

Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream

Gaston E. Small · Catherine M. Pringle

Received: 8 April 2009 / Accepted: 15 October 2009 / Published online: 15 November 2009
© Springer-Verlag 2009

Abstract A central tenet of ecological stoichiometry is that consumer elemental composition is relatively independent of food resource nutrient content. Although the P content of some invertebrate consumer taxa can increase as a consequence of P-enriched food resources, little is known about how ecosystem nutrient loading can affect the elemental composition of entire consumer assemblages. Here we examine the potential for P enrichment across invertebrate consumer assemblages in response to chronic high P loading. We measured elemental ratios in invertebrate consumers and basal food resources in a series of streams in lowland Costa Rica that range widely in P levels (2–135 $\mu\text{g l}^{-1}$ soluble reactive P). Streams with high P levels receive natural long-term (over millennia) inputs of solute-rich groundwater while low-P streams do not receive these solute-rich groundwater inputs. P content of leaf litter and epilithon increased fourfold across the natural P gradient, exceeding basal resource P content values reported in the literature from other nutrient-rich streams. Invertebrate consumers from the high-P study stream were elevated twofold in P content across multiple taxonomic and functional feeding groups, including predators. Our results strongly support the hypothesis that elevated P content in consumers feeding on P-enriched food resources is a consequence of deviation from strict homeostasis. In contrast

to prior studies, we found that between-stream variation in P content of a given taxon greatly exceeded within-stream variation among different taxa, suggesting that environment may be as important as phylogeny in controlling consumer stoichiometry. Relaxing the assumption of strict homeostasis presents challenges and opportunities for advancing our understanding of how nutrient limitation affects consumer growth. Moreover, our findings may provide a window into the future of how chronic anthropogenic nutrient loading can alter stoichiometric relationships in food webs.

Keywords Aquatic invertebrates · Ecological stoichiometry · Nutrients · Streams

Introduction

Ecological stoichiometry at the organismal level is an important component of nutrient dynamics within ecosystems. The elemental composition of organisms affects the storage and fluxes of nutrients within the food web. Likewise, the degree to which organisms maintain homeostasis influences the rates at which limiting nutrients are retained and excess nutrients are mineralized.

Differences in elemental composition among taxonomic and functional feeding groups are well documented (e.g., Cross et al. 2003; Evans-White et al. 2005), due to variation among different consumers in body nutrient demand and dietary nutrient supply. While the P content of vertebrate consumers is largely a function of “structural biomass” (e.g., bones and scales; Vanni et al. 2002), nearly all body P in invertebrate consumers is found in “metabolic biomass” (sensu Allen and Gillooly 2008). Ribosomal RNA and other intra-cellular pools of P may vary based on

Communicated by Robert Hall.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-009-1489-4) contains supplementary material, which is available to authorized users.

G. E. Small (✉) · C. M. Pringle
Odum School of Ecology, University of Georgia,
Athens, GA 30602, USA
e-mail: csmall@uga.edu

dietary P availability (Sternler and Elser 2002), potentially causing large changes in invertebrate body P content within a taxon. However, stoichiometric theory has assumed that, within a species, animals maintain a fixed elemental composition (i.e., strict homeostasis) that is independent of diet quality (e.g., Andersen and Hessen 1991; Urabe and Watanabe 1992; Sternler and Hessen 1994). Despite these assumptions of strict homeostasis, a growing body of evidence has demonstrated that the P content of some invertebrate taxa can vary substantially with diet P content (e.g., DeMott et al. 1998; Carrillo et al. 2001; Fink and Von Elert 2006; Shimizu and Urabe 2008). These deviations from strict homeostasis, observed for invertebrates feeding on high-P food, have been attributed to increased levels of RNA (Schade et al. 2003; Acharya et al. 2004; Elser et al. 2005) as well as P stored in hemolymph (Woods et al. 2002). However, there are few data on the extent to which ecosystem nutrient loading affects the elemental composition of entire consumer assemblages (e.g., Singer and Battin 2007). Only one study to date has examined effects of a multi-year, whole-stream nutrient enrichment on the elemental composition of invertebrate consumers. In that study, moderately elevated levels of N and P led to increased P content in a subset of the invertebrate assemblage, showing that at least some taxa (notably Trichoptera) deviated from strict homeostasis (Cross et al. 2003).

P loading is a ubiquitous problem in many freshwater ecosystems (Carpenter et al. 1998) and can greatly increase the P content of both algal epilithon and leaf litter (e.g., Cross et al. 2003), which constitute the base of stream food webs. If deviation from strict homeostasis is a widespread trait in aquatic invertebrates, then chronic high stream P loading could lead to the entire benthic consumer assemblage becoming P enriched as a consequence of feeding on high-P food resources.

To better understand how P loading affects stoichiometric relationships throughout a stream food web, we measured the elemental composition of invertebrate consumers and basal food resources in streams in lowland Costa Rica that vary widely in P levels due to natural, long-term (over millennia) inputs of solute-rich groundwater. We analyzed: (1) assemblage-level patterns between streams; (2) paired comparisons of similarly sized, conspecific taxa (in order to evaluate deviation from strict homeostasis); (3) comparisons between streams by insect order (to examine phylogenetic patterns in homeostasis); and (4) comparisons between streams by functional feeding group (in order to examine the effects of diet on homeostasis). We predicted that the historically high concentrations of dissolved P in our study streams would result in high P content across all taxa in the invertebrate consumer assemblage.

Materials and methods

Study site

La Selva Biological Station (10°26'N, 84°01'W) is situated on the Caribbean Slope of Costa Rica at the gradient break between the mountains and coastal plain, receiving almost 4,000 mm of rainfall each year (Sanford et al. 1994). Geomorphological features of the La Selva landscape result in natural interbasin transfers of solute-rich groundwater entering some streams (Pringle et al. 1990). These groundwater inputs are characterized by high solute concentrations (e.g., P, Na⁺, Cl⁻, HCO₃⁻; Pringle et al. 1993). Dense riparian vegetation results in light-limited algal communities (Pringle and Triska 1991) and high inputs of allochthonous material into streams.

We chose seven streams that differed widely in average soluble reactive P (SRP) concentration (2–135 µg l⁻¹) due to differential inputs of solute-rich groundwater (Table 1). These sites are second- to third-order streams, within close proximity (<2 km apart), and surrounded by dense forest. Channel widths range from 1 to 3 m, and the dominant substrata are detritus, silt, and clay, with boulders present at some sites. Our seven study streams are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle and Triska 1991; Triska et al. 2006), for which continuous monthly data have been available since 1997. We analyzed the elemental composition of leaf litter and epilithon at all seven sites. Insect assemblages vary widely among streams at La Selva, largely due to differences in geomorphology (Ramírez et al. 2006). In order to maximize the number of comparisons between similar taxa, we selected one high-P stream (Arboleda; SRP = 135 µg l⁻¹) and one low-P stream (Sura-60; SRP = 2.8 µg l⁻¹) to compare the elemental composition of insect consumers, based on their largely overlapping insect assemblages (Ramírez et al. 2006), as well as similarities in discharge and substrate (Table 1).

Sample collection and analysis

Leaf litter and epilithon were collected from our seven study streams in February and July 2006. At each sampling date, we collected three replicate grab samples of submerged leaves, so that individual leaf species were represented roughly in proportion to their abundance in the stream. Epilithon was collected from unglazed ceramic tiles after 4 weeks of incubation in each stream. Epilithon was scrubbed from the tiles, filtered (0.45 µm GF/F), and dried. In the Arboleda and Sura-60, we also sampled fine particulate organic matter (FPOM) and filamentous algae. FPOM samples consisted of deposited material from the upper 5 cm of substrate, and were collected with a syringe.

Table 1 Means (and ranges) of physicochemical variables in the seven study streams along the natural P gradient from monthly samples collected January 2006–August 2007

Stream	DIN ^a :SRP (molar)	SRP (μg l ⁻¹)	NO ₃ -N (μg l ⁻¹)	NH ₄ -N (μg l ⁻¹)	Temperature (°C)	Discharge (m ³ s ⁻¹)	Conductivity (μS cm ⁻¹ at 25°C)	pH
Arboleda	2.39	135 (27–397)	126 (63–162)	20 (7–42)	25.6 (24.7–26.4)	0.17 (0.09–0.21)	257 (173–310)	6.2 (5.9–6.5)
Sura-30	4.82	83 (39–150)	163 (60–277)	18 (0–87)	25.4 (24.7–26.7)	0.61 (0.43–0.86)	157 (73–188)	6.1 (5.8–6.8)
Salrito-60	7.84	33 (1.6–87)	98 (35–170)	19 (0–64)	25.0 (24.0–25.9)	0.11 (0.04–0.20)	110 (37–170)	6.1 (5.9–6.9)
Salto-60	44.2	10 (4.3–21)	180 (101–261)	20 (0–59)	24.7 (23.7–25.6)	0.45 (0.07–0.93)	32 (28–44)	5.9 (5.6–6.6)
Salrito-100	143	3.1 (0–7.2)	163 (78–460)	37 (18–60)	24.3 (23.6–25.6)	0.03 (0.02–0.06)	19 (17–24)	5.7 (5.3–6.7)
Sura-60	174	2.8 (0–9.0)	199 (58–353)	21 (0–51)	24.8 (24.0–26.2)	0.19 (0.05–0.55)	20 (16–26)	5.6 (4.7–6.5)
Piper	219	2.3 (0–6.8)	188 (99–404)	40 (6–166)	25.0 (24.3–25.9)	0.03 (0.01–0.10)	22 (19–26)	5.5 (4.8–6.2)

DIN Dissolved inorganic N, SRP soluble reactive P

^a NO₃-N + NH₄-N

Filamentous algae was collected by hand from submerged rocks.

Invertebrates were collected in the Arboleda and Sura-60 in May 2007 from all major stream habitats (leaf packs, boulders, depositional areas). Insects were sorted live under a dissecting microscope, length was measured to the nearest millimeter, and specimens were identified to family or genus. Guts were not removed prior to stoichiometric analysis due to the small size of many individuals (see Discussion). For smaller taxa, composite samples consisting of three to 50 individuals of a similar size class (within 2 mm) were used for C, N, and P analysis; otherwise, individual organisms were used. Some low-mass samples were analyzed only for P. Functional feeding groups (FFGs) were designated according to Merritt and Cummins (1996) and Jackson and Sweeney (1995). FFGs are based on mouthpart morphology and behavioral characteristics and include 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).

All samples were dried at 50°C for 48 h and homogenized. For C and N analysis, samples were analyzed on a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan). For P analysis, samples were acid-digested (Aqua Regia double acid; Jones et al. 1991), and analyzed spectrophotometrically (ascorbic acid method). 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. In each stream, nutrient concentrations were measured from filtered water samples (0.045 μm Millipore filters) for SRP (ascorbic acid method), NO₃-N (cadmium reduction method), and NH₄-N [phenate method; American Public Health Association (APHA) 1998].

Statistical analyses

Relationships among leaf litter and epilithon nutrient content and stream SRP were determined using regression analysis. Mean SRP values for each stream were calculated from monthly samples from January 2006 to August 2007. For each stream, we calculated mean %P, C:P, and N:P for leaf litter and epilithon. The relationship between SRP and leaf litter or epilithon %P was fit with a linear model. Because %C and %N showed no relation to SRP, C:P and N:P were inverse-transformed (i.e., to P:C and P:N, respectively) and fit with a linear model.

Overall differences in the elemental composition of insect assemblages between the high-P Arboleda and low-P Sura-60 were assessed using *t*-tests ($\alpha = 0.05$), based on

all samples collected for each stream (where each sample represents a certain size class of a species; sensu Cross et al. 2003). We compared 33 samples consisting of 16 taxa from the Arboleda and 41 samples consisting of 16 taxa from the Sura-60. Thirteen taxa overlapped between the two streams in this analysis, three taxa were found only in the Arboleda (Baetidae, Naucoridae, and Tricorythidae), and three taxa were found only in the Sura-60 (Calamoceratidae, Gomphidae, and Lutrochidae).

Differences in elemental composition between samples of similarly sized (within 2 mm), conspecific taxa between the two streams were analyzed using a paired *t*-test. Differences among FFGs and orders were analyzed with two-way ANOVA. Where stream identity and the interaction term (stream \times FFG or stream \times order) were both significant, Tukey's honest significant difference was used to compare differences between streams for each FFG or order. All analyses were conducted in SAS (SAS Institute 2001). All data are presented as either %C, N, and P of dry mass or as molar ratios.

Results

P content of basal food resources

Leaf litter %P increased with SRP from 0.05 to 0.22 %P ($r^2 = 0.91$, $P = 0.0008$), and led to subsequent decreases in C:P from 1986 to 466 ($r^2 = 0.88$, $P = 0.0016$) and N:P from 66 to 17 ($r^2 = 0.91$, $P = 0.0007$; Fig. 1). Leaf litter %C, %N, and C:N did not differ among streams.

The P content of epilithon was more variable than that of leaf litter, but %P increased significantly with SRP from 0.12 to 0.47 %P ($r^2 = 0.83$, $P = 0.0042$). Epilithon decreased in C:P from 245 to 41 ($r^2 = 0.90$, $P = 0.001$) with increasing stream SRP (Fig. 1). Epilithon N:P ranged from 5.4 to 28.9 but was not related to stream SRP. Epilithon %C, %N, and C:N did not differ among streams.

The P content of basal food resources was elevated in the high-SRP Arboleda relative to the low-SRP Sura-60. P content by dry weight increased fourfold for leaf litter (0.22 vs. 0.05 %P), more than twofold for epilithon (0.47

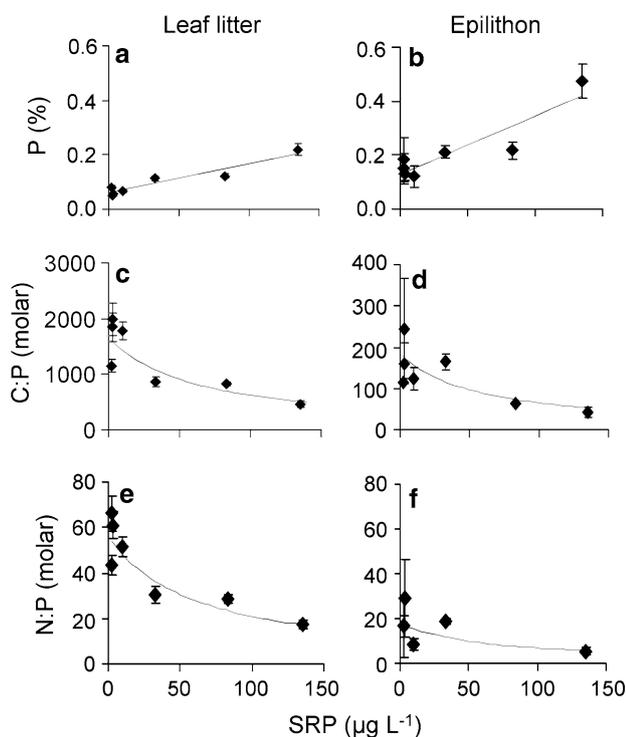


Fig. 1 Changes in mean P, C to P molar ratio (C:P), and N to P molar ratio (N:P) (\pm SE) of leaf litter (a, c, e) and epilithon (collected from tiles; b, d, f) from seven streams along a natural P gradient. Stream soluble reactive P (SRP) values are means from each stream studied in La Selva Biological Station, Costa Rica January 2006–August 2007. For C:P and N:P, linear regressions were plotted for P:C and P:N, respectively; see text for explanation. Regression statistics: a $r^2 = 0.91$, $P = 0.0008$, b $r^2 = 0.83$, $P = 0.0042$, c $r^2 = 0.88$, $P = 0.0016$, d $r^2 = 0.90$, $P = 0.001$, e $r^2 = 0.91$, $P = 0.0007$, f $r^2 = 0.15$, $P = 0.46$

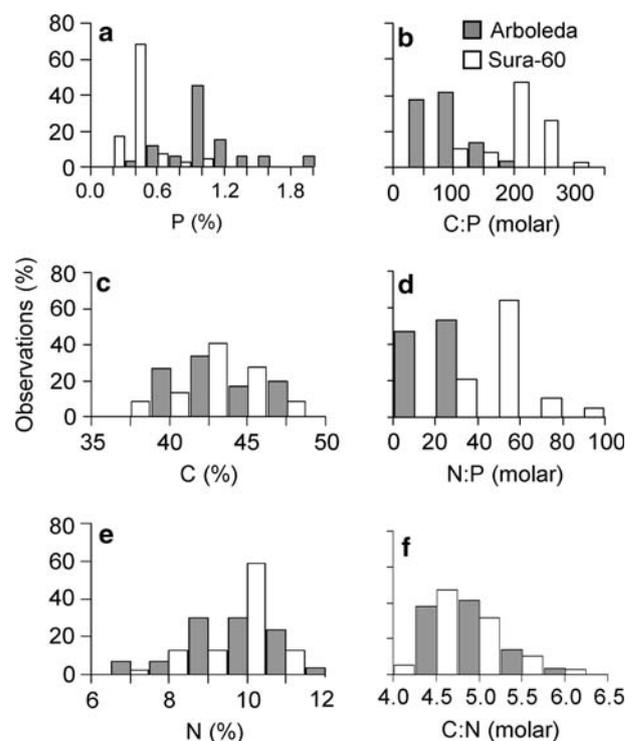


Fig. 2 Frequency histograms of invertebrate body P, C, N, C:P, N:P, and C:N for high-P Arboleda ($135 \mu\text{g SRP l}^{-1}$; shaded bars) and low-P Sura-60 ($2.8 \mu\text{g SRP l}^{-1}$; open bars) streams. Summary statistics (n , mean \pm SD): a Arboleda, 33, 1.1 ± 0.4 ; Sura-60, 41, 0.5 ± 0.2 ; b Arboleda, 29, 124.0 ± 83.8 ; Sura-60, 38, 238.8 ± 71.3 ; c Arboleda, 30, 44.4 ± 2.9 ; Sura-60, 37, 42.8 ± 7.7 ; d Arboleda, 30, 24.4 ± 17.6 ; Sura-60, 39, 47.2 ± 13.2 ; e Arboleda, 30, 10.3 ± 1.4 ; Sura-60, 38, 10.2 ± 0.9 ; f Arboleda, 29, 5.1 ± 0.4 ; Sura-60, 38, 5.1 ± 0.5

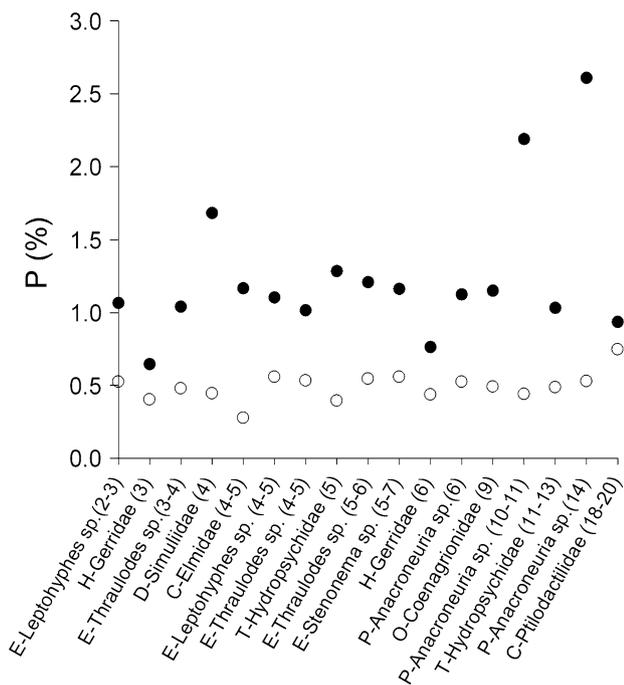


Fig. 3 P content (%P) of paired invertebrates of similar size from high-P Arboleda (solid circles) and low-P Sura-60 (open circles) streams. Numbers in parentheses indicate length of larvae in millimeters; taxa are arranged in order of increasing size. C Coleoptera, E Ephemeroptera, P Plecoptera, O Odonata, T Trichoptera, D Diptera, H Hemiptera

vs. 0.18 %P), sixfold for FPOM (0.18 vs. 0.03 %P), and fivefold for filamentous algae (0.30 vs. 0.06 %P).

P content of invertebrate consumers

P content in invertebrate consumer assemblage averaged twofold higher in the high-P Arboleda (mean %P = 1.15) relative to the low-P Sura-60 (mean %P = 0.51; Fig. 2). In the Arboleda, 79% of invertebrates sampled had a P content >1.0%. In contrast, in the Sura-60, only 5% of invertebrates sampled had a P content exceeding 1.0%. The Arboleda consumer assemblage was significantly higher in %P ($t = 8.05, df = 40.4, P < 0.0001$), and lower in C:P ($t = 6.10, df = 66, P < 0.0001$) and N:P ($t = 6.19, df = 68, P < 0.0001$; Fig. 2) relative to the Sura-60 assemblage. Invertebrate %C, %N, and C:N did not differ among streams ($P > 0.05$).

Paired comparisons of similar-sized conspecific taxa between the two streams showed that these taxa deviate from strict homeostasis, with a twofold average increase in P content in the high-P Arboleda. Invertebrates in the high-P Arboleda had significantly higher %P ($t = 6.70, df = 16, P < 0.0001$; Fig. 3) and lower C:P ($t = 7.04, df = 13, P < 0.0001$) and N:P ($t = 7.72, df = 13, P < 0.0001$) compared to the low-P Sura-60. Paired taxa did not differ in %C, %N, and C:N between the two streams ($P > 0.05$).

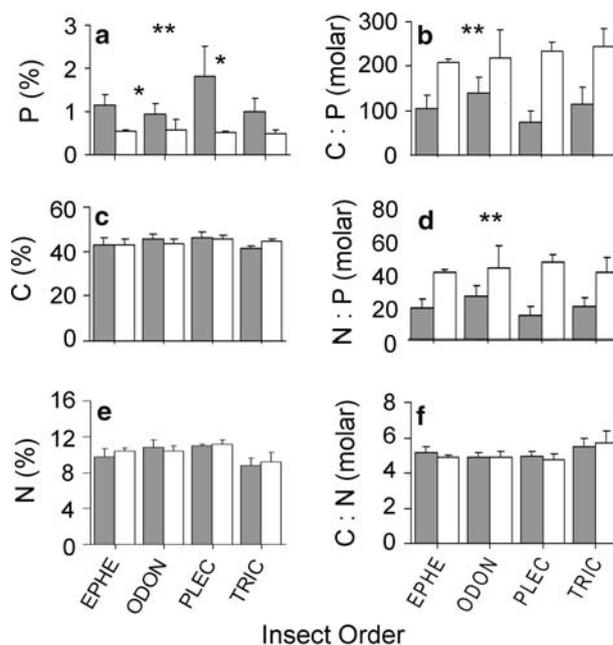


Fig. 4 P, C, N, C:P, N:P, and C:N (mean \pm 1 SE) for invertebrates of four dominant insect orders in the Arboleda (shaded bars) and Sura-60 (open bars) streams. Overall statistical differences in elemental composition between streams ($P < 0.05$) indicated by double asterisks. Differences in elemental composition within an order indicated by a single asterisk. EPHE Ephemeroptera, ODON Odonata, PLEC Plecoptera, TRIC Trichoptera

When grouped by order, %P was significantly higher among Arboleda invertebrates (mean 1.17) compared to Sura-60 invertebrates (mean 0.52, $F_{1,58} = 57.25, P < 0.0001$; Fig. 4). Mean %P differed among orders ($F_{8,58} = 3.15, P = 0.005$), ranging from 0.68 (Odonata) to 1.16 (Plecoptera). The difference in insect %P between streams varied by order (stream \times order interaction: $F_{8,58} = 3.42, P < 0.01$), with significant differences between streams observed in Diptera, Ephemeroptera, and Plecoptera. Insect C:P was significantly lower for orders in the Arboleda (stream effect: $F_{1,52} = 13.53, P = 0.0006$). No differences in C:P were observed among different orders ($F_{8,52} = 1.02, P = 0.43$), and differences between streams in C:P did not vary by order (stream \times order interaction: $F_{7,52} = 0.93, P = 0.49$). Likewise, insect N:P was lower for orders in the Arboleda ($F_{1,53} = 13.17, P = 0.0006$), but no differences were detected among different orders ($F_{8,53} = 0.70, P = 0.69$) and stream effects did not vary among orders (stream \times order interaction: $F_{8,53} = 0.95, P = 0.48$).

Insect %C varied among orders ($F_{7,52} = 47.04, P < 0.0001$), ranging from 43.0 (Trichoptera) to 48.6 (Hemiptera), but did not vary between streams ($F_{1,52} = 0.30, P = 0.58$), nor was there a significant stream \times order interaction ($F_{5,52} = 1.37, P = 0.25$). Insect %N did not vary between streams ($F_{1,53} = 0.36,$

$P = 0.55$) or among orders ($F_{8,53} = 1.60$, $P = 0.15$), and there was no stream \times order interaction ($F_{8,53} = 1.32$, $P = 0.25$). Insect C:N varied among orders ($F_{8,52} = 2.61$, $P = 0.02$), ranging from 3.38 (Diptera) to 5.61 (Trichoptera). Insect C:N did not vary between streams ($F_{1,52} = 0.10$, $P = 0.75$), nor was there a significant stream \times order interaction ($F_{7,52} = 1.10$, $P = 0.37$).

When grouped by FFG, insects were again significantly higher in %P ($F_{1,64} = 30.52$, $P < 0.001$) in the high-P Arboleda (mean = 1.11) relative to the low-P Sura-60 (mean = 0.51; Fig. 5). There was no difference in %P among different FFGs ($F_{4,64} = 0.17$, $P = 0.95$), nor was there a significant stream \times FFG interaction ($F_{4,64} = 0.16$, $P = 0.96$). Likewise, insect C:P was lower across insect FFGs in the Arboleda ($F_{1,57} = 17.12$, $P = 0.001$) but did not vary among different FFGs ($F_{4,57} = 0.24$, $P = 0.91$), and there was no stream \times FFG interaction ($F_{4,57} = 0.73$, $P = 0.57$). Insect N:P was also lower in the Arboleda (mean = 22.2) relative to the Sura-60 (mean = 45.8; $F_{1,59} = 21.93$, $P < 0.0001$). There were no differences in N:P among different FFGs ($F_{4,59} = 0.62$, $P = 0.65$), nor was there a stream \times FFG interaction ($F_{4,59} = 0.62$, $P = 0.65$).

There was no difference in insect %C between streams ($F_{1,57} = 0.04$, $P = 0.85$) or among FFGs ($F_{4,57} = 1.91$, $P = 0.12$), and the stream \times FFG interaction term was not

significant ($F_{4,57} = 1.09$, $P = 0.37$). Insect %N did not differ between streams ($F_{1,59} = 2.50$, $P = 0.12$), but there were differences among FFGs ($F_{4,59} = 10.45$, $P < 0.0001$), as %N ranged from 8.8 (shredders) to 11.0 (predators). There was a stream \times FFG interaction for %N ($F_{4,59} = 3.09$, $P = 0.02$), although there were no significant differences in %N within a given FFG between the two streams. Likewise, there was no difference in C:N between streams ($F_{1,57} = 3.51$, $P = 0.07$), but differences among FFGs were significant ($F_{4,57} = 6.35$, $P = 0.0003$), as mean C:N ranged from 4.88 (predator) to 5.84 (shredder). The stream \times FFG interaction term was not significant for C:N ($F_{4,57} = 1.12$, $P = 0.36$).

Discussion

Observed high P levels in basal resources and elevated P levels across the invertebrate assemblage indicate a general deviation from strict homeostasis among invertebrate consumers. Our results are unique in the generality of the observed response of all invertebrate consumers to P enrichment (i.e., across orders and FFGs). Even taxa identified as predators showed elevated P content in the high-P stream, the first such evidence for altered elemental composition in higher trophic levels. In contrast, other studies have reported highly variable invertebrate P content among taxa in nutrient-enriched streams, or no relationship between elevated stream P and invertebrate P content. For example, in a comparison of streams above and below municipal wastewater treatment plant inputs (3–29 $\mu\text{g l}^{-1}$ total P), Bowman et al. (2005) found an increase in the P content of heptageniid mayflies (Ephemeroptera) at only one of four high-nutrient sites, despite a tenfold increase in epilithon P content. In a survey of streams ranging in SRP from 3 to 99 $\mu\text{g l}^{-1}$, Evans-White et al. (2005) found no relationship between stream SRP and invertebrate P content. After 2 years of an experimental N + P addition in a detritus-based headwater stream (increasing SRP from 7 to 46 $\mu\text{g l}^{-1}$), Cross et al. (2003) only found significant increases in P content in one group of primary consumers (Trichoptera). In contrast, our findings show that insect consumers were enriched in P across all taxonomic and functional feeding groups in the high-P Arboleda.

The generality of P enrichment across invertebrate consumers in the naturally high-P Arboleda is a consequence of high historic levels of P loading, resulting in extremely high P content across all basal food resources. Basal food resources in the Arboleda were highly enriched in P relative to low-P La Selva streams and are higher than values reported from other nutrient-enriched streams. Mixed-species leaf litter collected from streams across the SRP gradient showed a fourfold increase in P content,

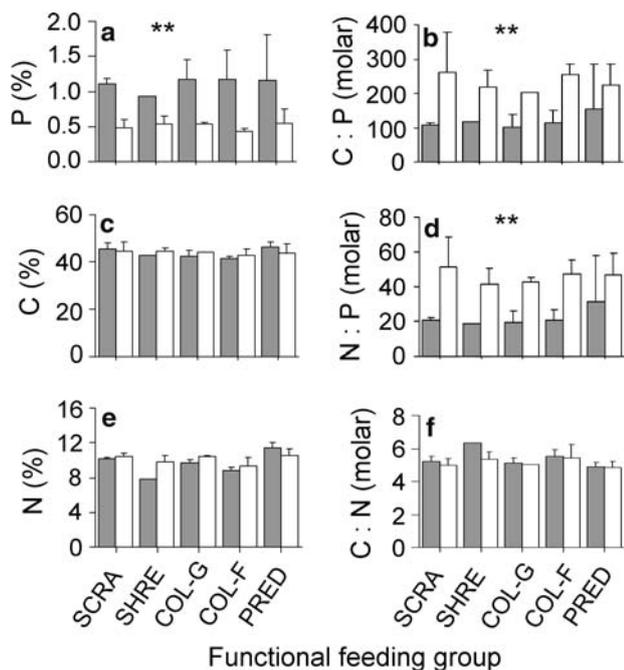


Fig. 5 P, C, N, C:P, N:P, and C:N (mean \pm 1 SE) for invertebrates from five functional feeding groups in the Arboleda (shaded bars) and Sura-60 (open bars) streams. Overall statistical differences in elemental composition between streams ($P < 0.05$) indicated by double asterisks. SCRA Scraper, SHRE shredder, COL-G collector gatherer, COL-F collector filterer, PRED predator

similar to values reported by Ardón et al. (2006) for single-species leaf packs incubated in the same study streams. Leaf litter P content in the Arboleda (0.22 %P) greatly exceeded the only other published values for leaf litter from a nutrient-enriched stream (0.05 %P; Cross et al. 2003). Increased microbial biomass on leaf litter in our high-P study stream likely accounts for this difference (Cross et al. 2003; Ardón et al. 2006). Epilithon P content increased fourfold over the SRP range exhibited by our seven study streams, and values from our high-P study streams exceed reported values from other P-enriched streams (Stelzer and Lamberti 2001; Cross et al. 2003; Bowman et al. 2005), which could result from luxury P uptake by algal cells or increased densities of P-rich heterotrophic bacteria.

Our findings support the hypothesis that deviation from strict homeostasis may be a general trait among invertebrate consumers, observable when food resources are sufficiently P enriched. The paired comparison of taxa between the Arboleda and Sura-60 shows that similar-sized individuals of the same taxon were uniformly enriched in P in the high-P stream. All 17 samples of similar-sized taxa increased in P content in the high-P stream, from 1.3 to 4.7-fold (mean = 2.7; Table S1). In contrast, Cross et al. (2003) found a more limited response, with 15 of 24 comparable samples (of similar-sized taxa) enriched in P, from 1.1 to 4.0-fold (mean = 2.1). The difference in responses between these two studies is likely due to the much greater degree of basal resource P enrichment in our high-P study streams. Primary consumers in high-P La Selva streams consume food resources that are 2–6 times greater in P content relative to those in low-P streams, and invertebrate predators in high-P streams consume prey items that are twofold greater in P. By contrast, primary consumers in the nutrient-enriched stream studied by Cross et al. (2003) consumed food resources that were on average 1.5 times higher in P, and invertebrate predators fed on prey items that were enriched only 1.2 times in P over reference conditions.

The strength of homeostasis can be evaluated as the change in consumer body elemental composition relative to the change in the elemental content of its food resources, according to the equation:

$$\log(y) = \log(c) + \frac{\log(x)}{H}$$

where y = consumer stoichiometry, x = resource stoichiometry, c is a constant, and H represents strength of homeostatic regulation (Sterner and Elser 2002). Values of $H > 1$ are consistent with some degree of homeostasis (i.e., the increase in consumer P content is smaller than the increase in food resource P content), and very large H values reflect strict homeostasis. While controlled feeding

experiments are required to accurately determine H for a given taxon, we can estimate this value based on field data. Across invertebrate taxa in our study, we estimated values of H ranging from 0.4 to 5.4 (Fig. 6; Table S1). For comparison, among those taxa that exhibited increased P content in the study by Cross et al. (2003), we calculated H values that ranged from 0.3 to 11.0. Taxa classified as shredders had the lowest H values (i.e., they were the least homeostatic) among primary consumers in our study and among all consumers in the Cross et al. (2003) study. The most striking difference between the two datasets is that invertebrate predators showed a high degree of P enrichment and had the lowest calculated H values in our study (range 0.4–1.4), whereas most predators in Cross et al. (2003) did not increase in P content in the nutrient-enriched stream. We suspect that this difference is due to the higher degree of P enrichment in food resources of invertebrate predators in the high-P La Selva stream, rather than a higher degree of homeostasis among predators analyzed by Cross et al. (2003). These values should be interpreted with caution, however, since we assume that our measurements of food resource stoichiometry accurately represent the nutrient content of food ingested by the consumer (e.g., no selective grazing). In a parallel study (G. E. Small, unpublished data), the P content of chironomid larvae from both the Arboleda and Sura-60 increased from 0.5 to 1.0 %P with increasing P content of leaf litter when groups of larvae from each source stream were reared on leaf litter conditioned in streams across the natural P gradient. These results add weight to our contention that differences in

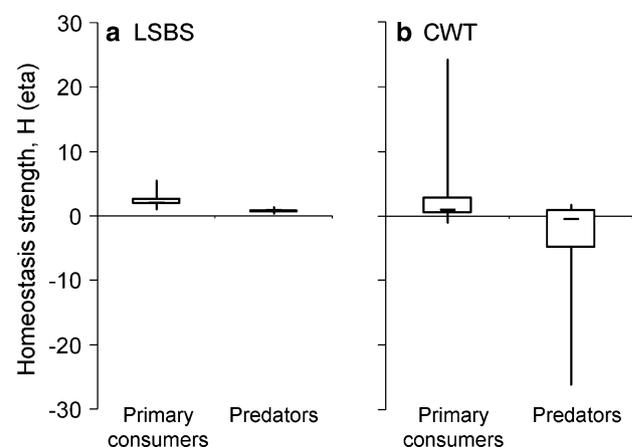


Fig. 6 Comparison of strength of homeostasis (H) for primary consumers and predators from **a** La Selva Biological Station (*LSBS*) and from a nutrient enrichment experiment at **b** Coweeta Hydrologic Laboratory (*CWT*; Cross et al. 2003). Higher H values denote strict homeostasis, whereas low, positive values of H reflect a high sensitivity of consumer stoichiometry relative to changes in food nutrient content. Negative values result from consumers decreasing in nutrient content in the presence of nutrient-enriched food resources. Details on calculations of H are provided in Table S1

invertebrate P content between streams are due to deviation from strict homeostasis.

Another potential mechanism for how the primary consumer assemblage could increase in response to P-enriched food resources is through a community shift toward species with higher P demand (Singer and Battin 2007). This is unlikely since our two study streams were purposely selected for their similarity in invertebrate species composition (Ramírez et al. 2006). It is also possible that populations of invertebrate consumers in naturally high-P streams could have evolved to have a higher P content. However, some degree of genetic isolation would also be required, which is very unlikely in this landscape. Our study sites are all within close proximity (within 2 km), and low-P streams become high-P at the gradient break (at 35 m above sea level) where high-solute springs emerge. In order to maintain genetic isolation sufficient for adaptation to very local conditions, ovipositing female insects would have to show high site fidelity, and invertebrate drift would have to be low. In contrast, lowland tropical streams of La Selva are characterized by large numbers of drifting invertebrates (Ramírez and Pringle 1999, 2001). Similarly, Peckarsky et al. (2005) found that phenotypic plasticity, rather than genetic differentiation, was the primary explanation for different invertebrate responses between adjacent streams with contrasting environmental conditions. Thus, while it is possible that invertebrate populations between streams may differ in their P demands, our results support the conclusion that increased P content in consumers is primarily due to deviation from strict homeostasis.

A fraction of the elevated P content measured in invertebrates in the high-P Arboleda is due to the elevated P content of food in their guts. To minimize the effect of undigested food, some studies have reported consumer biomass nutrient values that were based on measurements made after guts were removed (e.g., Cross et al. 2003) or after consumers were allowed time to clear their guts (e.g., Evans-White et al. 2005). Potential effect of actual gut contents on our nutrient measurements may be evaluated by a simple mixing model:

$$(GC_{\text{mass}} \times GC_{\%P}) + (B_{\text{mass}} \times B_{\%P}) = S_{\text{mass}} \times S_{\%P}$$

where GC is gut contents, *B* is body (i.e., insect tissue without guts), and *S* is the composite sample which includes gut contents. For example, a consumer feeding on leaf litter, whose gut contents constitute 20% of its dry mass (Cain et al. 1995), ingests food that is 0.22 %P in the Arboleda and 0.05 %P in the Sura-60. If similar-sized individuals (consumer tissue + gut contents) are 0.97 %P in the Arboleda and 0.62 %P in the Sura-60, then the consumer body %P (with gut contents excluded) would be 1.16 and 0.76 %P, respectively. Even with basal food

resources increasing up to fivefold in P content, the total-P represented in consumer gut contents is a small fraction of the total-P in the organism and should be a relatively small source of error in our measurements.

Primary consumers in many aquatic ecosystems face P-limited growth due to the large discrepancy between their body C:P ratio and the C:P ratio of their food resources (Cross et al. 2003). In contrast, the high degree of P enrichment in basal food resources in the high-P Arboleda is likely to release consumers from P limitation. Leaf litter C:P decreased from approximately 2,000 (molar ratio) in our low-P study streams to 500 in the high-P Arboleda, much lower than threshold elemental ratio ($TER_{C:P}$) values (i.e., the optimal value of food C:P for a given consumer) calculated for detritivorous aquatic invertebrates (mean $\sim 1,000$; Frost et al. 2006). Similarly, epilithon C:P values ranged from 200 in low-P streams to 50 in the highest-P stream, relative to a mean $TER_{C:P}$ of 200 for grazing invertebrates (Frost et al. 2006). This alleviation from P limitation in primary consumers is likely the cause of elevated growth rates that have been recorded for some primary consumers in high-P La Selva streams (Rosemond et al. 2002; Ramírez and Pringle 2006).

In contrast to prior studies, our study found that between-stream variation in P content within each taxon greatly exceeded within-stream variation among different taxa, suggesting that environment may be as important in controlling consumer stoichiometry as phylogeny. In fact, P content of some invertebrate taxa from our high-P stream was similar to values from some vertebrates reported by Vanni et al. (2002). Stoichiometric theory, under the assumption of strict homeostasis, has attributed variation in measured C:P values among taxa solely to differences in P demand by these organisms (Sterner and Elser 2002). However, if consumers are not strictly homeostatic, then high C:P values measured in invertebrate consumers could also reflect P limitation by these organisms (Hillebrand et al. 2008; Shimizu and Urabe 2008). The increase in P content of the entire consumer assemblage that we observed in the high-P Arboleda is consistent with this hypothesis, and adds to the weight of evidence that deviation from strict homeostasis may be a common trait in invertebrate consumers. Additionally, models predicting nutrient limitation in consumers typically assume strict homeostasis (Sterner and Elser 2002), and calculations of $TER_{C:P}$ values are highly sensitive to input values for consumer elemental composition (Frost et al. 2006). Relaxing the assumption of strict homeostasis presents challenges and opportunities for advancing our understanding of how nutrient limitation affects the growth of consumers.

The most significant ecological effects of P-enriched food resources would be expected for primary consumers, which are most likely to be P limited (Sterner and Elser 2002).

Secondary production of primary consumers in an experimentally nutrient-enriched stream was between 1.2 and 3.3 times higher than in a reference stream, and resulted in greater secondary production of invertebrate predators (Cross et al. 2006). Combined with increases in secondary production, elevated P content in primary consumers would have a multiplicative effect on the amount of P moving through the food web. While nutrient limitation may be common in primary consumers, it is not limited to this trophic level (Boersma et al. 2008). Invertebrate predators, feeding on relatively low-P aquatic insects, also have the potential to be P limited in low-P streams. The increased %P observed in invertebrate predators in the Arboleda suggests that any P limitation affecting this trophic level was alleviated in the high-P stream. If predator taxa are ingesting excess P in high-P streams, they should excrete this excess P at elevated rates. Elevated P content in aquatic invertebrates also suggests that P export to the terrestrial ecosystem through insect emergence may be elevated, especially if combined with increases in secondary production.

In this study, we document a twofold increase in P content in invertebrate consumers across multiple taxonomic and functional feeding groups in a stream which has received high P loading over millennia. Anthropogenic nutrient loading is a ubiquitous problem that is becoming exacerbated in many rivers due to the combination of increased effluent discharge and increased water withdrawals. If, as our results suggest, deviation from strict homeostasis is a common trait in freshwater invertebrates, then our study may represent a window into the future indicating how stoichiometric relationships throughout food webs may be altered by continuing anthropogenic nutrient loading in aquatic ecosystems.

Acknowledgments We are grateful to Minor Hidalgo for assistance in the field and Tom Maddox for laboratory assistance. Thanks to Wyatt Cross for graciously sharing data, and to Andrew Mehring and Darold Batzer for statistical advice. Additionally, this manuscript was greatly improved by discussion with Jon Benstead, John Davis, Amy Rosemond, Becky Bixby, Susan Kilham, Steve Thomas, John Duff, Frank Triska, and the Pringle lab. Research funding was provided by the National Science Foundation (DEB 0545463; C. M. Pringle, F. J. Triska, and A. Ramírez). G. E. Small is supported in part by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program. EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA. All experiments and sampling discussed here comply with both USA and Costa Rican regulations.

References

- Acharya K, Kyle M, Elser JJ (2004) Biological stoichiometry of *Daphnia* growth: an ecophysiological test of the growth rate hypothesis. *Limnol Oceanogr* 49:656–665
- Allen AP, Gillooly JF (2008) Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol Lett* 12:369–384
- American Public Health Association (APHA) (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, New York
- Andersen T, Hessen DO (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnol Oceanogr* 36:807–814
- Ardón M, Stallcup LA, Pringle CM (2006) Does leaf quality mediate the stimulation of leaf breakdown by phosphorus in Neotropical streams? *Freshwater Biol* 51:616–633
- Boersma M, Aberle N, Hantsche FM, Schoo KL, Wiltshire KH, Malzahn AM (2008) Nutritional limitation travels up the food chain. *Int Rev Hydrobiol* 93:479–488
- Bowman MF, Chambers PA, Schindler DW (2005) Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. *Freshwater Biol* 50:1836–1852
- Cain DJ, Luoma SN, Axtmann EV (1995) Influence of gut content in immature aquatic insects on assessments of environmental metal contamination. *Can J Fish Aquat Sci* 52:2736–2746
- Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol Appl* 8:559–568
- Carrillo P, Villar-Argaiz M, Medina-Sánchez JM (2001) Relationship between N:P ratio and growth rate during the life cycle of calanoid copepods: an in situ measurement. *J Plankton Res* 23:537–547
- Cross WF, Benstead JP, Rosemond AD, Wallace JB (2003) Consumer-resource stoichiometry in detritus-based streams. *Ecol Lett* 6:721–732
- Cross WF, Wallace JB, Rosemond AD, Eggert SL (2006) Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. *Ecology* 87:1556–1564
- DeMott WR, Gulati RD, Siewertsen K (1998) Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. *Limnol Oceanogr* 43:1147–1161
- Elser JJ, Schampel JH, Kyle M, Watts J, Carson EW, Dowling TE, Tang C, Roopnarine PD (2005) Response of grazing snails to phosphorus enrichment of modern stromatolitic microbial communities. *Freshwater Biol* 50:1826–1835
- Evans-White MA, Stelzer RS, Lamberti GA (2005) Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. *Freshwater Biol* 50:1786–1799
- Fink P, Von Elert E (2006) Physiological responses to stoichiometric constraints: nutrient limitation and compensatory feeding in a freshwater snail. *Oikos* 115:484–494
- Frost PC, Benstead JP, Cross WF, Hillebrand H, Larson JH, Xenopoulos MA, Yoshida T (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecol Lett* 9:774–779
- Hillebrand H, Frost P, Liess A (2008) Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. *Oecologia* 155:619–630
- Jackson JK, Sweeney BW (1995) Egg and larval development times for 35 species of tropical stream insects from Costa Rica. *J North Am Benthol Soc* 14:115–130
- Jones JB Jr, Wolf B, Mills HA (1991) Plant analysis handbook 1: methods of plant analysis and interpretation. Micro-Macro, Athens, pp 195–196
- Merritt RW, Cummins KW (1996) An introduction to the aquatic insects of North America, 3rd edn. Kendall/Hunt
- Peckarsky BL, Hughes JM, Mather PB, Hillyer M, Encalada AC (2005) Are populations of mayflies living in adjacent fish and fishless streams genetically differentiated? *Freshwater Biol* 50:42–51

- Pringle CM, Triska FJ (1991) Effects of geothermal waters on nutrient dynamics of a lowland Costa Rican stream. *Ecology* 72:951–965
- Pringle CM, Triska FJ, Broder G (1990) Spatial variation in basic chemistry of streams draining a volcanic landscape on Costa Rica's Caribbean slope. *Hydrobiologia* 206:73–85
- Pringle CM, Rowe GL, Triska FJ, Fernandez JF, West J (1993) Landscape linkages between geothermal activity and solute composition and ecological response in surface waters draining the Atlantic slope of Costa Rica. *Limnol Oceanogr* 38:753–774
- Ramírez A, Pringle CM (1999) Invertebrate drift and benthic community dynamics in a lowland neotropical stream, Costa Rica. *Hydrobiologia* 386:19–26
- Ramírez A, Pringle CM (2001) Spatial and temporal patterns of invertebrate drift in streams draining a Neotropical landscape. *Freshwater Biol* 46:47–62
- Ramírez A, Pringle CM (2006) Fast growth and turnover of chironomid assemblages in response to stream phosphorus levels in a tropical lowland landscape. *Limnol Oceanogr* 51: 189–196
- Ramírez A, Pringle CM, Douglas M (2006) Temporal and spatial patterns in stream physicochemistry and insect assemblages in tropical lowland streams. *J North Am Benthol Soc* 25:108–123
- Rosemond AD, Pringle CM, Ramírez A, Paul MJ, Meyer JL (2002) Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnol Oceanogr* 47:278–289
- Sanford RL, Paaby P, Luvall JC, Phillips E (1994) Climate, geomorphology, and aquatic systems. In: McDade LA, Bawa KS, Hespenheide HA, Hartshorne GS (eds) *La Selva: ecology and natural history of a neotropical rainforest*. University of Chicago Press, Chicago, pp 19–33
- SAS Institute (2001) SAS version 8.2 for Windows. SAS Institute, Cary
- Schade JD, Kyle M, Hobbie SE, Fagan WF, Elser JJ (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecol Lett* 6:96–101
- Shimizu Y, Urabe J (2008) Regulation of phosphorus stoichiometry and growth rate of consumers: theoretical and experimental analyses with *Daphnia*. *Oecologia* 155:21–31
- Singer GA, Battin TJ (2007) Anthropogenic subsidies alter stream consumer-resource stoichiometry, biodiversity, and food chains. *Ecol Appl* 17:376–389
- Stelzer RS, Lamberti GA (2001) Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnol Oceanogr* 46:356–367
- Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton
- Sterner RW, Hessen DO (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annu Rev Ecol Syst* 25:1–29
- Triska FJ, Pringle CM, Duff JH, Avanzino RJ, Ramírez A, Ardón M, Jackman AP (2006) Soluble reactive phosphorus transport and retention in tropical, rainforest streams draining a volcanic and geothermally active landscape in Costa Rica: long-term concentration patterns, pore water environment, and response to ENSO events. *Biogeochemistry* 81:131–143
- Urabe J, Watanabe Y (1992) Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnol Oceanogr* 37:244–251
- Vanni MJ, Flecker AS, Hood JM, Headworth JL (2002) Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. *Ecol Lett* 5:285–293
- Woods HA, Perkins MC, Elser JJ, Harrison JF (2002) Adsorption and storage of phosphorus by larval *Manduca sexta*. *J Insect Physiol* 48:555–564

Copyright of *Oecologia* is the property of Springer Science & Business Media B.V. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.