SRP L^{-1}) and black symbols indicate sites with high P (>50 μg SRP L^{-1}).

sation was not observed for *Castilla* or *Zygia* (Table 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 5).

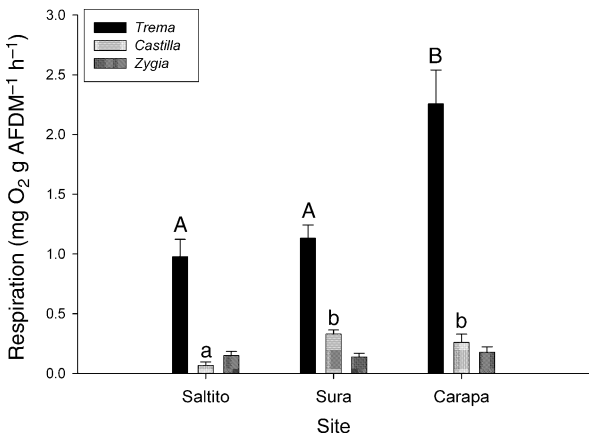

Fig. 3 Microbial respiration on three species of leaves in three sites in La Selva Biological Station. Saltito has low ambient P concentrations (>10 μg SRP L^{-1}), Sura has high ambient P (49 μg SRP L^{-1}) and Carapa is the P-enriched stream (188 μg SRP L^{-1}). Capital letters denote significant differences within *Trema* and lower case letter denote significant differences within *Castilla*, there were no significant differences within *Zygia*.

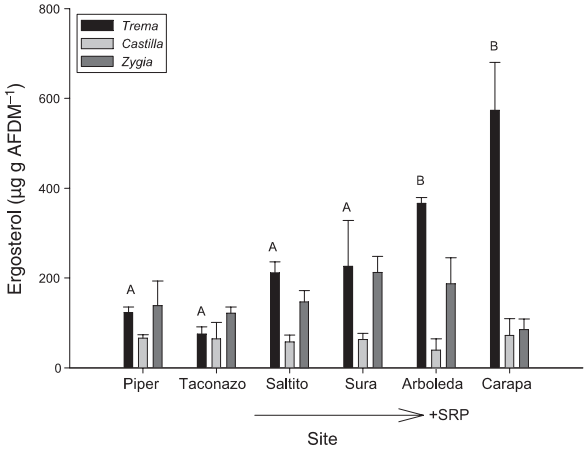

Fig. 4 Mean ergosterol concentration (±1 SE) on leaves of three riparian trees incubated in six streams in La Selva Biological Station. Samples were collected on day 11 for *Trema* and day 44 for *Castilla* and *Zygia*. Sites are arranged in order of increasing SRP. Letters denote significant differences within species using ANOVA and *post hoc* Tukey. There were no significant differences in *Castilla* or *Zygia*.

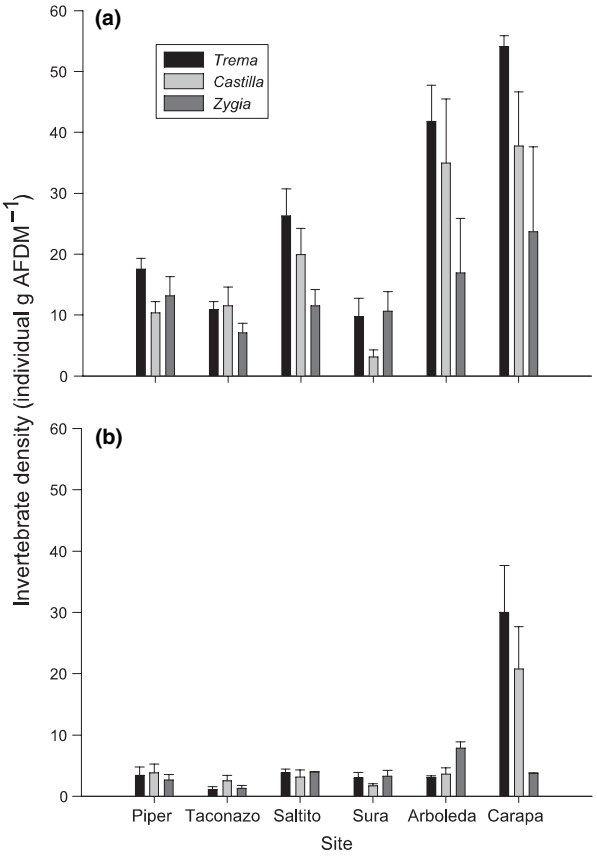

Fig. 5 Mean density (±1 SE) of: (a) chironomids and (b) non-chironomids on leaves of three riparian trees incubated in six streams in La Selva Biological Station. Invertebrates were collected on day 11 for *Trema* and day 44 for *Castilla* and *Zygia*.

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 (M. Ardón, unpublished data). As we selected our three species to represent extremes of quality from eight 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 $k = 0.005$ day^{-1}) is comparable to the average breakdown rate reported for *Rhododendron* (low quality species) in North Carolina streams (average $k = 0.0046$ 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, although 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 chironomid 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 2 & 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.

Because of 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). As 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) can slow down decomposition rates

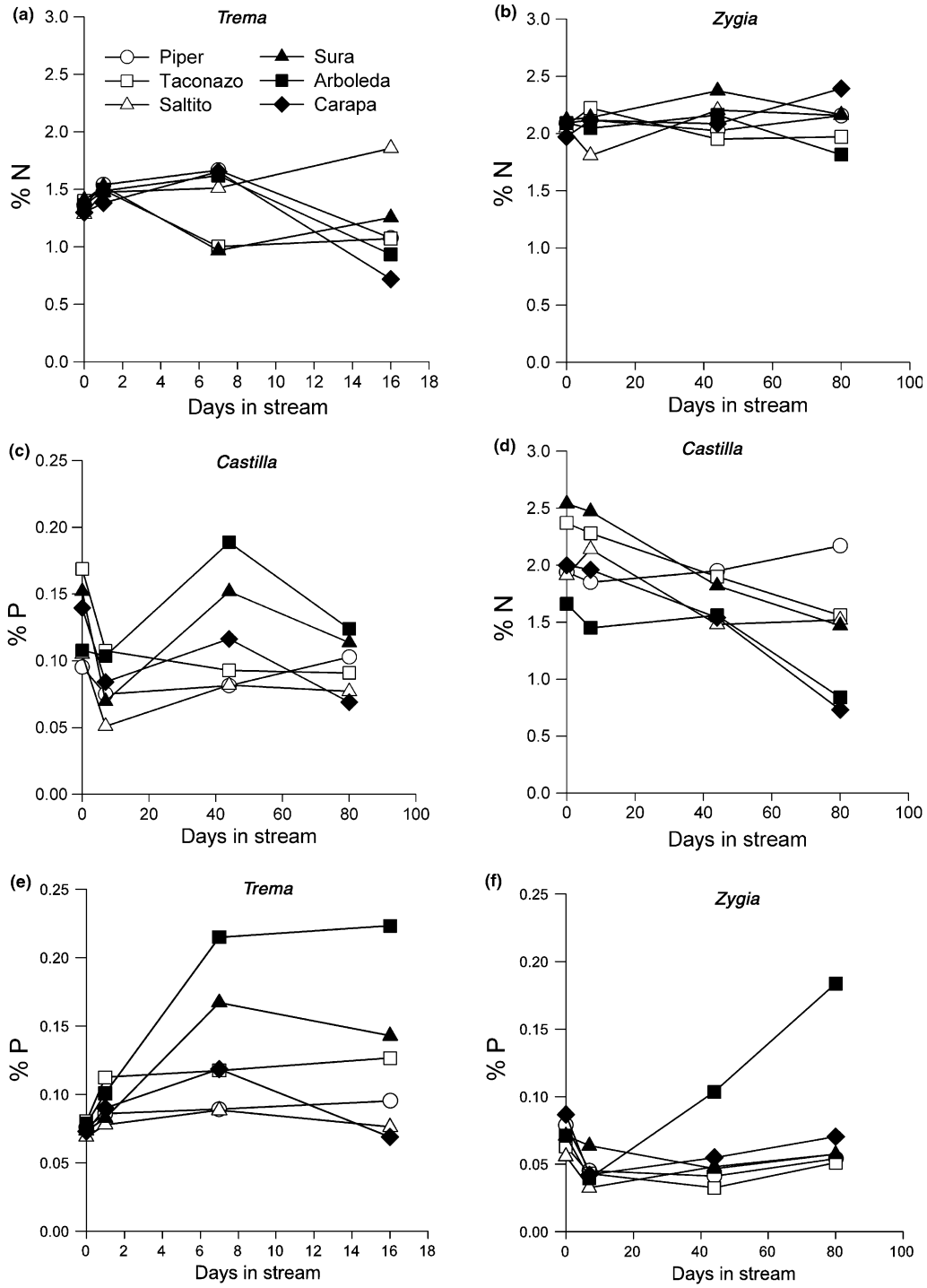

Fig. 6 Leaf nutrient content during breakdown of three species of leaves incubated in six streams in La Selva Biological Station. Percent nitrogen in: (a) *Trema*, (b) *Castilla* and (c) *Zygia* over time. Percent phosphorus in: (d)*Trema*, (e) *Castilla* and (f) *Zygia* over time. Note the different scale on the X axis for (a) & (d) *Trema*.

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

Table 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

	%N		%P	
	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**	**0.8**
Saltito	27.32	0.34	149.89	0.06
Sura	9.94	0.08	−93.2	0.0485
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

Bold indicates $P < 0.05$.

et al. (1984) reported that breakdown of alder wood (*Alnus rugosa*) (Du Roi.) Spreng (low lignin concentration 13.1% dry weight, DW) was stimulated by P enrichment, while breakdown of spruce wood (*Picea mariana* (P. Mill.); 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 (≤12%) in response to N enrichment, than leaves with high lignin concentrations (≥18%). In a North Carolina wetland, Bridgham & Richardson (2003) hypothesised that low quality (lignin ≥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 with 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 (Bridgham, Updegraff & Pastor, 1998; Vervaet *et al.*, 2002), 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.

Table 5 Relative response (slope) of breakdown rates, ergosterol, chironomid and non-chironomid density to increasing stream water P concentration in leaves of *Trema*, *Castilla* and *Zygia*. The regressions are run with and without the Piper site, which had higher flow velocity than other sites. Numbers in parentheses are the r^2-values of the regressions.

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

Bold numbers indicate $P \leq 0.05$.
w, with; w/o, without.

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). *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. 6d,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 did not predict 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 1187.93, N : P 44.1) or *Castilla* (C : P 819.33, 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 probably because of seasonal changes in leaf chemistry (Table 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, Lawrence & Clark, 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, as 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 3 & 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 O_2 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 concentration in the water (Table 2; Fig. 5b). This suggests that the 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 that of 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 with 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 a high 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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