REPORT

Consumer-resource stoichiometry in detritus-based streams

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Abstract

Stoichiometric relationships between consumers and resources in detritus-based ecosystems have received little attention, despite the importance of detritus in most food webs. We analysed carbon (C), nitrogen (N), and phosphorus (P) content of invertebrate consumers, and basal food resources in two forested headwater streams (one reference and the other nutrient-enriched). We found large elemental imbalances between consumers and food resources compared with living plant-based systems, particularly in regard to P content, which were reduced with enrichment. Enrichment significantly increased nutrient content of food resources (consistent with uptake of N and P by detritus-associated microbes). P content of some invertebrates also increased in the enriched vs. reference stream, suggesting deviation from strict homeostasis. Nutrient content varied significantly among invertebrate functional feeding groups, orders and, to some extent, size classes. Future application of stoichiometric theory to detritus-based systems should consider the potential for relatively large consumer-resource elemental imbalances and P storage by insect consumers.

Keywords

Carbon : nitrogen : phosphorus, detritus, ecological stoichiometry, experimental enrichment, food web, insect, invertebrate, stream.

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INTRODUCTION

Ecological stoichiometry is a conceptual framework that considers the relative balance of key elements in trophic interactions (Reiners 1986; Elser *et al.* 1996; Elser & Urabe 1999; Elser *et al.* 2000c; Sterner & Elser 2002). Within this framework, food items consumed are essentially packages of elements that may or may not be in balance with a consumer's elemental requirements. Limiting nutrients [e.g. nitrogen (N) or phosphorous (P)] are retained at higher efficiencies by the consumer, while others are consumed in excess and are egested or excreted. Data collected from a wide variety of invertebrates suggest that a consumer's chemical composition [i.e. carbon : nitrogen : phosphorus (C : N : P) ratio] is relatively homeostatic regardless of the chemical composition of its food (e.g. Hessen & Lyche 1991; Elser *et al.* 2000a; Sterner & Elser 2002).

According to stoichiometric theory, and considerable empirical support, the nutrient content and relative growth rate of a given consumer ultimately determine its nutritional requirements (e.g. Sterner & Elser 2002). Consumers (or specific life stages) with high body N or P content and high

growth rates require food that is high in N or P, respectively, to maintain optimal growth. These nutrient-rich consumers are also most susceptible to reductions in growth or fitness if food resources are low in N or P relative to body demand (e.g. Sterner *et al.* 1993). Alternatively, consumers (or life stages) with low body N or P or low relative growth rates have lower requirements for these elements, and are less likely to suffer from reduced food quality (e.g. Schulz & Sterner 1999).

Several key factors are known to contribute to intra- and inter-specific variation in consumer nutrient content. For example, a number of studies have linked variation in organism P content to differences in specific growth rate (e.g. Main *et al.* 1997; Elser *et al.* 2000c). Rapid growth rates during early stages of development or among species with fast turnover rates (i.e. r-selected species) have been correlated with high levels of P-rich ribosomal RNA and associated high body P demand (Elser *et al.* 2000c). In addition, variation in body nutrient content can arise from differences among organisms (or life stages) in the relative allocation of structural biomolecules such as chitin (high N : P ratio) or bone (high P) (Elser *et al.* 1996). Thus, to

some degree, differences in ontogeny, life history strategy and relative allocation of structural biomolecules may all contribute to the nutritional requirements of a given consumer.

In essence, stoichiometric theory implies that different food types do not have inherent 'qualities' *per se*; food quality is relative based on the nutritional requirements of individual consumers. For this reason, attention should be focused on the relative imbalances between consumer C : N : P and that of their food, instead of relying solely on measures of food quantity or nutrient content. Such knowledge of elemental imbalance may allow predictions about which taxa or life stages are most limited by nutrients (e.g. Urabe & Watanabe 1992), which taxa should respond positively (in terms of growth and secondary production) to increased nutrient content of food, and which taxa should dominate high- vs. low-nutrient environments (e.g. Elser *et al.* 1988).

Important questions remain, however, concerning the importance of other evolutionary or ecological characteristics in determining consumer nutrient content (Frost *et al.* 2003). For example, little is known about relationships between phylogenetic position and nutrient content (but see Fagan *et al.* 2002; Vanni *et al.* 2002), or whether organisms adapt, via natural selection, to low or high-nutrient food resources. Moreover, basic information is still lacking about how consumer trophic level or feeding mode (i.e. functional feeding group, sensu Cummins 1973) may affect body nutrient content. However, some evidence suggests that nutrients tend to concentrate among consumers at higher trophic levels (e.g. Fagan *et al.* 2002; Sterner & Elser 2002).

Most empirical support for stoichiometric theory has come from algal-based food webs in lentic environments (Sterner & Elser 2002). In these systems, a stoichiometric perspective has led to considerable progress in understanding trophic dynamics, nutrient cycling and competitive interactions (see Sterner & Elser 2002 and references therein). However, we still lack basic information about consumer-resource stoichiometry in other types of ecosystems, such as those based on detritus (but see Higashi *et al.* 1992). Study of detritus-based food webs is crucial because they represent the dominant pathway of energy flow in most ecosystems (i.e. >80% of plant biomass ultimately ends up in detritus-based food webs; O'Neill & Reichle 1980; Wetzel & Ward 1992). Moreover, the nutritional quality of detritus is likely to be extremely low relative to the demands of detritivores (e.g. Enriquez *et al.* 1993), leading to potentially large elemental imbalances and growth constraints. In addition, there has been little research on benthic communities that are typically dominated by insect taxa (but see Frost & Elser 2002; Frost *et al.* 2002a,b; Stelzer & Lamberti 2002; Frost *et al.* 2003).

Our primary objective in this paper was to test some of the basic tenets of ecological stoichiometry in a detritus-based ecosystem by contrasting elemental composition of larval insects and basal resources in two adjacent headwater streams of different trophic status (a nutrient poor vs. an experimentally nutrient-enriched stream). We determined the effects of nutrient enrichment on elemental composition of basal resources, and then examined how this enrichment affected the elemental composition of higher trophic levels and consumer-resource elemental imbalances. We also compared elemental composition of invertebrates among major taxonomic (orders), functional [functional feeding groups (FFG)] and life history (length of larval lifespan and body size) categories to extend our general understanding of consumer-resource stoichiometry to these nutrient-poor detritus-based food webs.

METHODS

Study site

This study was conducted in two adjacent headwater streams at the Coweeta Hydrologic Laboratory in Macon Co., North Carolina, USA. Coweeta is a large (1626 ha) heavily forested basin located in the Blue Ridge physiographical province of the southern Appalachian Mountains (see Swank & Crossley 1988). A network of small first to third order streams drains the Coweeta basin. Vegetation is dominated by mixed hardwoods (primarily maple, poplar and oak) and a dense understory of *Rhododendron*, which shades the streams for most of the year. The two streams used in this study drain the relatively small catchments (C) 53 and 54 (hereafter, C53 and C54). In general, C53 and C54 have similar physical and chemical characteristics (i.e. catchment area, elevation, slope, discharge, temperature, pH and conductivity; Lugthart & Wallace 1992) but differ considerably in their concentrations of inorganic N and P as a result of an experimental nutrient enrichment. Nutrients (NH_4NO_3, K_2HPO_4 and KH_2PO_4) were dripped into C54 via a solar-powered metered-dose pump roughly every 10 m along the entire length of the stream for 2 years (July 2000–August 2002). Before enrichment, stream water in both catchments was extremely low in N and P [*c.* 29 μg (NH_4 + NO_3)-N/L, 7 μg/L soluble reactive phosphorus (SRP), biweekly samples, September 1999–June 2000]. After enrichment, nutrient levels in the enriched stream, C54, averaged 383 μg (NH_4 + NO_3)-N/L and 46 μg/L SRP (A. D. Rosemond, unpublished data). Invertebrate community structure was similar in both streams and has been extensively described elsewhere (e.g. Lugthart & Wallace 1992). Headwater streams at Coweeta are extremely heterotrophic and detritus, mostly in the form of leaf litter from the surrounding catchment, provides >90% of the energy base for microbial and invertebrate

production (e.g. Wallace *et al.* 1997b; Hall *et al.* 2000). In-stream primary production is minimal (*c.* 4–8 g C/m^2/y) and generally constitutes <1% of the total C entering these streams (Wallace *et al.* 1997a).

Basal resources

Leaf litter

Submerged leaf litter was collected from C53 and C54 on a monthly basis from June 1999 to August 2002 for analysis of C, N and P content. Five leaves were collected at five random points along each stream on each date (i.e. 25 total leaves/date) without regard to taxon. Monthly estimates of leaf C : N : P, therefore, represent a mixture of leaf species roughly in proportion to their natural abundance each month. Litter samples were transported to the laboratory, dried and homogenized. Samples were subsequently weighed on a microbalance to the nearest μg. For C and N analysis, samples were weighed in tin capsules and analysed with a Carlo Erba NA 1500 CHN analyser (Carlo Erba, Milan, Italy). For P analysis, samples were weighed into acid-washed and pre-ashed ceramic crucibles, ashed at 500 °C, acid digested and analysed spectrophotometrically (ascorbic acid method; APHA 1998). Ground pine needles (US National Institute of Standards and Technology, 1575a) and poplar leaves (Analytical Chemistry Laboratory, University of Georgia) were used as external standards for P and N analyses (recovery 99% for P, 102% for N). All data are presented as either %C, N and P of dry mass or as molar ratios.

Epilithon

Unglazed ceramic tiles (190 × 190 mm) were placed in C53 and C54 on 2 April 2002 to allow natural colonization of epilithon. We chose this pre-leaf-out period because it encompasses the time of peak chlorophyll *a* standing crop in Coweeta streams (typically c. 10 mg/m^2; J. Greenwood, University of Georgia, personal communication). After 6 weeks, tiles were removed and immediately frozen. When tiles were thawed, epilithon was removed, filtered onto a glass fibre filter (pore size 45 μm), dried at 50 °C for >72 h, and analysed for C, N and P as for other organic material. Freezing and thawing of tiles can potentially lyse algal cells, causing nutrient-rich cell contents to be lost upon filtration. Nonetheless, relative differences between streams in epilithon nutrient content remain valid.

Fine particulate organic matter

Deposited inorganic and organic material (upper 5–10 cm) was collected haphazardly by hand from the entire length of C53 and C54 on 2 April 2002. FPOM retained on metal sieves (pore size <4.75 mm and >125 μm) was dried, homogenized, and analysed for total C, N and P as above.

Invertebrates

Invertebrates were collected from C53 and C54 between 14 March and 2 April 2002. Organic and inorganic substratum was collected from all dominant stream habitats (i.e. leaf packs, cobble, depositional areas and bedrock outcrops) along the entire length of each stream. Substratum was rinsed onto stacked sieves with pore sizes ranging from 125 μm to 4.75 mm. Large invertebrates were removed live from material retained on sieves >1 mm. Chironomids and early instars of other taxa (all >1 mm in length) were removed live under a dissecting microscope from material retained on smaller sieves (125–250 μm mesh size). Invertebrates were frozen within 4 h of collection. Upon thawing, invertebrates were identified, measured in length to the nearest mm, their gut contents carefully removed (except chironomids and first instars of a few other taxa), refrozen at −80 °C, lyophilized, homogenized, weighed, and analysed for total C, N and P as above. Analysis of C, N and P content was generally conducted on composite samples of a given taxon consisting of three to 100 individuals of similar size (within 1 mm size classes). Otherwise, if enough material was present, chemical analysis was performed on individual larvae. The majority of samples contained enough material for C, N and P analysis; however, when sample weight was limited, we restricted our analysis to %P. Biomass of individuals was obtained using previously established length-weight regressions for Coweeta stream taxa (Benke *et al.* 1999). FFGs were designated according to Merritt and Cummins (1996) and knowledge of the local fauna based on gut-content analyses (J. B. Wallace, unpublished data). FFGs, which are based primarily on mouth-part morphology and behavioural characteristics (Cummins 1973), included scrapers (scrape biofilm from hard surfaces), shredders (consume primarily leaf material), collector-gatherers (gather fine organic particles), collector-filterers (filter fine particles) and predators (consume other animals).

Statistical analyses

Leaf litter

We analysed the time series of leaf litter nutrient content using randomized intervention analysis (RIA, Carpenter *et al.* 1989). RIA uses paired, before-and-after time-series data from a manipulated and a reference system to detect changes caused by the manipulation. In this study, RIA was used to test the null hypothesis that no change in leaf litter nutrient content occurred in the treatment stream relative to the reference stream following the initiation of nutrient enrichment.

Invertebrates

Overall differences between streams in invertebrate %C, %N, %P, C : P, N : P and C : N were assessed with Kolmogorov–Smirnov goodness-of-fit tests. To test the null hypothesis of no difference in invertebrate nutrient content between streams (i.e. chemical homeostasis), samples of similarly sized taxa (within 2 mm) from both streams were compared with paired *t*-tests. Differences among FFG and orders were analysed with two-way analysis of variance; significant ANOVAs were followed by Tukey's HSD multiple comparisons. Relationships between body size (mg ash-free dry mass (AFDM)) or length of larval lifespan (days) and invertebrate nutrient content were examined with linear regression. All such regressions were performed on the entire data set, within FFGs, and within orders. Data were either $\log(x + 1)$ or arcsin-square root transformed to meet assumptions of normality and homoscedasticity. No adjustments were made to α for protection against experimentwise error (e.g. Bonferroni) because of relatively low statistical power, and the potential for increased type II error (Perneger 1998).

RESULTS

Basal resources

Nutrient enrichment of C54 resulted in significantly higher nutrient content of leaf litter, including increases in %P, and consequent decreases in N : P and C : P ratios relative to leaf litter in the control stream ($P < 0.0001$ for all three tests, RIA; Fig. 1, Table 1). Post-enrichment values of P content were, on average, 59% higher in C54 compared with C53 (range 14–111%). There was also a small, but significant ($P = 0.04$, RIA), increase in %C in the enriched stream (data not shown). No change in %N or C : N ratio of leaf litter was observed (see Table 1 for C : N; %N data not shown).

Fine particulate organic matter (FPOM) contained slightly more P and N, and less C in the enriched stream than in the reference stream (P: 0.1% vs. 0.07%; N: 0.9% vs. 0.8%; C: 23% vs. 25%). These minor differences were reflected in FPOM elemental ratios (Table 1).

Epilithon in the enriched stream contained c. 4× more P and c. 5.5× more N than in the reference stream (P: 0.08% vs. 0.02%; N: 12.1% vs. 2.2%). Enrichment also increased %C in the enriched stream relative to the reference (27.7% vs. 16.5%), presumably because of differences in standing crop, and a higher proportion of organic (including microbes) vs. inorganic matter on tiles in the enriched stream. Epilithon C : P, N : P and C : N ratios differed correspondingly (Table 1).

Invertebrates

We analysed the nutrient content of 40 invertebrate taxa from C53 and C54, ranging from 1 to 36 mm in length and 0.002–86 mg AFDM in weight. Larval lifespan of these taxa ranges from <2 weeks to >3 years (see Wallace *et al.* 1999). Invertebrates exhibited considerable variability in %P, %N and %C (Fig. 2). Overall, the coefficient of variation in %P (CV = 52%) was much higher than %N (CV = 13%) or %C (CV = 8%). Coefficients of variation for %P, %N and %C were, on average, 30–45% lower in the enriched stream (C54) than in the reference stream (C53). High variability of %P was reflected in invertebrate C : P, N : P and C : N ratios, where overall variability was much higher in C : P (CV = 60%) and N : P (CV = 51%) ratios than in the C : N (CV = 15%) ratio (Fig. 2). Coefficients of variation for C : P, N : P and C : N were also 30–40% lower in the enriched stream (C54) than in the reference stream (C53). Overall, there was no difference in %P, %C, %N, C : P, N : P and C : N ratios of invertebrates between C53 and C54 (Kolmogorov–Smirnov tests, $P > 0.05$).

Paired comparisons of similar-sized taxa between C53 and C54 revealed patterns that indicate some invertebrate taxa may not be strictly homeostatic with regard to body nutrient content. Invertebrates in the enriched stream had significantly higher %P ($P = 0.03$) and lower C : P ($P = 0.01$) and N : P ($P = 0.02$) ratios than in the reference stream (Fig. 3). No difference was detected in %C, %N or C : N ratios among paired taxa between streams ($P > 0.05$).

Significant differences in %P, %N and %C were found among major insect orders when data from both streams were considered together (two-way ANOVA, order: $P < 0.0001$, Fig. 4). Trichoptera and Ephemeroptera were generally lower in %P and %N than Diptera, Odonata and Plecoptera (Fig. 4). Significant differences also existed in C : P, N : P and C : N among the major insect orders (two-way ANOVA, order: $P < 0.001$, Fig. 4). The only significant difference found in invertebrate nutrient content between streams was among Trichoptera, in which C : P ratios were lower in the enriched stream (C54) than in the reference stream (C53) (two-way ANOVA, stream: $P = 0.03$, stream x order interaction: $P = 0.002$, Fig. 4).

Percent P, %N and %C varied significantly among invertebrate FFGs when considering all data together (two-way ANOVA, FFG: $P < 0.0001$, Fig. 5). Again, most of the variation was associated with P content. On average, predators contained more P and N than the other FFGs. There were no significant effects of enrichment on %P, %N and %C for any FFG (two-way ANOVA, stream and stream x FFG interaction non-significant: $P > 0.05$). C : P and C : N ratios also differed significantly among FFGs
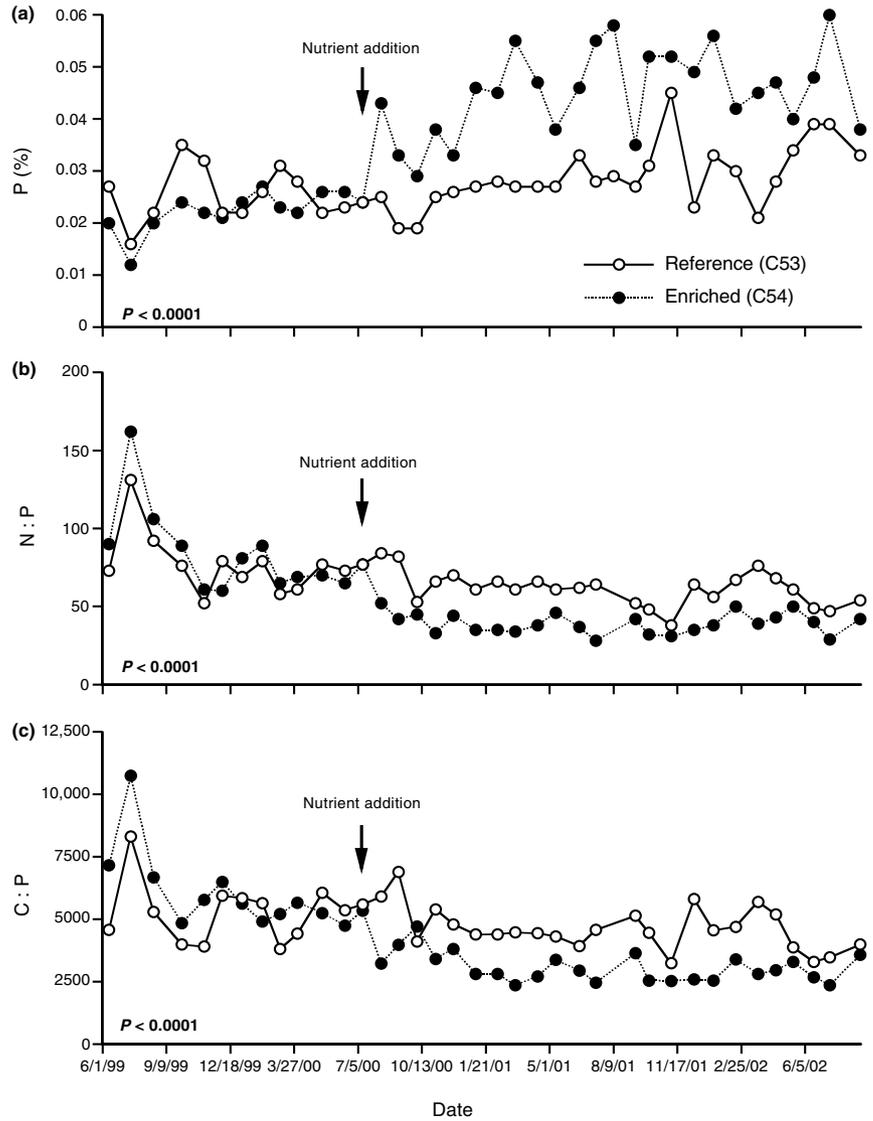
Figure 1 Changes in (a) %P, (b) N:P and (c) C:P of leaf litter in the reference stream (C53, open symbols) and the enriched stream (C54, closed symbols) from June 1999 to August 2002. Arrow indicates start of nutrient enrichment of C54. All ratios are molar. Data are mean values ($N = 5$, except first date where $N = 3$).

(two-way ANOVA, FFG: $P < 0.001$, Fig. 5). C:P ratios of shredders were higher than those of collector-gatherers and predators. C:N ratios were higher among shredders and collector-filterers than predators. No difference in N:P ratios was apparent among FFGs. Additionally, there were no overall differences in C:P, N:P and C:N ratios between C53 and C54 (two-way ANOVA, stream and interaction non-significant: $P > 0.05$).

Invertebrate body size (mg AFDM) did not explain any significant variation in invertebrate nutrient content when all data were considered together (linear regression, all P values >0.05) and when invertebrate orders were examined separately for each stream. However, when body size-nutrient content relationships were examined by FFG irrespective of stream, some significant patterns emerged.

Collector-gatherer C:P ($r^2 = 0.58$, $P < 0.01$), N:P ($r^2 = 0.48$, $P < 0.01$) and C:N ($r^2 = 0.21$, $P = 0.1$) ratios all increased with body size (mg AFDM); %P decreased with body size ($r^2 = 0.25$, $P = 0.04$). In addition, collector-filterers exhibited an increase in N:P ratio ($r^2 = 0.27$, $P = 0.08$) and a concomitant decrease in %P ($r^2 = 0.24$, $P = 0.05$) with body size. No such relationships existed for scrapers, shredders or predators.

No significant relationships were found between larval lifespan (d) and any measure of body nutrient content.

DISCUSSION

Basal food resources in streams at Coweeta had very low nutrient content (Table 1). Leaf litter, which forms the basis

Table 1 Comparisons of C : P, N : P and C : N among invertebrate trophic groups and food resources from lake, stream and terrestrial habitats[1]. Values in bold are from this study. C53 (reference), C54 (enriched). All ratios are molar

	Trophic group						Food resource		Elemental imbalance[2]	
	C53			C54[3]			C53	C54[3]	C53	C54
	Mean	Median	Range	Mean	Median	Range	Mean	Mean		
Stream shredders							Leaf detritus			
C : P	**498**	**493**	**(136–877)**	**252**	**221**	**(123–610)**	**4858**	**3063**	**4360**	**2565**
C : N	**6.7**	**6.4**	**(5.4–8.9)**	**6.4**	**6.3**	**(5.0–7.7)**	**73**	**82**	**66**	**75**
N : P	**73**	**76**	**(17–125)**	**39**	**30**	**(19–97)**	**67**	**39**	–	–
Stream collectors							FPOM			
C : P	**277**	**208**	**(93–574)**	**227**	**219**	**(80–358)**	**1015**	**673**	**738**	**396**
C : N	**6.4**	**6**	**(5.2–9.0)**	**6**	**5.8**	**(5.3–7.2)**	**34**	**29**	**28**	**23**
N : P	**43**	**38**	**(14–78)**	**37**	**37**	**(14–59)**	**28**	**23**	–	–
Stream scraper-herbivores							Stream epilithon			
C : P	**369**	–	–	**287**	**304**	**(155–371)**	**1741**	**845**	**1372**	**476**
C : N	**6.2**	–	–	**5.8**	**5.4**	**(5.1–7.1)**	**8.7**	**4.6**	**2.5**	**−1.6**
N : P	**59**	–	–	**51**	**56**	**(22–68)**	**201**	**318**	–	–
Stream predators							Stream prey			
C : P	**223**	**215**	**(102–351)**	**227**	**215**	**(78–430)**	**324**	**236**	**101**	**13**
C : N	**5.1**	**5.2**	**(4.9–5.4)**	**5.6**	**5.5**	**(5.0–6.8)**	**6.1**	**5.9**	**1**	**0.8**
N : P	**43**	**42**	**(20–65)**	**40**	**37**	**(15–75)**	**52**	**40**	–	–
Terrestrial herbivores							Terrestrial plants			
C : P				116			968	852		
C : N				6.5			36	29.5		
N : P				26			28	–		
Lake zooplankton							Lake phytoplankton			
C : P				124			307	183		
C : N				6.3			10.2	3.9		
N : P				22			30	–		
Lake benthic invertebrates							Lake benthic algae			
C : P				148			(98–1496)	(−50–1348)		
C : N				5.5			–			
N : P				27			–			

[1]Terrestrial herbivores and plants, lake phytoplankton and zooplankton from Elser *et al.* 2000a; lake benthic algae and benthic invertebrates from Frost & Elser 2002 and Frost *et al.* 2003.
[2]Elemental imbalance is calculated as the arithmetic difference between a consumer and its food resource.
[3]Post-enrichment values.

for most heterotrophic production (Wallace *et al.* 1997b), was extremely low in N and P. C : P and N : P ratios were among the highest reported for vascular plant detritus (Enriquez *et al.* 1993). These values are also considerably higher than those of living terrestrial foliage (Table 1), suggesting nutrient loss via absorption by trees before leaf abscission, the dominance of structural C compounds in leaf litter, and leaching of soluble N and P upon contact with stream water. Increases in P of leaf litter after enrichment coincided with significant increases in bacterial and fungal production on leaves (K. Suberkropp, University of Alabama, unpublished data), suggesting increased microbial

biomass associated with leaf litter as the primary cause of changes in nutrient content.

Nitrogen and P content of epilithon was much higher than that of leaf litter, but still low relative to many previously reported values for streams [Table 1, e.g. Kahlert 1998; C : P range (99–603), N : P range (10–49)]. FPOM (<4 mm), which is also directly consumed by many stream invertebrates, generally had a higher nutrient content than large leaf particles (Table 1; Sinsabaugh & Linkins 1990); this was probably a result of increased surface : volume ratios leading to a higher proportion of nutrient-rich microbes (i.e. bacteria) on smaller particles, and the presence
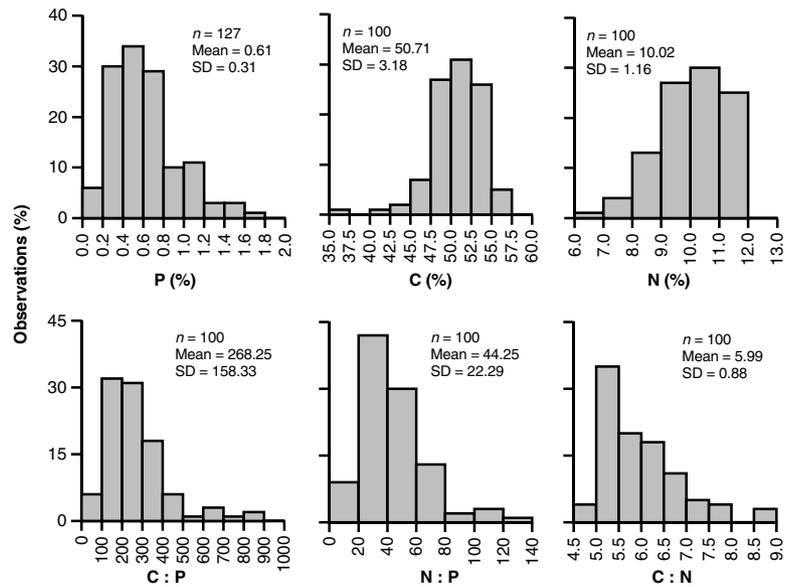
Figure 2 Frequency histograms of invertebrate body %P, %C, %N, C:P, N:P and C:N. Histograms include all data from C53 and C54 together; no significant difference was found between streams for any measure of nutrient content when all data were considered together (Kolmogorov–Smirnov tests, $P > 0.05$). All ratios are molar.

of microbial and animal exudates within stream FPOM (Findlay *et al.* 2002).

C:P and N:P ratios of stream consumers were considerably higher than those of invertebrate consumers in littoral benthic, planktonic and terrestrial environments (Table 1). C:N ratios of consumers did not differ among these environments (Table 1). Differences in P content were unexpected, as invertebrate nutrient content has been thus far reported as relatively constant across a wide range of systems with very different basal resource elemental composition (Table 1, e.g. Elser *et al.* 2000a; Frost *et al.* 2003). Such low relative P content may be characteristic of detritus-based systems. Nutritional constraints in these systems may be severe enough to cause evolutionary adaptation to low nutrient food resources via lowered body nutrient content and hence lowered requirements for P (e.g. Elser *et al.* 2000b; Fagan *et al.* 2002). In addition, some prominent detritivores at Coweeta (e.g. *Tipula* spp., Diptera) harbour gut flora or fauna that potentially aid in the assimilation of low nutrient leaf litter (e.g. Klug & Kotarski 1980), an adaptation also seen among wood-eating termites (Higashi *et al.* 1992).

To estimate simple consumer-resource elemental imbalances, we calculated arithmetic differences between the C:P and C:N of consumers and their food resources (Table 1). This provides a comparison of the relative stoichiometric constraints among trophic groups in detritus-based streams vs. systems based on living plants or algae. It is evident that stream consumers dependent on leaf litter (i.e. shredders) in C53 are far out of balance with their food resources (C:P 4360, C:N 66, Table 1). In comparison, the elemental imbalances between terrestrial herbivores and living plants

(C:P 852, C:N 30) are c. 5× and 2× lower than those of stream shredders, in terms of P and N, respectively. Thus, limits to growth and production of leaf-eating consumers are potentially more severe in detritus-based food webs than in those based on living plant tissue. Imbalances of other stream consumers (i.e. collectors and scrapers) were also relatively high in comparison with lake zooplankton, but fell within the range of some lake benthic invertebrates (Table 1). Stream predators were generally the least out of balance with their food, based on consumption of N and P-rich prey. For all stream trophic groups, nutrient enrichment reduced consumer-resource imbalances, potentially alleviating nutrient limitation for some taxa (C54, Table 1).

A central tenet of ecological stoichiometry is that consumers maintain elemental homeostasis within a relatively small range (Elser *et al.* 1996; Sterner & Elser 2002), regardless of the elemental composition of their food. Here we have shown that this may not be strictly true for some invertebrates that exhibited up to fourfold differences in C:P and N:P ratios between C53 and C54. This deviation from strict homeostasis may have been due to either increased P storage in insect tissue or haemolymph (Woods *et al.* 2002) or increased rRNA allocation associated with higher growth rates (e.g. Schade *et al.* 2003). Woods *et al.* (2002) demonstrated that a larval lepidopteran (*Manduca sexta*) was capable of storing excess P as α−glycerophosphate in haemolymph. The authors suggested that P storage might act as a potential buffer against short-term decreases in P availability. Interestingly, the taxa in our study that consistently showed the largest differences in P content between C53 and C54 were Trichoptera, which share a close evolutionary history with
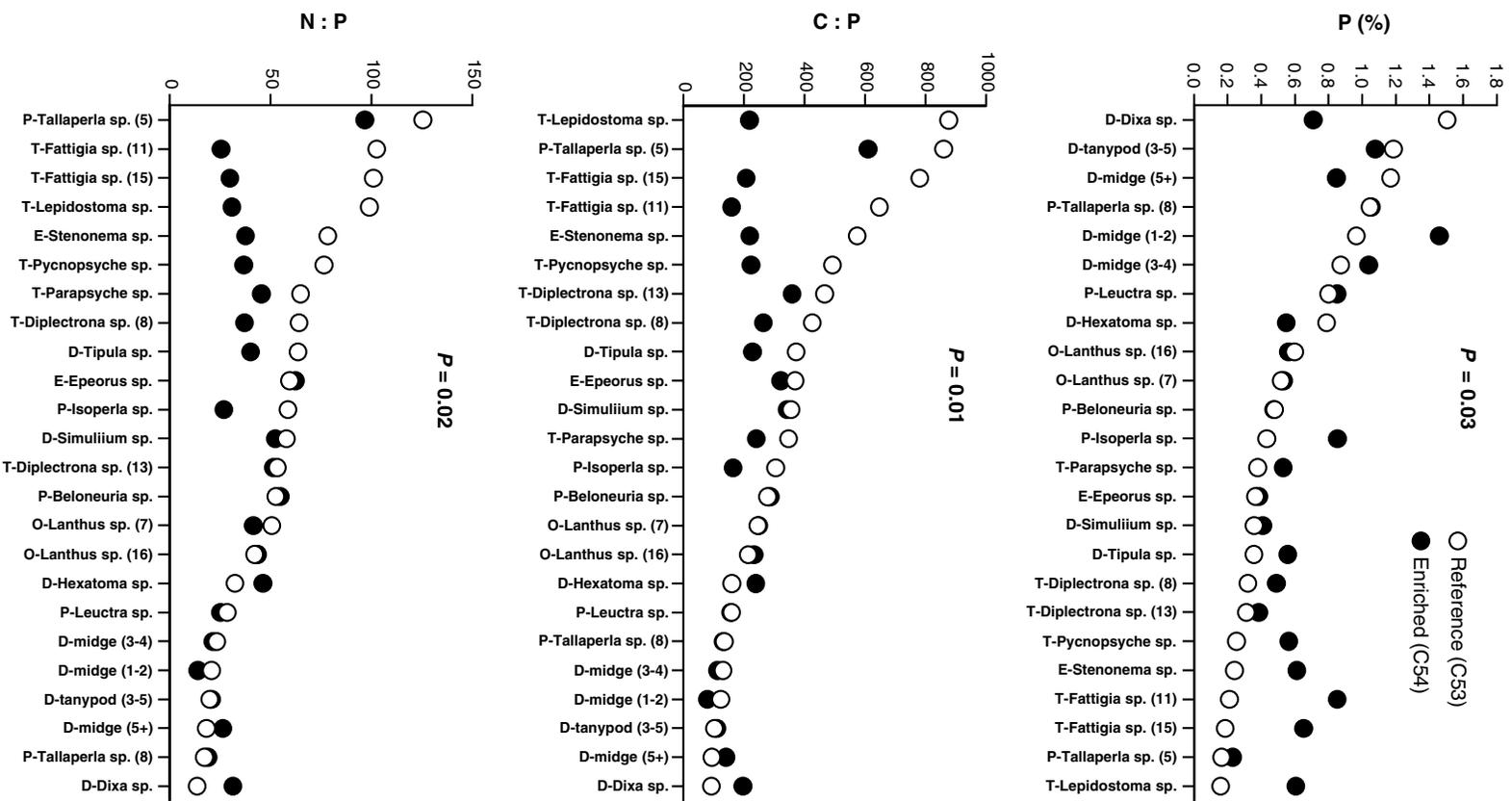
N : P

C : P

P (%)

○ Reference (C53)
● Enriched (C54)

P = 0.02

P = 0.01

P = 0.03

Figure 3 Percent P, C : P and N : P ratios of paired invertebrates of similar size from C53 (reference) and C54 (enriched). Capital letters before the genus indicate insect order. D = Diptera; E = Ephemeroptera; O = Odonata; P = Plecoptera; T = Trichoptera. Where more than one size class of a given taxon is presented, numbers in parentheses indicate length of larvae in mm. *P* values are shown for paired *t*-tests between streams. All ratios are molar.
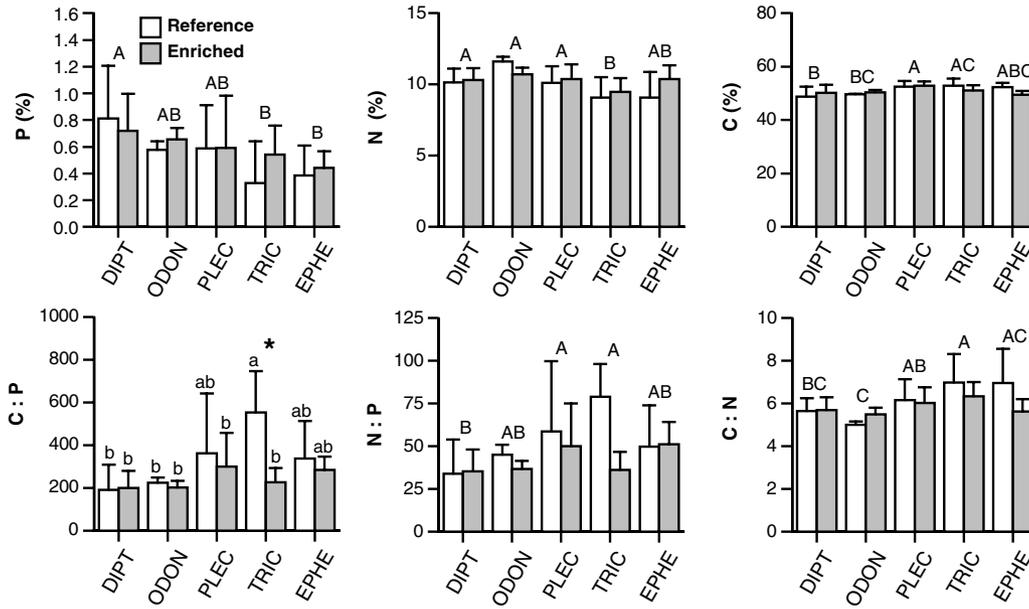
Figure 4 Mean %P, %N, %C, C:P, N:P and C:N ratio (+1 SD) of invertebrates from five dominant insect orders in the reference stream (C53, white bars) and the enriched stream (C54, grey bars). DIPT = Diptera; ODON = Odonata; PLEC = Plecoptera; TRIC = Trichoptera; EPHE = Ephemeroptera. Results of two-way ANOVA: order factor significant for all variables: %P****, %N****, %C****, C:P****, N:P***, C:N****, stream factor significant for C:P*, order × stream interaction significant for C:P** and N:P**. Different capital letters above bars indicate significant differences among orders (Tukey's HSD) using data from both streams (i.e. stream factor not significant). Different lower-case letters above bars (as for C:P) indicate significant differences (Tukey's HSD) considering the reference and enriched streams separately (i.e. stream factor significant). *$P < 0.05$, **$P < 0.01$, *** $P < 0.001$, ****$P < 0.0001$. For %P in C53 and C54, DIPT ($N = 17, 23$), ODON ($N = 5, 6$), PLEC ($N = 9, 14$), TRIC ($N = 12, 23$) and EPHE ($N = 6, 10$). For other graphs in C53 and C54, DIPT ($N = 11, 17$), ODON ($N = 5, 6$), PLEC ($N = 8, 12$), TRIC ($N = 8, 20$) and EPHE ($N = 4, 7$). All ratios are molar.

Lepidoptera (Wheeler *et al.* 2001), and may be similarly capable of P storage. An alternative explanation is that higher P content of some consumers in the enriched stream was due to higher growth rates and greater cellular allocation to rRNA (e.g. Schade *et al.* 2003). Frost & Elser (2002) showed that mayfly larvae (*Ephemerella* sp.) fed P-enriched epilithon harboured significantly more P in their body tissue than those that were fed low-P periphyton. In this case, growth rates were higher on P-rich epilithon, suggesting a growth-rRNA effect on mayfly P content. A thorough test of these alternative hypotheses for organisms that deviate from strict homeostasis will require careful estimates of daily growth rate, rRNA content and C:N:P content of consumers and basal resources.

Our analyses relating body size and larval lifespan to nutrient content were made in an effort to test the 'growth rate hypothesis' (i.e. rapid growth associated with P-rich rRNA, Elser *et al.* 1996; Main *et al.* 1997). Although we did not find that body size was consistently related to nutrient content, we did find some patterns that support previous stoichiometric theory. For example, N and P content

decreased with body size among collector-gatherers and collector-filterers. These results suggest that, for some taxa, later instars contain less N and P, have decreased growth rates (as shown for Chironomidae, Huryn 1990; W. F. Cross, unpublished data) and may have lower physiological requirements for N and P than early instars. Although no relationship was found between larval lifespan (a proxy for turnover rate) and P content, patterns may be difficult to detect among organisms that vary in size or life stage. For example, relationships may be obscured when comparing early instars of long-lived taxa (i.e. high P for that taxon due to high growth rates at small size, low P relative to other taxa due to long-lived life history) and late instars of short-lived taxa (i.e. low P for that taxon and high relative P).

We found significant differences among stream invertebrate functional and taxonomic groups. N and P content were generally highest among predators, which is consistent with the results of Fagan *et al.* (2002), who found that terrestrial invertebrate predators contained higher amounts of N than invertebrate herbivores. Multiple valid hypotheses exist for this trophic difference, including the effect of higher nutrient content in the diet of predators (Fagan *et al.*
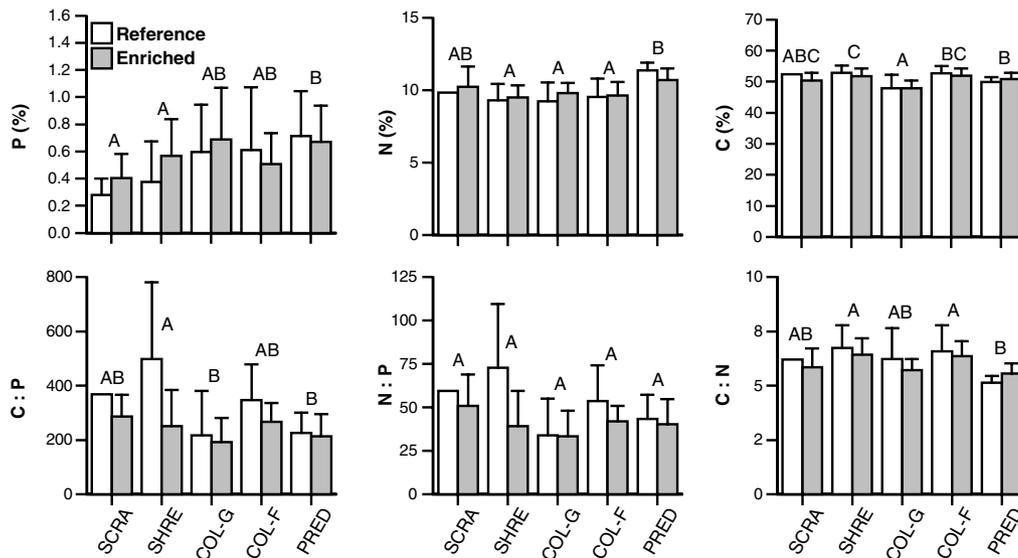
Figure 5 Mean %P, %N, %C, C:P, N:P and C:N ratio (+1 SD) of invertebrates from four FFGs in the reference stream (C53, white bars) and the enriched stream (C54, grey bars). SCRA = scraper; SHRE = shredder; COL-G = collector-gatherer; COL-F = collector filterer; PRED = predator. Results of two-way ANOVA: FFG factor significant for all variables except N:P: %P**, %N****, %C****, C:P****, C:N****, stream factor not significant for any variables, FFG × stream interaction not significant for any variables. Different capital letters above bars indicate significant differences (Tukey's HSD) based on results from both streams combined. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, **** $P < 0.0001$. For %P in C53 and C54, SCR ($N = 2, 10$), SHRE ($N = 13, 20$), COL-G ($N = 9, 9$), COL-F ($N = 9, 8$) and PRED ($N = 16, 29$). For other graphs in C53 and C54, SCR ($N = 1, 5$), SHRE ($N = 11, 16$), COL-G ($N = 7, 7$), COL-F ($N = 6, 6$) and PRED ($N = 11, 28$). All ratios are molar.

2002), but proper understanding will require further analysis. Among orders, Diptera consistently harboured the highest amount of P, which may be related to a disproportionate number of fast-growing taxa (i.e. chironomids) within this order (Huryn 1990). We found no apparent relationship between nutrient content and invertebrate phylogenetic position (i.e. ancient Ephemeroptera vs. recently derived Diptera) (e.g. Fagan *et al.* 2002).

A large disparity exists between the temporal scale of leaf-litter sampling in this study (monthly for 3 years) and that of other food web components (once). This disparity raises important questions about seasonal differences in consumer-resource elemental imbalance. For example, temporal changes in resource elemental composition could potentially alleviate nutrient imbalances for some invertebrates during critical times of the year (i.e. during growth spurts). Although our long-term leaf litter data showed no obvious seasonal trends, this does not preclude the potential for seasonal variability in FPOM, epilithon, or, to a lesser extent, invertebrates. Future studies geared towards understanding temporal variability of consumer-resource C:N:P content will be important in recognizing the potential for seasonal differences in stoichiometric constraints.

We sampled most food web components from both streams after the experimental enrichment had begun in one

of them. As a consequence, it is not certain that stoichiometric differences between streams were actually due to the enrichment. However, both of the study streams were similarly low in inorganic N and P before the enrichment. In addition, large changes in leaf-litter nutrient content coincided directly and significantly with experimental enrichment (Fig. 1). Therefore, it is highly likely that between-stream elemental differences in epilithon, FPOM and invertebrates were caused by the enrichment.

This study is one of the first to describe stoichiometric relationships among consumers and basal resources in a detritus-based ecosystem. We have shown that larval invertebrates in these systems harbour low relative amounts of P in their body tissue compared with invertebrates in other food webs based on living plant tissue. We have also shown that some invertebrate taxa do not exhibit strict elemental homeostasis and may have the potential to avoid nutrient limitation via storage of P. Indeed, insects may cope with physiological nutrient constraints in a different manner (e.g. store nutrients) than some well-studied crustaceans (i.e. copepods and cladocerans). Nutrient enrichment of detritus-based ecosystems may alleviate nutrient limitation of some invertebrate taxa, and potentially lead to long-term alteration of community structure, secondary production and food web

dynamics. More research in detritus-based systems will be necessary to determine whether stoichiometric relationships in these systems are fundamentally different from those in systems based on living plants.

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