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Does nitrogen become limiting under high-P conditions in detritus-based tropical streams?

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SUMMARY

1. We examined effects of nutrients on leaf breakdown in interior forest streams at La Selva Biological Station, Costa Rica. We tested the hypothesis that dissolved inorganic nitrogen (DIN) becomes limiting when ambient phosphorus (P) concentration is high. We also compared the breakdown of relatively 'low quality' leaves (lower C : N, *Trema integerrima*) with that of 'higher quality' leaves (higher C : N, *Ficus insipida*) in a high-P stream.
2. Litterbags were incubated in two streams: one enriched experimentally with P [target concentration 200 μg soluble reactive phosphorus (SRP) L^{-1}] and one control (naturally low P concentration approximately 10 μg SRP $L^{-1)}$. Ammonium enrichment was achieved by adding fertiliser upstream of half of the litterbags in each stream.
3. Phosphorus addition stimulated leaf breakdown, microbial respiration, ergosterol and leaf %P. Leaf breakdown rate was consistent with those in La Selva streams with naturally high P concentration.
4. Nitrogen (N) addition had no effect on leaf breakdown, microbial respiration, ergosterol or leaf chemistry in either the P-enriched or the reference stream, in spite of low N : P ratios. We conclude that N is probably not limiting in streams at La Selva that are naturally high in P. This may be due to moderately high ambient N concentration (>200 μg DIN L^{-1}) prevailing throughout the year.
5. The species with a lower C : N decomposed more rapidly and supported higher microbial activity than that with a higher C : N. Subtle differences in leaf N content, as well as dissolved P concentration, may be important in determining microbial colonisation and subsequent leaf breakdown.

Keywords: decomposition, leaf chemistry, microbial respiration, nutrient limitation, phosphorus

Introduction

Terrestrially derived organic matter constitutes an important basal resource in forested stream ecosystems (Cummins, 1974; Petersen & Cummins, 1974; Webster & Benfield, 1986; Wallace *et al*., 1997). This may be particularly true in lowland tropical rainforests, where dense year-round shading limits algal primary production (Paaby & Goldman, 1992). Breakdown of leaf litter after it enters streams is influenced by a variety of abiotic factors, including temperature (Gessner, Robinson & Ward, 1998), pH (Griffith & Perry, 1994; Suberkropp, 2001), altitude (Fabre & Chauvet, 1998) and dissolved nutrients (Elwood *et al*., 1981; Meyer & Johnson, 1983; Grattan & Suberkropp, 2001).

Dissolved phosphorus and nitrogen are often limiting to leaf breakdown in freshwater ecosystems. A number of studies have examined the effects of dissolved nutrients on leaf litter breakdown by experimentally adding nitrogen (N) and/or phosphorus (P). Several studies have demonstrated a stimulatory effect of increased nitrogen concentration on organic matter processing (e.g. Meyer & Johnson,

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1983; Robinson & Gessner, 2000). Other studies have found no effect of nutrient enrichment on the biotic variables measured, despite dissolved N : P ratios that indicate N limitation (Triska & Sedell, 1976; Newbold *et al*., 1983; Royer & Minshall, 2001).

The chemical properties of leaf litter also determine colonisation of leaves by microbes and invertebrates, and subsequent leaf breakdown. The N content of leaf litter may influence breakdown by affecting its nutritional value to microbial (Chadwick & Huryn, 2003) and invertebrate (Irons, Oswood & Bryant, 1988) consumers. Carbon : N ratio has been shown to be a good predictor of leaf breakdown rate (Webster & Benfield, 1986). An emerging body of research indicates that interactions between leaf and water chemistry are also important (Royer & Minshall, 2001; Stelzer, Heffernan & Likens, 2003; Ardón, Stallcup & Pringle, 2006). Microbes associated with decaying leaf material can obtain nutrients from the water (Kaushik & Hynes, 1971; Mulholland *et al*., 1984; Suberkropp & Chauvet, 1995; Suberkropp, 1997) as well as from the leaf litter itself (Sinsabaugh *et al*., 1993; Suberkropp, 1998). Microbes may rely on N and P derived from leaf litter, particularly when nutrient concentrations in the surrounding water are limiting (Sinsabaugh *et al*., 1993).

Streams at La Selva Biological Station, Costa Rica, present a good opportunity to study nutrient dynamics in tropical streams. Geothermal activity along the volcanic spine of Central America is a source of solutes (P, Ca, Mg, Fe, Na, Cl and SO_4) in the groundwater, which emerges at ambient temperature into lowland streams draining La Selva (Pringle & Triska, 1991; Pringle *et al*., 1993; Pringle & Triska, 2000). Geothermally modified waters account for up to 50% of discharge in some streams, while other streams receive no geothermal inputs at all (Genereux, Wood & Pringle, 2002), resulting in considerable natural variation in stream P concentration (Pringle *et al*., 1993).

There are conflicting lines of evidence as to whether N is limiting in high-P streams at La Selva Biological Station. N : P ratios across a range of streams with naturally high P concentrations are far below the Redfield ratio of 16 : 1, indicating the potential for N limitation (Redfield, Ketchom & Richards, 1963). In a study of streams exhibiting a natural range of phosphorus concentrations, Rosemond *et al.* (2002) demonstrated an asymptotic relationship between soluble reactive phosphorus (SRP) and leaf breakdown rate, fungal biomass and invertebrate biomass. This finding suggests that when dissolved P reaches a certain concentration, N may become secondarily limiting. Additions of N and P in a laboratory microcosm study accelerated leaf mass loss, with combined N and P additions having the strongest effects (Rosemond *et al*., 2002). However, there is also reason to suspect that N may not be limiting in high-P streams, as background dissolved inorganic nitrogen (DIN) concentrations are moderately high (>200 μg DIN L^{-1}) in most streams at La Selva (Pringle & Triska, 1991).

This study addresses three main questions: (i) does P-enrichment of a first-order stream simulate the rapid leaf breakdown observed in naturally P-rich streams at La Selva, which are also rich in other geothermally introduced solutes? (ii) is nitrogen secondarily limiting to the process of leaf breakdown in the P-enriched stream? and (iii) does leaf chemistry (specifically, %N and C : N) play a role in determining leaf breakdown in the P-enriched stream?

Methods

Study area

La Selva Biological Station is a 1536-ha reserve in the Caribbean lowlands of Costa Rica, at the base of the Barva volcano (10°26′N, 84°01′W). La Selva borders Braulio Carrillo National Park and is part of the last intact altitudinal gradient (35–1500 m a.s.l.) of protected land draining the Caribbean slope of Central America. The region receives approximately 4000 mm of precipitation annually and is classified as lowland tropical wet forest (Sanford *et al*., 1994). The reserve incorporates many streams, which exhibit natural variation in phosphorus (5–350 μg SRP L^{-1}) and other solutes due to inputs of geothermally modified groundwater (Pringle & Triska, 1991).

Interior forest streams draining La Selva are detritus-based; most trees are non-deciduous, resulting in continuous leaf fall throughout the year (Hartshorn, 1983). Algal production tends to be low due to heavy shading (Paaby & Goldman, 1992). The benthic insect community is dominated by Diptera (Chironomidae) and Ephemeroptera (Pringle & Ramírez, 1998).

This study was conducted in two first-order streams: a whole-stream P-enrichment experiment

(the Carapa, background concentration approximately 10 μg SRP L^{-1}; target concentration approximately 200 μg SRP L^{-1}) designed to isolate the effects of P; and a reference stream with naturally low P concentrations (the Saltito, approximately 10 μg SRP L^{-1}). Phosphorus enrichment in the Carapa began in July 1998 and was accomplished by adding a continuous drip of dilute phosphoric acid (K_2HPO_4) into the main channel (Ramírez, 2001). Regular water samples were taken at specified distances downstream to determine the extent of enrichment. Background DIN concentrations are moderately high (>200 μg DIN L^{-1}) in both streams and stream temperature (24–27 °C) tends to vary little throughout the year (Ramírez, Pringle & Molina, 2003).

Water chemistry and nitrogen enrichment

We enriched NH_4 concentrations using slow-releasing nitrogen fertiliser (31N-0K-0P; Scotts-Sierra Horticultural Products Co., Marysville, OH, U.S.A.). Nylon bags containing fertiliser were tethered immediately upstream of treatment litterbags using cable ties. Each of the streams had +N and ambient N (i.e. control) treatments, with +N treatments located downstream of control treatments in order to prevent inadvertent enrichment of control treatments. Fertiliser bags were replaced approximately every 3 days to insure continuous release throughout the duration of the experiment. Water samples were collected weekly to determine the extent of N-enrichment; however, results from these samples were inconclusive. In order to confirm that N enrichment actually occurred and to track the pattern of fertiliser release over time, an additional study was conducted in January 2006. Using a method identical to that of the original experiment, two fertiliser bags were placed in the P-enrichment stream. Unfiltered water samples were taken upstream and immediately downstream, of the fertiliser bags over a period of 3 days (two samples each at 0, 1, 2, 8, 24, 32, 48 and 72 h).

Water samples were collected monthly at both study sites from January 2001 to August 2002. These samples were collected as part of an ongoing study and the data are presented here to put our experiment in context of long-term water chemistry at the study sites. Intensive water sampling was also conducted in the P-enrichment stream from March to June 2003 (a period encompassing both dry and rainy seasons) in order to determine if N concentrations decreased downstream from the P-enrichment. All water samples were kept frozen and transported to the University of Georgia for analysis. Ascorbic acid/molybdenum blue, cadmium reduction and phenate-hypochlorite methods were used to determine SRP, NO_3 and NH_4 concentrations, respectively [American Public Health Association (APHA), 1998].

Leaf breakdown and nutrient dynamics

Leaves from two common riparian tree species were used: *Ficus insipida* Willd. (Family: Moraceae) and *Trema integerrima* (Beurl) Standl (Family: Ulmaceae), referred to hereafter as *Ficus* and *Trema*. The species were chosen for the differences in their leaf C : N ratios and total %N and similarity in secondary (condensed tannins, total phenolics and hydrolysable tannins) and structural (lignin, cellulose and hemicellulose) compounds, based on a comparison of eight common riparian species from La Selva (Ardón *et al.*, 2006). Newly fallen leaves were collected during May and June 2002, air dried at ambient temperature and stored in an air-conditioned room prior to use.

A total of 192 five-gram litter bags (96 per species) were assembled in the laboratory using plastic mesh bags with a mesh size of approximately 5 × 8 mm (Cady Industries, Pearson, GA, U.S.A.). Half of the bags were taken to each stream, where they were randomly arranged into groups of six (three per species) and fastened using cable ties. Each group was anchored to the stream bottom using a metal stake. Half of the litterbags received control treatment and half received nitrogen enrichment treatment via a nitrogen-releasing bag (described above) fastened to the stake using a cable tie.

On each of eight collection days (0, 1, 2, 4, 7, 12, 17 and 21) between 29 May and 19 June 2002, we retrieved three litter bags per species and nitrogen treatment from each stream (in total, 12 litter bags per stream per collection day). Leaves from the day 0 collection were taken back to the laboratory immediately and used to calculate handling losses and initial leaf chemistry. A net was placed downstream of each litterbag during collection to retrieve any dislodged invertebrates. Samples were placed in individual Ziploc bags for transport to the laboratory, where they were refrigerated until processing. Leaves were rinsed over a 250-μm sieve to remove insects and

debris and leaf discs were removed for analysis of ergosterol.

Leaf material was dried at 40 °C for a minimum of 24 h, weighed and ground in a Wiley Mill. A subsample of ground material was combusted at 550 °C for 1 h to determine ash-free dry mass (AFDM). Remaining leaf material was frozen and transported to the University of Georgia, where it was milled to a fine powder using a Spex Certiprep 8000-D Mixer Mill (Spex Certiprep, Meutchen, NJ, U.S.A.) Total carbon and nitrogen were analysed using a Carlo Erba NA 1500 CHN analyser (Carlo Erba, Milan, Italy). For total phosphorus analysis, leaf material was weighed into acid-washed, pre-ashed ceramic crucibles, ashed at 500 °C, digested in acid and analysed spectrophotometrically (ascorbic acid method; APHA, 1998). Leaf %C and %N were analysed for days 0, 1, 2, 4, 7 and 12; total %P was analysed for days 0, 4, 7 and 12.

Fungal biomass

Using a standard hole punch, we removed 40 discs from several leaves in each litter bag for analysis of ergosterol as a measure of fungal biomass. Five discs were dried at 40 °C, weighed and ashed for 1 h at 550 °C to determine AFDM, which was used to correct for mass loss and to calculate ergosterol per gram AFDM. The remaining 35 discs were preserved in methanol and transported to the University of Georgia. Samples were kept frozen prior to extraction and analysis. Ergosterol was extracted in high-performance liquid chromatography grade methanol by refluxing for 30 min, partitioning into pentane, drying and re-dissolving in methanol. The amount of ergosterol was determined by comparing absorbance at 282 nm to known quantities after separation with HPLC (Suberkropp & Weyers, 1996). Ergosterol was measured for days 4, 7 and 12.

Microbial respiration

Study 1. Microbial respiration on leaves was measured on days 6 and 12 in the P-enrichment stream and days 7 and 13 in the reference stream. Because of logistical constraints, respiration could not be measured in both streams on the same day. Respiration measurements were made using custom-made Plexiglas metabolism chambers equipped with pumps to maintain recirculating flow (Rapid Creek Research, Boise, ID, U.S.A.; Ramírez *et al.*, 2003). Four replicate litter bags were chosen at random, rinsed in stream water to remove invertebrates and placed inside the chamber. The chamber was then filled with stream water, sealed and covered with black plastic to inhibit photosynthesis. Respiration was measured as the change in dissolved oxygen over a period of 1 h using a YSI dissolved oxygen meter (YSI Inc., Yellow Springs, OH, U.S.A.). Two replicate litterbags were used for each species and N treatment in each stream (i.e. eight litter bags per stream per day).

Study 2. In order to further assess the potential for nitrogen limitation in the P-enrichment stream, we carried out a follow-up study of microbial respiration during July 2003. Sixteen 5-g *Ficus* litter bags were incubated for an initial period of 8 days in the Carapa: eight litter bags at a site 10 m below the site of enrichment (i.e. +P) and eight litter bags at a site upstream of the P-enrichment (i.e. control). After the initial 8-day incubation period, all litterbags were removed from the stream. Of the eight replicate litterbags from each P treatment, four litterbags were transferred to individual streamside containers (20 L) containing stream water enriched with nitrogen. Nitrogen enrichment was accomplished by adding $NaNO_3$ to the container to achieve a target concentration of 3 mg $NaNO_3$ L^{-1}. Water samples were taken to demonstrate the extent of nutrient enrichment. The other four replicates were placed in identical plastic containers without nitrogen enrichment. Water for all containers ($N = 16$) was taken from the area of the stream in which the litterbags had been incubated in order to achieve consistent P concentrations. Litterbags were incubated in the streamside containers for 8 additional days.

Following incubation in the streamside containers, we removed several leaf discs from each leaf pack for measurement of microbial respiration using small (22 mL) chambers. Leaf discs were then dried, weighed and ashed in order to estimate biomass. Smaller respiration chambers were chosen for this experiment because we have found that they exhibit less variability than the larger chambers described above (M. Ardón, unpublished data). Respiration was measured as the change in dissolved oxygen over a period of 30 min using a YSI dissolved oxygen meter (YSI Inc.).

Statistical analysis

The slopes of leaf breakdown were compared using ANCOVA (Proc GLM; SAS Version 8.0, SAS Institute, Cary, NC, U.S.A.). Changes in the nitrogen, carbon and phosphorus content of leaves during breakdown were estimated by examining the slopes of the regression 'percent AFDM remaining versus percent nutrient content' (Melillo *et al*., 1984). Ergosterol data were $\log_{10}(x + 1)$ transformed to meet assumptions of normality and compared using repeated measures ANOVA. All microbial respiration data were $\log_{10}(x + 1)$ transformed to meet assumptions of normality. Data from microbial respiration study 1 were analysed using three-way ANOVA with leaf species, P treatment and time (day 6/7 or 12/13) as model effects. Significant independent variables were examined using least squared differences Tukey HSD. Time did not have a significant effect on respiration ($P = 0.88$) and was subsequently removed from the model. Data from microbial respiration study 2 were analysed using two-way ANOVA and least squared differences Tukey HSD. Unless otherwise stated, all data analyses were performed using JMP Version 4 (SAS Institute Inc.).

Results

Water chemistry and nitrogen enrichment

Background DIN concentrations were moderately high in both study streams during the study period (≥180 μg DIN L^{-1}). Mean SRP exceeded the target concentration in the P-enrichment stream (480 μg SRP L^{-1}) and was relatively low in the reference stream (9 μg SRP L^{-1}; Table 1). Molar DIN : SRP ratios ranged from 0 to 11 in the P-enriched stream and 16–198 in the reference stream (Fig. 1). During March to June 2003, there was no difference in NO_3-N concentrations between sites upstream and 10, 50 and 100 m downstream of the P-enrichment (Fig. 2).

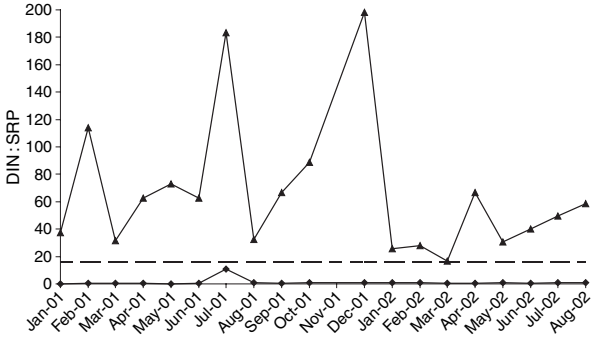

Fig. 1 Molar DIN : SRP ratios in the P-enrichment stream (diamonds) and reference stream (triangles) from January 2001 to August 2002. The Redfield ratio (16N : 1P) is shown as a dotted line.

During the study period, NH_4-N concentration was slightly higher in the +N treatments than in the controls (paired *t*-test, $P = 0.03$; Table 2). As our low water sampling frequency (i.e. once per week) did not appear to capture the full extent of N-enrichment, we took water samples at increased frequency during a follow-up study (January 2006) and subsequent analyses demonstrated consistent NH_4 enrichment. Although the extent of enrichment varied considerably (1.4–16.9 × ambient concentration), we observed an overall increase in NH_4 during the first 72 h following placement of fertiliser bags in the stream (Table 3). As fertiliser bags were changed approximately every 3 days during the experiment, nitrogen enrichment was likely continuous for the +N litter bags during the study period.

Leaf breakdown

There was no difference in the rate of leaf breakdown between +N and control treatments ($P = 0.29$–0.91), and N treatment was subsequently removed from the model. *Trema* decayed more rapidly in the P-enriched stream as compared to the reference stream ($P < 0.0001$). Breakdown of *Trema* was faster than *Ficus* across both P treatments ($P < 0.0001$; Fig. 3). Leaf breakdown was fastest for *Trema* leaves in the +P

Table 1 Water chemistry for the two study streams from January 2001 to August 2002

Stream	Mean SRP	SRP range	Mean DIN	DIN range	Mean N : P	N : P range
Carapa (+P)	855	59–2065	205	112–369	0.5	0.2–10.7
Saltito (low-P)	12	2–40	230	148–549	42.7	16.4–198.3

All nutrient concentrations are reported as μg L^{-1}. N : P represents the molar ratio of DIN (NO_3 + NO_2 + NH_4) to SRP.

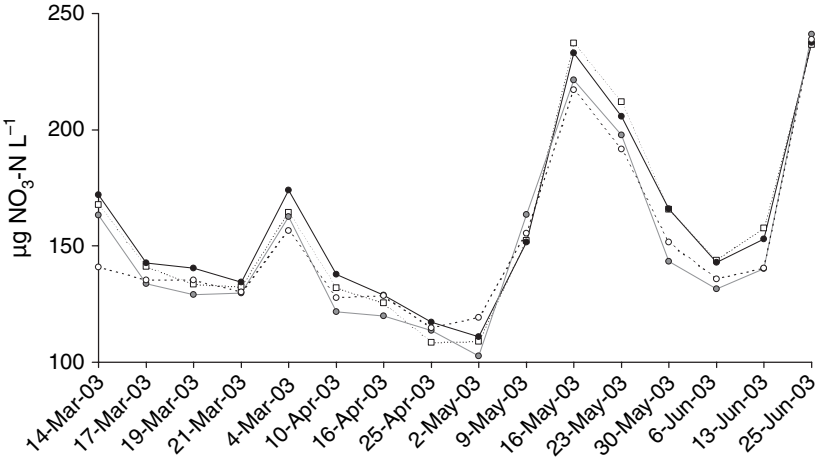

Fig. 2 NO_3-N concentrations (µg NO_3-N L^{-1}) in the P-enrichment stream from March to June 2003. Sites sampled were upstream of enrichment (squares); 10 m below enrichment (black circles); 50 m below enrichment (gray circles); 100 m below enrichment (open circles).

Table 2 Nutrient concentrations in the P-enrichment and reference streams, including results of N enrichment on NH_4 and NO_3 concentrations

	NH_4–N		NO_3–N		
Stream	Control	+N	Control	+N	SRP
Carapa (+P)	9	15*	184	179	480
Saltito (low-P)	17	23*	197	191	9

NH_4 was significantly higher in N-enriched treatments (paired *t*-test, asterisks indicate $P < 0.05$).There was no measured difference in NO_3 concentration between control and +N treatments. All nutrient concentrations are reported as µg L^{-1}.

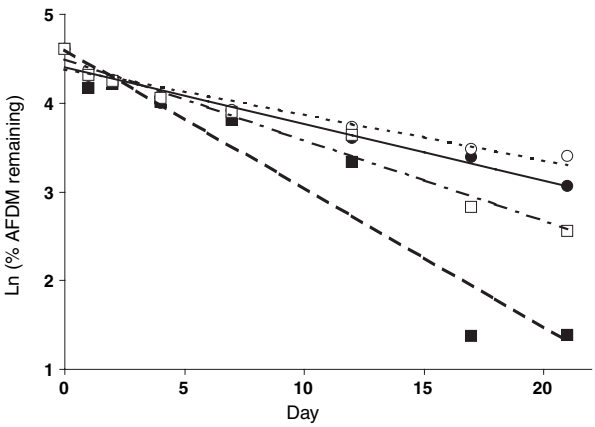

Fig. 3 Mean ln of %AFDM remaining over time. *Ficus* + P (shaded circles); *Ficus* low-P (open circles); *Trema* + P (shaded squares); *Trema* low-P (open squares). Nitrogen treatments are combined.

stream (3.99% AFDM remaining after 21 days, $k = 0.153$ day^{-1}) and slowest for *Ficus* leaves in the reference stream (30.05% AFDM remaining, $k = 0.053$ day^{-1}; Table 4).

Leaf nutrient dynamics

There was no effect of N enrichment on %N, %C or C : N of either species. Data from N-enriched and control treatments were therefore pooled for further analysis. Total %N increased markedly for both species and sites immediately following incubation in the stream, then steadily declined. We observed no trend in the relationship between percent AFDM remaining and leaf %N with respect to leaf species or P treatment (Table 5). *Trema* always maintained higher total %N than *Ficus* (Fig. 4a).

Leaf %C decreased with respect to percent AFDM remaining in all instances (Table 5). *Ficus* maintained

Table 3 Nitrogen (NH_4) enrichment over time following placement of nitrogen fertiliser bags in Carapa (January 2006)

	Hour (following placement of fertiliser in stream)							
	0	1	2	8	24	32	48	72
Control (µg NH_4 L^{-1})	183	128	9	14	11	72	30	31
Nitrogen (µg NH_4 L^{-1})	580	298	118	61	186	92	223	44
NH_4 enrichment	3.2×	2.3×	13.1×	4.4×	16.9×	1.3×	7.4×	1.4×

NH_4 enrichment is expressed as level above ambient concentration.

Table 4 Breakdown rates and percent AFDM remaining for leaves in P-enrichment and reference streams

Leaf and P treatment	Breakdown rate (k day^{-1})	r^2	% AFDM remaining (day 21)	Days to 95% mass lost	ANCOVA
Ficus (+P)	0.066	0.88	21.30	45.4	a
Ficus (low-P)	0.053	0.86	30.05	56.8	a
Trema (+P)	0.153	0.89	3.99	19.5	b
Trema (low-P)	0.098	0.84	12.90	30.7	c

All P-values for regressions are <0.0001. Letters indicate significant ($P < 0.001$) differences between breakdown rates (k) using ANCOVA.

Table 5 Slopes and correlation coefficients of the inverse linear function relating ash-free dry mass (AFDM) remaining and percent nitrogen, carbon and phosphorus of leaf litter

	%N		%C		C : N		%P	
	slope	r^2	slope	r^2	slope	r^2	slope	r^2
Ficus (+P)	27.72	0.05	2.65*	0.20*	1.48*	0.02*	−214.55*	0.34*
Ficus (low-P)	17.47	0.02	3.16*	0.16*	0.26*	<0.01*	−81.88*	0.06*
Trema (+P)	−3.46	<0.01	2.66*	0.42*	7.13*	0.80*	−187.53*	0.24*
Trema (low-P)	8.90	0.01	2.70*	0.40*	6.30*	0.58*	100.41*	0.02*

*$P < 0.05$.

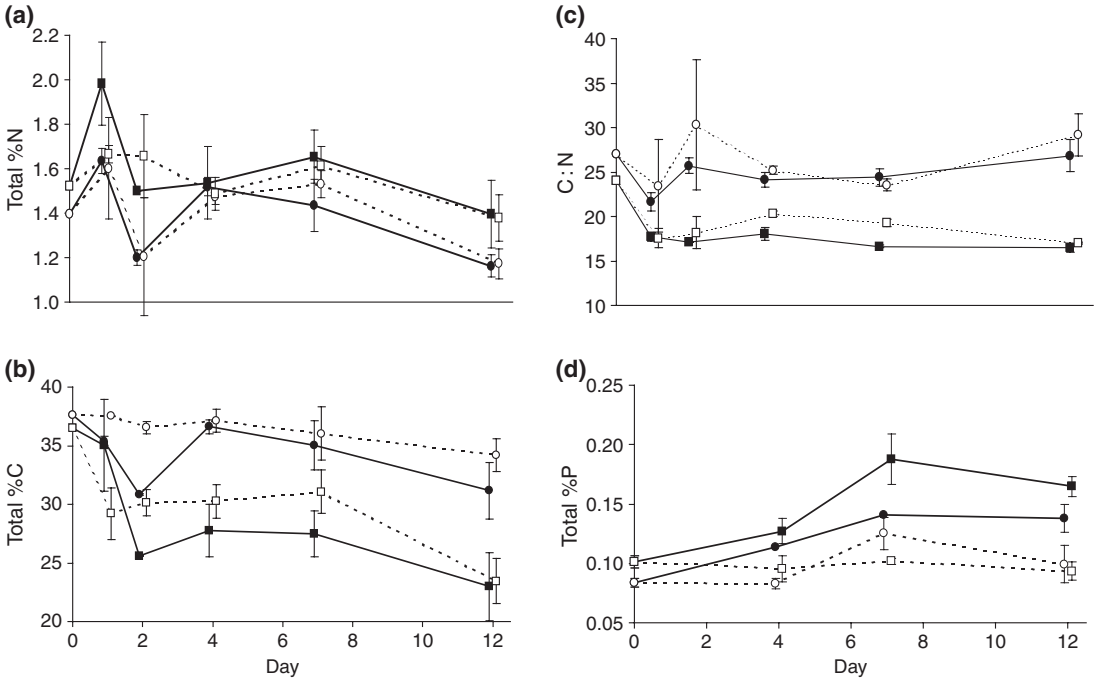

Fig. 4 Leaf litter (a) total %N, (b) total %C, (c) C : N ratio and (d) total %P *Ficus* + P (shaded circles); *Ficus* low-P (open circles); *Trema* + P (shaded squares); *Trema* low-P (open squares). Nitrogen treatments are combined. Bars are ±1 SE.

higher total %C than *Trema* in both the P-enriched and reference stream (Fig. 4b). There was a negative relationship between *Trema* percent AFDM remaining and C : N ratio; we observed no such relationship for *Ficus* leaves (Table 5). Consistent with our initial predictions, *Ficus* C : N was always higher than *Trema* C : N (Fig. 4c).

We observed a positive relationship between percent AFDM remaining and total %P for both *Ficus* and *Trema* in the P-enriched stream, but not in the low-P stream (Table 5). Furthermore, *Trema* leaves had

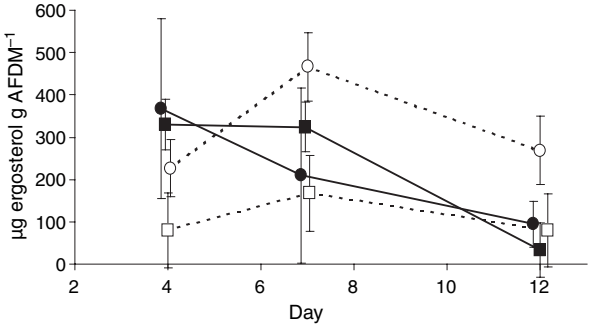

Fig. 5 Ergosterol. *Ficus* + P (shaded circles); *Ficus* low-P (open circles); *Trema* + P (shaded squares); *Trema* low-P (open squares). Nitrogen treatments are combined. Bars are ±1 SE.

higher %P than *Ficus* across both P treatments throughout the study (Fig. 4d).

Fungal biomass

Repeated measures ANOVA revealed no effect of P treatment or leaf species on ergosterol ($P = 0.37$ and 0.29, respectively). We also observed no interaction between stream P concentration and leaf species ($P = 0.07$; Fig. 5), probably due to the large amount of variability among replicates. Visual inspection of the pattern of ergosterol accrual suggests that fungal biomass peaked earlier in the P-enriched stream than in the reference stream, indicating more rapid fungal colonisation of leaves in the P-enriched stream. From the existing data (days 4, 7 and 12), we are able to reasonably conclude that fungal biomass peaked sometime prior to day 7 in the P-enriched stream, and between days 4 and 12 in the reference stream.

Microbial respiration

Using whole litter bags and large respiration chambers (study 1), we found no difference in microbial respiration between days 6/7 and days 12/13 for either leaf species or P treatment ($P = 0.88$). This indicated fairly stable microbial activity on litterbags during the first 2 weeks of incubation. There was no effect of N-enrichment on microbial respiration ($P = 0.79$). We observed significant effects of P concentration ($P = 0.02$) and leaf species ($P < 0.0001$) on microbial respiration. Respiration was highest on *Trema* leaves in the P-enriched stream and lowest on *Ficus* leaves in the reference stream (Fig. 6a).

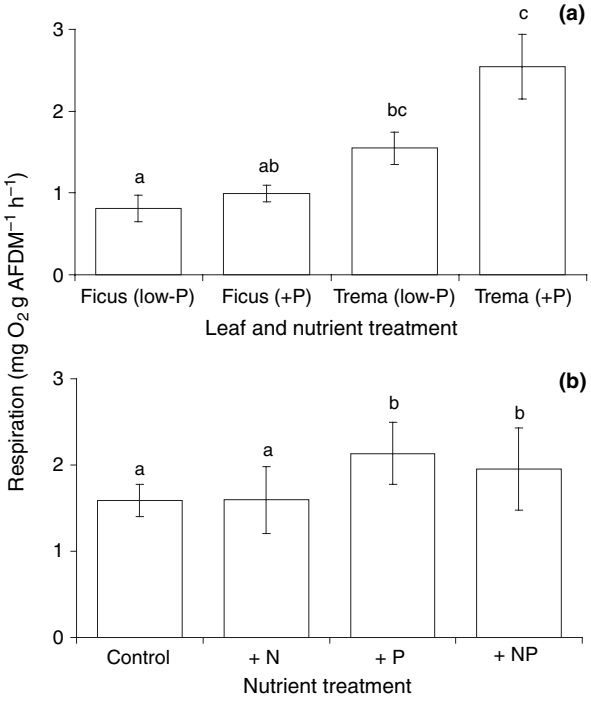

Fig. 6 Microbial respiration on leaves during (a) study 1 and (b) study 2, follow-up study conducted in July 2004. Nitrogen treatments are combined. Bars are ±1 SE.

Table 6 Water chemistry from microbial respiration study 2 (streamside chambers)

	Nutrient treatment			
	C	N	P	NP
NH_4 (µg L^{-1})	21	54	50	69
NH_4 enrichment	2.6×		1.4×	
NO_3 (µg L^{-1})	252	2895	210	2395
NO_3 enrichment	11.5×		11.4×	

Enrichment is expressed as level above ambient concentration. C, control; N, nitrogen added; P, phosphorus added; NP, nitrogen and phosphorus added.

In study 2 (using leaf discs and smaller respiration chambers), we achieved NH_4 enrichment similar to that in the *in situ* fertiliser addition; NO_3 enrichment was far greater (approximately 11.5× above ambient concentration; Table 6). We observed a positive effect of P-enrichment on microbial respiration on *Ficus* leaves ($P = 0.01$). There was no effect of N enrichment on microbial respiration in the follow-up study ($P = 0.051$, *post hoc* Tukey test of least squared means not significant) and no interaction between P- and N- treatments ($P = 0.08$, *post hoc* Tukey test of least squared means not significant; Fig. 6b).

Discussion

P-enrichment stimulates leaf breakdown processes

The whole-stream P-enrichment allowed us to isolate experimentally the effect of the P enrichment from that of the other solutes (Ca, Mg, Fe, Na, Cl and SO_4) that are also present in streams with naturally high P concentrations at La Selva. Phosphorus addition directly stimulated leaf breakdown, microbial respiration and leaf total %P. Differences in leaf breakdown between the P-enriched and reference streams were consistent with results from studies in streams with a range of P concentrations (Ramírez, 2001; Rosemond *et al*., 2002). For example, breakdown of *Trema* leaves in a naturally high-P (137 μg SRP L^{-1}) stream was 2.04 times faster than rates observed in a low-P (12 μg SRP L^{-1}) stream (Ardón *et al*., 2006). Here we found a 1.56-fold greater rate of breakdown of *Trema* leaves in the P-enriched stream than in the reference stream.

We found direct evidence that P-enrichment leads to an increase in leaf total %P, which provides additional evidence that the microbial community responded positively to the whole-stream P-enrichment in the Carapa. Ramírez *et al.* (2003) demonstrated increased microbial respiration on *Ficus* leaves in the P-enriched stream; here we show that the trends observed for *Ficus* can be extended to other high-quality (i.e. fast-decomposing) leaf species such as *Trema*.

These findings suggest increased microbial uptake and immobilisation of dissolved phosphorus (Table 5). Studies in temperate streams have demonstrated increased leaf %P in high-nutrient streams (Molinero, Pozo & Gonzalez, 1996). Moreover, shifts in leaf %P are sometimes accompanied by concomitant changes in C : N : P ratios of invertebrate consumers, indicating that the effects of increased dissolved phosphorus can span multiple trophic levels and may result in shifts in elemental composition of invertebrate consumers (Cross *et al*., 2003). Whether high P concentration in lowland tropical streams affects consumer-resource stoichiometry warrants further investigation.

Because of the rapid leaf breakdown observed (particularly in the P-enriched stream), future studies examining leaf breakdown in the tropics should examine the microbial community (i.e. fungal biomass, microbial respiration and leaf chemistry) within 1–2 days after placing leaves in the stream. Studies tracking the accumulation of fungal biomass have demonstrated that, following an initial lag, ergosterol typically increases to a peak and then declines (e.g. Sridhar & Barlocher, 2000). Our first measurement of ergosterol on day 4 in the high-P stream may have missed a peak in fungal biomass accrual (Fig. 5), in which case the microbial response might have been even more rapid than we expected. Decay rates for *Ficus* fell within the range reported by Rosemond *et al.* (2002) and both species decayed rapidly and would be classified as 'fast' ($k > 0.01$ day^{-1}; Petersen & Cummins, 1974). However, both *Ficus* and *Trema* leaves decayed far more rapidly than fast-decomposing species from the temperate zone. For example, in studies of leaf breakdown in North America, breakdown of yellow poplar (*Liriodendron tulipifera* L.) leaves is considered to be rapid. In nitrate-rich, hardwater streams, yellow poplar can be expected to lose 95% mass within 88 days (based on a *k*-rate of 0.0342 day^{-1}; Suberkropp & Chauvet, 1995). In the present study, *Trema* leaves in the P-enrichment stream lost 95% mass after just 19 days and even the slowest-decomposing species (*Ficus* in the low-P stream) would have lost 95% mass in 58 days. This may be due in part to the effects of water temperature. Regardless, future researchers should consider measuring microbial respiration and fungal biomass on leaf litter as soon as day 1, in order to capture the full extent of the initial microbial response.

No evidence of nitrogen limitation in a high-P stream

We found no effect of localised nitrogen addition on any of the variables measured in the experimentally P-enriched stream. This was also true for the microbial respiration study using streamside chambers and more controlled nutrient additions. As measurements of nitrogen release from fertiliser bags and in streamside chambers indicated that our enrichment technique was successful in both studies, yet we observed no effects of this enrichment, we conclude that nitrogen is not limiting to microbial activity or leaf breakdown in this P-enriched tropical stream.

Furthermore, if N were limiting in the P-enriched reach of the stream, we would expect uptake of dissolved NO_3 to increase (and dissolved NO_3 concentrations to decrease) with respect to P concentration. However, we observed no difference in dissolved NO_3 concentration with increasing down-

stream distance from the site of P-enrichment (0, 10, 50 and 100 m; Fig. 2). While N : P ratios (Redfield *et al.*, 1963) and laboratory microcosm studies (Rosemond *et al.*, 2002) indicated the potential for nitrogen limitation in high-P streams, background DIN concentrations in both streams were relatively high. It has been suggested that the ideal N : P ratio for leaf breakdown in freshwater systems may be as low as 4 : 1 (Triska & Sedell, 1976); even so, N : P ratios in the P-enrichment stream fall below this threshold. It is likely that with moderate to high ambient nitrogen concentrations, a factor other than nitrogen may be limiting to leaf breakdown processes. Based on our observation that P-enrichment leads to accelerated leaf breakdown similar to that observed in streams exhibiting naturally high P concentrations (e.g. Rosemond *et al.*, 2002), it is possible that N is not limiting to leaf breakdown in other high P streams at La Selva.

The possibility that N may not be limiting (in spite of N : P concentrations indicating the strong potential for N limitation) is supported by results of other investigations. Triska & Sedell (1976) observed no effect of nitrogen addition on leaf breakdown, although N : P ratios in the control stream suggested N limitation. The lack of response to N-addition was similarly attributed to high ambient N concentrations. In another study, addition of NH_4 to Walker Branch (Tennessee, U.S.A.) did not stimulate leaf breakdown despite low (<10 μg NH_4-N L^{-1}) background concentrations (Newbold *et al.*, 1983). Royer & Minshall (2001) found that added nutrients (N + P) did not stimulate leaf breakdown in a stream with moderately high nutrient concentrations, despite N : P ratios suggesting N limitation. They hypothesised that, when background nutrient concentrations are high, other factors (such as leaf quality) may be more important in determining leaf breakdown rates. It appears that this may also be the case in P-rich streams at La Selva, where we have found that leaf quality can limit the magnitude of P stimulation of leaf breakdown (Ardón *et al.*, 2006).

The role of nitrogen content of leaf litter

Subtle differences in leaf chemistry (%N, C : N) between *Ficus* and *Trema* may be important to microbial consumers. We found that *Trema* leaves (i.e. lower C : N) decomposed more rapidly, had higher %P and supported higher microbial respiration than *Ficus* (i.e. higher C : N) leaves. Higher leaf %P resulting from immobilisation of nutrients by microbes supports the hypothesis that *Trema* leaves, with proportionally more nitrogen, are more accessible to microbes. Increased microbial activity may also render leaves more palatable to invertebrates, with cascading effects on higher trophic levels.

Factors other than %N and C : N also affect leaf breakdown rates and recent research indicates that leaf breakdown in high-P streams is mediated by structural compounds within leaf litter (Ardón *et al.*, 2006). For example, lignin appears to be a particularly good predictor of leaf breakdown in terrestrial ecosystems (Aber & Melillo, 1982; Melillo, Aber & Muratore, 1982). While *Ficus* and *Trema* were chosen for their similarity with respect to structural and secondary compounds, further exploration using species with a range of leaf C : N would allow us to determine whether the effects observed in our study can be explained by C : N ratio.

Microbial response to nutrients in leaf litter can be mediated by water chemistry (Molinero *et al.*, 1996) and the importance of leaf nutrients may increase when background nutrients are at limiting levels. It is interesting that we observed no limitation in terms of dissolved nitrogen and yet we did observe a positive microbial response to leaves with a higher intrinsic nitrogen content (i.e. lower C : N). A similar trend was demonstrated in a whole-catchment N-addition experiment carried out in Maine, U.S.A. (Chadwick & Huryn, 2003), where dissolved nitrate concentrations, although substantially higher in the N-enriched catchment, did not affect leaf breakdown. However, leaves collected from trees in the N-enriched catchment had higher %N and decomposed more rapidly than leaves of the same species collected from the reference catchment. Hence, leaf litter chemistry may be an important determinant of leaf breakdown rate and microbial colonisation, even when dissolved nutrients are not limiting. In the present study, we observed a response to P-addition and leaf nutrient concentrations, indicating that microbes in lowland tropical streams may obtain nutrients from both water and substrate.

In conclusion, P enrichment of a forested tropical stream stimulated leaf breakdown, microbial respiration and leaf total %P. Breakdown rates observed for the two study species were similar to rates observed in streams exhibiting naturally high levels of SRP. There

was no evidence of N limitation in the P-enriched stream, suggesting that N is probably not limiting in naturally high-P streams throughout La Selva. Subtle differences in leaf nitrogen content appeared to be important in determining leaf breakdown, indicating that nutrients derived from leaf litter may influence leaf breakdown even when background nutrient concentrations are relatively high. Further study of the effects of extrinsic (dissolved) and intrinsic (leaf litter) nutrients on leaf breakdown processes is important if we are to understand the implications of increasing nutrient loading in tropical streams.

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