Nitrogen versus phosphorus demand in a detritus-based headwater stream: what drives microbial to ecosystem response?

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Introduction

An irrefutable aspect of global change is the increased mobilization of nutrients (Schlesinger 2004), which potentially affects many trophic pathways in aquatic and terrestrial ecosystems. Previous work on the effects of nutrients has focused largely on primary-producer−based pathways (Elser et al. 2007). However, detrital pathways may be even more sensitive to nutrients because detrital food resources are typically more nutrient poor than those derived from living plants (Cebrian & Lartigue 2004).

The primary ways that nutrient availability affects detrital food webs are (1) changes in cellular nutrient content of basal resources and (2) consequent stimulation of biomass, growth rates, and production of heterotrophic consumers. We observed evidence for both of these effects in a long-term nitrogen (N) and phosphorus (P) enrichment of a detritus-based headwater stream. Specifically, we observed increased N (Cross et al. 2003, Gulis et al. 2004, Greenwood et al. 2007) and P content (Cross et al. 2003) of allochthonous leaves, wood, fine particulate organic matter (FPOM, fine particulate organic matter) and associated microorganisms, as well as epilithon. We also observed changes in the biomass, growth rates, and production of organisms that spanned levels of organization from microorganisms to metazoans. These changes included increased biomass (Gulis & Suberkropp 2003) and production (Suberkropp, unpublished data) of fungi and bacteria; increased growth rates and biomass of epilithon (Greenwood & Rosemond 2005); increased growth rates, biomass and production of invertebrates (Cross et al. 2005, Cross et al. 2006); and increased growth rates of salamanders (Johnson et al. 2006).

Although we found significant changes in nutrient content of resources and production of organisms, our study design did not allow us to examine separately the effects of N versus P loading. We used 2 lines of evidence from our study to evaluate the potential relative importance of N versus P in driving the changes we observed in multiple resources and consumers. First, dissolved inorganic nutrient concentrations in stream water were used to determine ambient N:P molar ratios (to suggest potential N vs. P limitation, but see Dodds 2003). We also compared predicted N:P ratios (based on background stream water concentrations plus our added enrichment) versus observed N:P in our treatment stream. Dissolved N:P ratios that differ from predicted in either direction would suggest potential selective uptake of N or P by biota. Second, we determined the magnitude of the relative response of C:N, C:P, and N:P of basal resources to enrichment, as well as the trends in the changes in these ratios over 2 years of enrichment.

Key words: co-limitation, detritus-based food web, nitrogen, nutrient demand**,** nutrient enrichment, phosphorus, stream

Study site

This study was conducted in 2 first-order streams at the Coweeta Long Term Ecological Research site, Macon County, N.C., USA, draining catchments 53 (reference) and 54 (treatment). The streams are similar in physical characteristics and are located in heavily forested watersheds on the same south-facing slope. Allochthonous detritus provides >90% of the energy base for invertebrate production in these streams (Wallace et al. 1997, Hall et al. 2000, Cross et al. 2007). A more complete description of these streams can be found in Greenwood & Rosemond (2005).

Methods

Stream water samples. We used a paired watershed approach to examine the effects of 5 years of continuous enrichment with NH_4NO_3, KH_2PO_4, and K_2HPO_4 along a 145-m reach of stream (see detailed methods in Greenwood & Rosemond 2005). Samples were taken for dissolved nutrient analyses (NH_4-N, NO_3-N, and soluble reactive phosphorus (SRP)) for 1 year of pretreatment (July 1999–July 2000) and 5 years during nutrient enrichment (July 2000−July 2005) every 2 weeks from both the reference and treatment stream. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4-N and NO_3-N. Samples were filtered in the field using a 0.45-μm nitrocellulose

membrane filter and then frozen until analyzed on an Alpkem autoanalyzer at the Analytical Chemistry Laboratory, Odum School of Ecology, Athens, GA. When replicate samples were taken, N:P ratios were based on averaged nutrient concentrations from that date. Elevated data points in the nitrate and SRP dataset, likely due to sample contamination, were omitted if values exceeded one standard deviation above the mean of each nutrient (resulting in omission of 6 data points in each case). Ammonium concentrations were much more variable, and therefore, no outliers were removed. Elevated concentrations in the treatment stream may have resulted from occasionally high solute delivery through the irrigation system, and these data were also retained. We determined molar N:P ratios as DIN:SRP in the reference stream and determined predicted N:P ratios in our treatment stream by summing target concentrations from our added nutrient solution (500 μg/L DIN and 100 μg/L SRP) with upstream reference stream water concentrations. Actual N:P ratios found in our treatment stream were then compared to predicted. We also ran linear regressions through time for the reference and treatment streams to determine whether there were consistent temporal changes in N:P ratios.

   Nutrient content of basal resources. Nutrient content of basal resources was determined as described in Cross et al. (2003), Gulis et al. (2004) and Greenwood et al. (2007). Most samples were collected throughout a 3 year period (pretreatment and the first 2 years of enrichment). Here, we contrasted the percentage change observed in C:N and C:P of various resources. For samples taken over multiple years, we determined whether the magnitude of change in nutrient content of resources differed through time.

Results

Stream water samples. We found that reference stream water N:P ratios were widely variable, but averaged 29.3 (n = 98) for the 6-year study period, suggesting potentially greater P than N limitation under ambient conditions. We found that there was a weak but significant positive increase in N:P through time (Fig. 1), consistent with slightly higher nitrate concentrations over the last 2 years of the study (unpublished data). In contrast, the treatment stream had N:P ratios that were lower than the reference and more consistent through time, due to the added nutrients dominating nutrient concentrations (Fig. 2). Ratios of N:P averaged 15.1 (n = 115) over the treatment period, which was higher than predicted values based on our added nutrients (overall mean = 11.1, n = 91) (Fig. 2), suggesting relatively greater demand for P versus N. There was a small but significant decline in N:P in the treatment stream over time, suggesting a trend toward less disproportionate uptake of P, increased P use efficiency, or saturation of abiotic uptake of P through the enrichment period (Fig. 2).
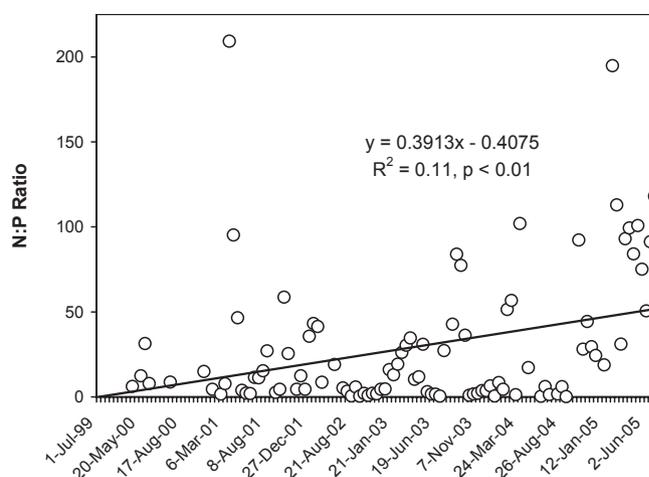
Fig. 1. Dissolved streamwater molar N:P ratios (DIN:SRP) from the reference stream. There was a weak but significant increase in the N:P ratio in stream water over time, indicating relatively greater N vs. P availability through the study period.
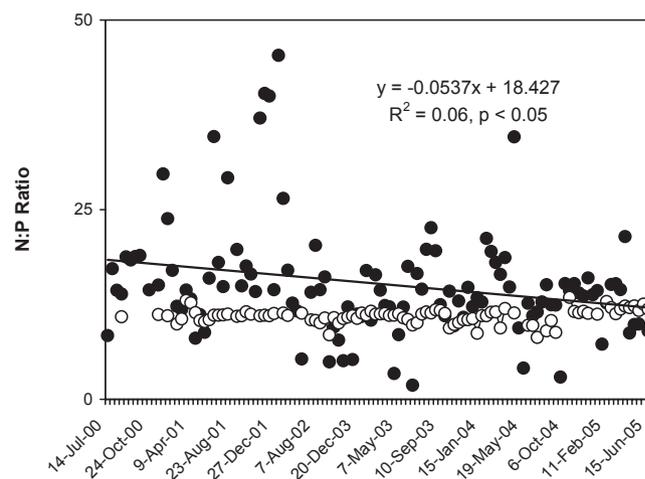
Fig. 2. Dissolved streamwater molar N:P ratios from the treatment stream both measured (solid symbols) and predicted (open symbols) based on the baseline nutrient concentrations (upstream in treatment) and experimentally added concentrations. Data are shown for the period of enrichment, which began 11 July 2000. Measured streamwater ratios that were greater than those predicted indicate relatively greater demand for P vs N. There was a weak but significant decline in the measured N:P ratio in our treatment stream, suggesting less preferential uptake of P through time.

Nutrient content of basal resources. When basal resources (leaves, FPOM, epilithon) were compared for relative change in C:N or C:P, there was a relatively greater reduction in C:P, as indicated by reductions in N:

Table 1. Basal resources collected in the reference stream (Ref) and treatment stream (Treat) with the percentage differences (% Diff) between streams calculated for each parameter (from CROSS et al. (2003)). Leaves represent bulk collections from the stream, FPOM (fine particulate organic matter) was collected from sediment cores, and epilithon was determined from colonized ceramic tiles.

		Ref	Treat	% Diff
Leaves	C:P	4858	3063	–37%
	C:N	73	82	+12%
	N:P	67	39	–42%
FPOM	C:P	1015	673	–34%
	C:N	34	29	–15%
	N:P	28	23	–18%
Epilithon	C:P	1741	845	–51%
	C:N	8.7	7.6	–13%
	N:P	201	110	–45%

Table 2. Nutrient ratios of basal resources for the pretreatment year (1999–2000) (PRE), the first year of enrichment (2000–2001) (YR1) and the second year of enrichment (2001–2002) (YR2). Data on white oak (*Quercus alba* L.) wood veneers and red maple (*Acer rubrum* L.) sticks are from GULIS et al. (2004) based on annual averages, data on leaves of *Rhododendron maximum* L. ('Leaves Rhodo') and *Acer rubrum* L. ('Leaves Acer') from GREENWOOD et al. (2007) based on maximum differences, and data on C:N, C:P, and N:P of bulk leaf litter from CROSS et al. (2003). Leaf litter values are those presented in Table 1, but in this case separated by year to compare to the temporal response of other basal resources. Ref, Treat, and % Diff are defined as in Table 1.

		Ref	Treat	% Diff
A. C:N				
Wood veneers	PRE	256.6	207.2	–19%
	YR1	237.9	57.9	–76%
	YR2	292.4	53.3	–82%
Sticks	PRE	283.6	259.9	–8%
	YR1	265.1	234.8	–11%
	YR2	415.3	120.2	–71%
Leaves Rhodo	PRE	81.4	64.2	–21%
	YR1	84.5	49.5	–41%
	YR2	130.6	52.9	–59%
Leaves Acer	PRE	53.3	60.6	+14%
	YR1	76.8	42.3	–45%
	YR2	80.1	42.0	–47%
B. Bulk leaf litter				
C:N	PRE	69.2	75.3	+9%
	YR1	72.0	81.9	+14%
	YR2	79.3	74.5	–6%
C:P	PRE	5263.5	6088.3	+16%
	YR1	4859.9	3380.1	–30%
	YR2	4454.6	2921.2	–34%
N:P	PRE	76.7	83.9	+9%
	YR1	67.2	42.0	–37%
	YR2	56.7	39.2	–31%

P (Table 1). Bulk samples of leaves showed no reduction in C:N in the treatment stream relative to the reference (Table 1), but showed a 37% decline between the 2 streams in C:P (however C:N of single species leaf litter was reduced with enrichment, and bulk samples became more reduced with time, Table 2). The greatest change observed in C:P was for epilithon (compared to FPOM and leaves), but these results should be viewed with caution, as they were determined from a single sampling period.

The degree of change in basal resource nutrient content varied slightly over time. We observed similar responses in C:N content of detrital substrates in years 1 and 2 of the experiment, which indicated reduced C:N overall, but slightly greater reductions in C:N of wood and leaves and a large reduction in C:N of sticks in year 2 compared to year 1 (Table 2A). Differences in the pretreatment year were assumed to be due to natural variation. Likewise, in bulk collections of leaf litter, C:N and C:P were reduced more in year 2 than year 1, suggesting that nutrient content of basal resources was not saturated after 1 year of enrichment (Table 2B).

Discussion

Our results showed that both N and P were likely limiting to stream heterotrophs, for which we observed increased immobilization of both N and P when added at elevated concentrations to our treatment stream. Likewise, previous work in headwater streams in southern Appalachian indicate a relatively high demand for N and P, which limit biological processes. Higher leaf decomposition rates were linked to higher nitrate concentrations (MEYER & JOHNSON 1983) and both N and P limited microbial activity and biomass on wood (but fungal biomass did not respond to N or P alone; TANK & WEBSTER 1998). Demand for P (as uptake rates) appears to be greater than nitrate-N in these streams (MUNN & MEYER 1990), but uptake rates of ammonium-N were greater than rates for P, which were both rapid (WEBSTER et al. 2000). Although P limitation in our system possibly stimulated N immobilization or vice versa, either would likely have become limiting if not supplied in our experiment. A recent meta-analysis of N and P limitation across freshwater, marine and terrestrial systems found that simultaneous N + P additions yielded strong synergistic responses, above those found with either N or P addition alone (ELSER et al. 2007). Results from heterotrophic-based systems cited above are

consistent with those of ELSER et al. (2007), which illustrate the tight coupling between N and P and the high demand for both nutrients by biota in these systems.

Multiple lines of evidence of our study, however, suggest greater P than N limitation. First, the ambient N:P ratios of the stream water in the reference stream suggested possible P limitation (averaging 29.3). Similarly, the mean N:P of stream water from these 2 streams taken over 1985–1990 was 29.0 (n = 74; J.B. Wallace & S.L. Eggert, unpublished data). However, the median values were only 11.8 and 13.6, respectively, for the current study and the 1985–1990 study, and as recently pointed out by DODDS (2003), there are many problems with the use of ratios of dissolved inorganic N and P to predict nutrient deficiencies. More robust evidence of greater P than N limitation comes from the comparison of N:P ratios measured in the treatment stream and those predicted based on our added nutrients. The relatively higher measured values suggest that there was preferential uptake of P relative to N by biota early in the enrichment period, and that the decline in N:P in our treatment stream through time may be indicative of a progressive reduction in disproportionate uptake. Interestingly, N:P ratios in the treatment stream eventually approached our enrichment ratio of 11:1, which is lower than the Redfield ratio of 16:1 (REDFIELD 1958). This result is consistent with findings by WEBSTER et al. (2000) that may suggest relatively greater P versus N demand by bacteria and fungi than phytoplankton, on which Redfield ratios are based. Changes in the nutrient content of basal resources were also consistent with potentially greater biotic responses to P versus N enrichment, but do not consider potential physiological constraints on microbial storage of P versus N.

Relative nutrient content of basal resources and trends in stream water N:P ratios suggest there may have been relatively greater demand for P than N in this system. Nitrogen deposition is increasing globally (GALLOWAY et al. 2004) and even headwater streams, in otherwise undisturbed watersheds, are likely receiving higher inputs of N. In this study, N:P ratios in the reference stream increased over the experimental period, suggesting that N may have become more available in this undisturbed system through time. In response to our experimental N + P enrichment, we observed profound changes in the production and growth rates of organisms and have determined that these changes have altered carbon dynamics in these streams (BENSTEAD et al., unpubl. manuscript). For similar heterotrophic streams, there is a high likelihood of very close co-limitation between N and P as well as the prospect of continued N loading via atmospheric deposition. Thus, to avoid fundamental changes in such systems, we suggest that policy applicable to headwater streams should specifically address land-use alterations that potentially mobilize P.

Acknowledgements

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