

## Effects of suburbanization on foodweb stoichiometry of detritus-based streams

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**Abstract.** Changes to nutrient loads may affect the stoichiometry of urban or suburban stream food webs. We quantified foodweb stoichiometry in 9 detritus-based headwater streams draining forested or suburban watersheds in northeastern, coastal Massachusetts (USA). We measured C, N, and P content (%C, %N, %P) and relative ratios (C:N:P) of basal food resources and macroinvertebrate consumers in each stream. Greater dissolved nutrient concentrations in suburban streams did not cause significant changes in overall elemental content (%C, %N, %P) of basal food resources, but their N:P ratios decreased in high-nutrient streams, indicating that higher nutrients do affect food quality. The nutrient content of most macroinvertebrate taxa consuming these altered food resources was unaffected by suburban nutrient enrichment. However, some taxa deviated from strict homeostasis. Samples of these stoichiometrically variable taxa from the most enriched suburban streams had variable %N, a result suggesting that shifts in %P may not be the only means by which body stoichiometry is altered by nutrient enrichment. Moreover, many of the taxa with nutrient contents that differed across stream groups are typically more abundant in suburban than in forested streams, a result suggesting a potential link between consumer–resource stoichiometry and the tolerance of certain taxa for nutrient enrichment. Overall, our results are consistent with those of other studies on ecosystem properties or functions in urban streams in which the complex nature of urban effects does not always yield general patterns seen in less-affected systems.

**Key words:** food webs, stoichiometry, detritus, suburban, urban, stream.

Landuse change strongly affects global ecosystems (Grimm et al. 2008a). Conversion of undeveloped areas to urban and suburban areas greatly modifies the structure, function, and dynamics of aquatic ecosystems (Paul and Meyer 2001, Walsh et al. 2005, Grimm et al. 2008b). Resulting effects on stream ecosystems include changes in geomorphology, hydrology, water chemistry, and stream biota, collectively known as the urban stream syndrome (Paul and Meyer 2001, Walsh et al. 2005), which affects local community structure and ecosystem function, typi-

cally with cascading effects on downstream ecosystems (Meyer et al. 2005).

One common symptom of the urban stream syndrome is an increase in nutrients (Paul and Meyer 2001, Meyer et al. 2005, Walsh et al. 2005) associated with point and nonpoint sources (Malmquist and Rundle 2002) and with decreased efficiency in removing and retaining N and P (Wollheim et al. 2005, Withers and Jarvie 2008). In the USA, point-source pollution has declined significantly since the implementation of the Clean Water Act in 1972, leaving nonpoint source pollution as the most important cause of lake, stream, and coastal eutrophication (Carpenter et al. 1998). Greater nonpoint source pollution in urban and suburban than in forested watersheds is driven by a combination of high impervious area and more nutrient sources, e.g.,

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fertilizers and human waste (Withers and Jarvie 2008).

One way to assess the effects of altered nutrient inputs into an ecosystem is to use the organizing principles of ecological stoichiometry. Ecological stoichiometry considers the flow and transfer of multiple key elements through ecological interactions (Reiners 1986, Sterner and Elser 2002). Evaluating the chemical composition (nutrient content and relative nutrient ratios) of ecosystem components provides information on physiological, behavioral, and environmental stresses on organisms in an ecosystem (Sterner and Elser 2002, Cross et al. 2005). Ecological stoichiometry also can be used to understand the feedback mechanisms that mediate how consumers affect their environment (Sterner et al. 1992, Elser et al. 1998, Vanni 2002).

Alterations in nutrient content of consumers and basal food resources affect foodweb structure and function. In an experimental study in North Carolina (USA), addition of N and P to a forested stream shifted the elemental stoichiometry of some macroinvertebrate taxa (Cross et al. 2003). In the Canadian Rockies, effluent from a sewage treatment plant affected stoichiometry of basal food resources, and both C:P and C:N were lower downstream than upstream of the effluent discharge. Invertebrate community composition differed between upstream and downstream reaches and total biomass was higher in the downstream reach (Bowman et al. 2005). P enrichment from a wastewater treatment plant in Austria led to a shift in macroinvertebrate community structure toward faster-growing, P-rich consumers and a shift in C:N of some consumer taxa, indicating a lack of elemental homeostasis for certain species (Singer and Battin 2007).

Research on stoichiometric changes to nutrient-enriched stream ecosystems has been focused mostly on point-source inputs (but see Tsoi et al. 2011). The main objective of our study was to quantify the stoichiometric consequences of nonpoint-source nutrient pollution for detritus-based, headwater streams draining suburban areas. We sampled 9 streams, representing 3 groups based on type of nutrient enrichment, to quantify the stoichiometry of their foodweb components. We hypothesized that higher in-stream nutrient concentrations in suburban streams would cause higher nutrient content of basal food resources. We also hypothesized that increases in nutrient content of basal resources would be propagated up the food chain, resulting in higher P content in consumer taxa sampled from streams under higher nutrient loading (i.e., deviation from strict elemental homeostasis).

## Methods

### *Study site*

We collected samples of foodweb components including basal resources and benthic macroinvertebrates during spring and summer 2008 from 9 headwater stream reaches with diverse N and P inputs caused by different degrees of suburbanization in their watersheds. All sampling sites were in the Parker and Ipswich River basins in northeastern Massachusetts (USA) and are part of the Plum Island Ecosystem Long-Term Ecological Research site. Both rivers drain low- to moderate-relief coastal lowlands and their watersheds have relatively poor drainage (Baker et al. 1964). Rainfall averages 1150 mm/y, spread evenly throughout the year (Claessens et al. 2006).

We selected streams to fit into 1 of 3 stream groups based on an earlier study (Wollheim et al. 2005). Reference (Ref) streams had watersheds with little suburban development and correspondingly low dissolved inorganic N (DIN) and  $\text{PO}_4^{3-}$  concentrations. The remaining 6 streams were selected from more urbanized watersheds and represented a range of suburban development. Three streams had high DIN concentrations but low to moderate  $\text{PO}_4^{3-}$  concentrations (N stream group), and 3 had high DIN and high  $\text{PO}_4^{3-}$  concentrations (N+P stream group) (Table 1). We chose these 9 streams for sampling based on similarities in physical structure and riparian cover. All stream reaches were wadeable during base flow, of moderate gradient, and had gravel/cobble substrate and dense deciduous riparian-zone vegetation. We sampled each stream 3 times during the growing season (April, May, July) to characterize the stoichiometry of leaf litter, fine benthic organic matter (FBOM), epilithon, and benthic macroinvertebrates. We chose sampling dates to maximize macroinvertebrate diversity by sampling when different species were likely to be in the stream.

### *Water chemistry*

We collected monthly water samples during base-flow conditions from October 2007 through September 2008 to characterize the nutrient regimes of our study streams. These samples included unfiltered samples and samples filtered through 0.7- $\mu\text{m}$  pore-size, precombusted Whatman GF/F filters. We analyzed all filtered samples for  $\text{NO}_3^-$  with a Dionex ICS-1000 Dual Channel Ion Chromatograph (Dionex, Sunnyvale, California). We measured  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  with colorimetric methods on a SmartChem

TABLE 1. Watershed characteristics for the 9 study streams in coastal Massachusetts (USA). Ref = reference.

Stream group	Stream name	% impervious surface in watershed	Population density <sup>a</sup> (persons/km <sup>2</sup> )	Watershed area <sup>a</sup> (km <sup>2</sup> )
N+P	IS_102	25.2	979.2	3.8
	IS_169	11.5	174.8	0.6
	IS_122	6.0	309.3	1.4
N	IS_103	26.4	1149.2	0.6
	IS_152	11.7	418.7	1.5
	IS_128	7.3	346.1	0.9
Ref	PS_109	6.9	157.6	1.3
	IS_140	4.0	124.1	1.5
	CC	8.2	130.4	3.8

<sup>a</sup> From Wollheim et al. (2005)

robotic autoanalyzer (Westco Scientific Instruments, Brookfield, Connecticut) on filtered stream samples. We measured total N (TN) and total P (TP) by digesting the unfiltered stream samples with an alkaline digestion method (USGS 2003) followed by colorimetric analysis on the SmartChem. We measured total dissolved N (TDN) by digesting filtered stream samples and estimated dissolved organic N (DON) by subtracting DIN (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) concentrations from TDN.

#### Particulate organic matter

We sampled major basal food resource components 3 times at each study site throughout the growing season to quantify the influence of in-stream nutrients on food-resource stoichiometry. We collected suspended particulate organic matter (SPOM) concurrently with monthly water samples by filtering stream water through a preweighed 0.7- $\mu$ m pore-size GF/F filter. We removed epilithon from cobbles and large gravel with a wire brush during macroinvertebrate sampling (see below). We sampled FBOM from the stream bottom by agitating the top layer of organic matter and sampling the resuspended organic matter. All epilithon and FBOM samples were composites from 3 or 4 locations throughout each site. We filtered epilithon and FBOM samples in the laboratory through preweighed 0.7- $\mu$ m pore-size GF/F filters.

At each site we collected 10 natural, in-situ leaf-litter samples at randomly chosen locations during macroinvertebrate sampling (see below). In addition, we placed 5 litter bags (35  $\times$  15 cm with 5-mm polypropylene mesh) containing 5 g ( $\pm$ 0.1 g) of naturally senesced red maple (*Acer rubrum*) leaves collected from a single riparian location in each stream  $\sim$ 2 mo before each sampling event to quantify the influence of in-stream nutrient concentrations on leaf-litter stoichiometry. We weighed and dried all natural litter samples and the contents of the litter bags. We combined the

natural litter samples and homogenized them with a Wiley mill, and prepared a separate sample from the litter bags in the same manner.

#### Macroinvertebrates

We used the protocol described in the US Environmental Protection Agency Environmental Monitoring and Assessment Program for Surface Waters (Baker et al. 1997) with minor modifications when collecting macroinvertebrates. We separated large invertebrates immediately in the field and froze them. We retained smaller invertebrates on stacked sieves (125- to 250- $\mu$ m mesh size), placed each fraction on ice, separated the invertebrates from benthic material with the aid of a dissecting microscope within 12 h, and froze them. We identified all individuals to the lowest possible taxonomic level and measured them to the nearest 1 mm.

#### C, N, P analysis

We used the same methods to analyze C, N, and P content of all organic matter and macroinvertebrate samples. We dried leaf-litter samples and filters for a minimum of 48 h at 60°C and weighed them to the nearest 0.1 mg. We froze all macroinvertebrates individually. We lyophilized, weighed, and homogenized all samples to a fine powder with a spatula. We used a Perkin-Elmer Series II CHNS/O Analyzer 2400 (Perkin-Elmer, Waltham, Massachusetts) to measure C and N content. We processed samples for P content with alkaline persulfate digestion followed by molybdate blue colorimetry. We analyzed bovine muscle and pine needle standards (US National Institute of Standards and Technology, US Department of Commerce) concurrently with each set of samples. Percent recovery of P and N from the standards averaged 94% and 93%, respectively. All data are presented as %C, %N, or %P of dry mass or as molar ratios.

TABLE 2. Mean ( $\pm 1$  SE) nutrient concentrations in study streams during the study period (October 2007–September 2008). N:P is presented as a molar ratio. Values of each variable did not differ among groups with the same letters ( $p > 0.001$ ). DIN = dissolved inorganic N.

Stream group	Stream name	DIN ( $\mu\text{g/L}$ )		$\text{PO}_4^{3-}$ ( $\mu\text{g/L}$ )		N:P
N+P	IS_102	1249.98 (138.39)	A	20.75 (3.41)	A	217.12 (52.86)
	IS_169	873.76 (189.09)		19.51 (3.41)		172.06 (51.85)
	IS_122	280.70 (115.56)		22.30 (3.10)		66.60 (13.51)
N	IS_103	1166.92 (170.61)	A	13.63 (2.17)	B	303.24 (64.53)
	IS_152	1298.73 (161.36)		17.04 (2.79)		265.95 (56.81)
	IS_128	1206.42 (177.89)		13.63 (2.17)		298.91 (56.72)
Ref	PS_109	199.88 (54.07)	B	8.67 (2.17)	C	193.76 (53.42)
	IS_140	79.42 (27.73)		7.74 (2.17)		235.22 (82.88)
	CC	125.50 (37.40)		10.53 (2.17)		91.77 (16.77)

### Statistical analyses

We tested all data for normality with the Shapiro–Wilk goodness-of-fit test. We either  $\log(x)$ - or  $\arcsin\sqrt{x}$ -transformed data that did not meet goodness-of-fit criteria. We used a 2-way multivariate analysis of variance (MANOVA) with stream group (Ref, N, and N+P) and sampling month as the independent variables to test for the effect of ambient nutrient regime on %C, %N, %P, C:N, C:P, and N:P of food resources (epilithon, FBOM, SPOM, leaves from litter bags, and natural leaf litter). We used a 4-way MANOVA with stream group, sampling month, taxonomic order, and genus as the independent variables to test for the effect of ambient nutrient regime on %C, %N, %P, C:N, C:P, and N:P on macroinvertebrates (Evans-White et al. 2005). We used Wilk's likelihood ratio test to detect significance. For variables with significant multivariate tests, we followed the MANOVA with a univariate analysis of variance (ANOVA). If the univariate ANOVA was significant, we used Tukey's Honestly Significant Difference (HSD) multiple comparisons to test explicitly the influence of the significant variable (e.g., stream group) on specific variables (e.g., individual taxa). We calculated coefficients of variation (CV) of nutrient contents and ratios of macroinvertebrates within stream groups to assess overall variation in stoichiometry within stream communities. We used regression analysis to quantify the relationship between body size and nutrient content.

We used stepwise multiple regressions to test the influence of stream nutrients on nutrient contents and ratios within each foodweb component. We included surface-water TN, TP, DIN, TDN,  $\text{PO}_4^{3-}$ , DOC:TDN, DOC: $\text{PO}_4^{3-}$ , TN:TP, and TDN: $\text{PO}_4^{3-}$  as potential independent variables to assess their influence on

patterns in stream foodweb stoichiometry. We analyzed significant factors from the stepwise multiple regression in individual linear regressions to explore patterns of significant influence on nutrient content.

## Results

### Dissolved nutrient concentrations

During the 10-mo sampling period, mean DIN concentrations were  $>5\times$  higher in N and N+P streams than in Ref streams ( $p < 0.0001$ ; Table 2). Mean  $\text{PO}_4^{3-}$  concentrations were  $\sim 2\times$  higher in N+P streams than in Ref streams ( $p < 0.0001$ ) and  $\sim 1.5\times$  higher in N+P streams than in N streams ( $p < 0.0001$ ). Across all streams, DIN ranged from below detection to 2179.49  $\mu\text{g/L}$  whereas  $\text{PO}_4^{3-}$  ranged from 0.62 to 41.81  $\mu\text{g/L}$ . Stream-water N:P ratios ranged from 30 to 711, with significant differences among all stream groups ( $p < 0.001$ ; Table 2). In-stream DIN concentrations were not related to  $\text{PO}_4^{3-}$  concentrations ( $R^2 = 0.01$ ,  $p = 0.40$ ).

### Basal resources

Basal food resources had variable nutrient content, but nutrient content generally did not differ among stream groups (Table 3, Fig. 1). Nutrient content of epilithon and FBOM differed among stream groups (MANOVA,  $p = 0.01$  and  $0.02$ , respectively), and nutrient content of natural leaf litter differed among months (MANOVA,  $p = 0.0006$ ). Only nutrient content of epilithon was affected by a stream group  $\times$  month interaction ( $p = 0.025$ ). Epilithon had higher %N and N:P in Ref streams than in N and N+P streams (HSD, all  $p < 0.01$ ; Fig. 1). FBOM had higher N:P in Ref streams than in N+P streams (HSD,  $p = 0.03$ ). Nutrient content of SPOM, natural leaf litter, and leaves from litter bags did not differ among

TABLE 3. Results of multivariate analysis of variance (MANOVA) for effects of stream group (reference, N enriched, N+P enriched) and month on nutrient content of epilithon, fine benthic organic matter (FBOM), suspended particulate organic matter (SPOM), natural leaf litter, and standardized leaves in litter bags and univariate analysis of variance (ANOVA) results for significant MANOVAs. Significant differences are in bold.

Food resource	Independent variable	ANOVA																								
		MANOVA			%C			%N			%P			C:N			C:P			N:P						
		Wilks's	p		F	p		F	p		F	p		F	p		F	p		F	p		F	p		
Epilithon	Stream group	41.52	<b>0.010</b>	0.21	0.89	8.39	<b>0.0009</b>	0.90	0.46	1.59	0.23	0.82	0.50	9.04	<b>0.0006</b>											
	Month	34.00	0.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
FBOM	Stream group × month	45.27	<b>0.025</b>	1.54	0.23	2.33	0.093	2.03	0.13	2.60	0.069	3.44	<b>0.028</b>	1.28	0.31											
	Stream group	0.31	<b>0.015</b>	0.53	0.67	1.50	0.25	1.40	0.27	1.79	0.19	1.46	0.26	6.05	<b>0.005</b>											
SPOM	Month	0.64	0.28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Stream group × month	0.43	0.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Natural leaf litter	Stream group	65.54	0.39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Month	94.00	0.10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Litter bags	Stream group × month	121.87	0.48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Stream group	34.43	0.48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Litter bags	Month	24.00	<b>0.0006</b>	0.45	0.64	22.09	< <b>0.0001</b>	6.15	<b>0.010</b>	14.37	<b>0.0002</b>	7.04	<b>0.006</b>	0.88	0.43											
	Stream group × month	43.07	0.40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Litter bags	Stream group	42.91	0.051	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Month	30.00	0.85	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Litter bags	Stream group × month	53.54	0.97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

stream groups (MANOVA, all  $p > 0.05$ ), but %N, %P, C:N, and C:P of natural leaf litter differed among months (MANOVA, all  $p < 0.01$ ). ANOVAs on natural litter data did not reveal any consistent patterns in differences among months (e.g., increasing or decreasing nutrient content throughout the sampling period). Initial nutrient content might have differed among natural litter samples, so henceforth, we report results using standardized leaf litter from the litter bags (Fig. 1).

Stream nutrient concentrations affected nutrient content of leaves from litter bags less than nutrient content of other basal food resources. Only %P of leaves from litter bags was significantly related to stream nutrient concentrations. However, stream nutrient concentrations explained significant variation in %N, %P, C:N, C:P, and N:P of epilithon, FBOM, and SPOM. The models with the best fit were for epilithon %N ( $r^2 = 0.47$ ), epilithon C:N ( $r^2 = 0.45$ ), epilithon N:P ( $r^2 = 0.47$ ), FBOM N:P ( $r^2 = 0.56$ ), SPOM %N ( $r^2 = 0.44$ ), and SPOM C:N ( $r^2 = 0.55$ ). The independent variables most commonly included in the models of best fit were stream-water TDN,  $PO_4^{3-}$ , TN:TP, and  $DOC:PO_4^{3-}$ .

N:P ratios of basal resources declined across gradients of increasing stream-water TDN and  $PO_4^{3-}$  concentrations (Fig. 2A–F). N:P of epilithon (Fig. 2A, B) and FBOM (Fig. 2C, D) were negatively related to TDN and  $PO_4^{3-}$ , whereas N:P of SPOM was only weakly related to TDN and was not related to  $PO_4^{3-}$  (Fig. 2E, F). N:P of leaves from litter bags was not related to stream-water TDN or  $PO_4^{3-}$ . The strongest relationship was between N:P of FBOM and TDN ( $R^2 = 0.37$ ; Fig. 2C).

Macroinvertebrates

Stream group, order, genus, and month significantly affected macroinvertebrate stoichiometry (MANOVA, all  $p < 0.01$ ; Table 4), and no interaction terms were significant (all  $p > 0.05$ ). Macroinvertebrate phylogeny had a greater influence on body stoichiometry than did stream group. Macroinvertebrate %P (mean across all invertebrates = 0.9%) did not differ among stream groups, but %N was lower in the N+P streams (mean = 7.6%) than in the Ref (8.5%) or N (8.2%) streams (HSD, Ref vs N+P,  $p = 0.004$ ; N vs N+P,  $p = 0.016$ ).

Macroinvertebrate %P was more variable (CV range: 29–37%) than %N (CV range: 16–20%) or %C (CV range: 15–16%) among stream groups and was more variable in N and N+P streams than in Ref streams (Table 5). Percent N differed most strongly among stream groups (Table 4) and was more

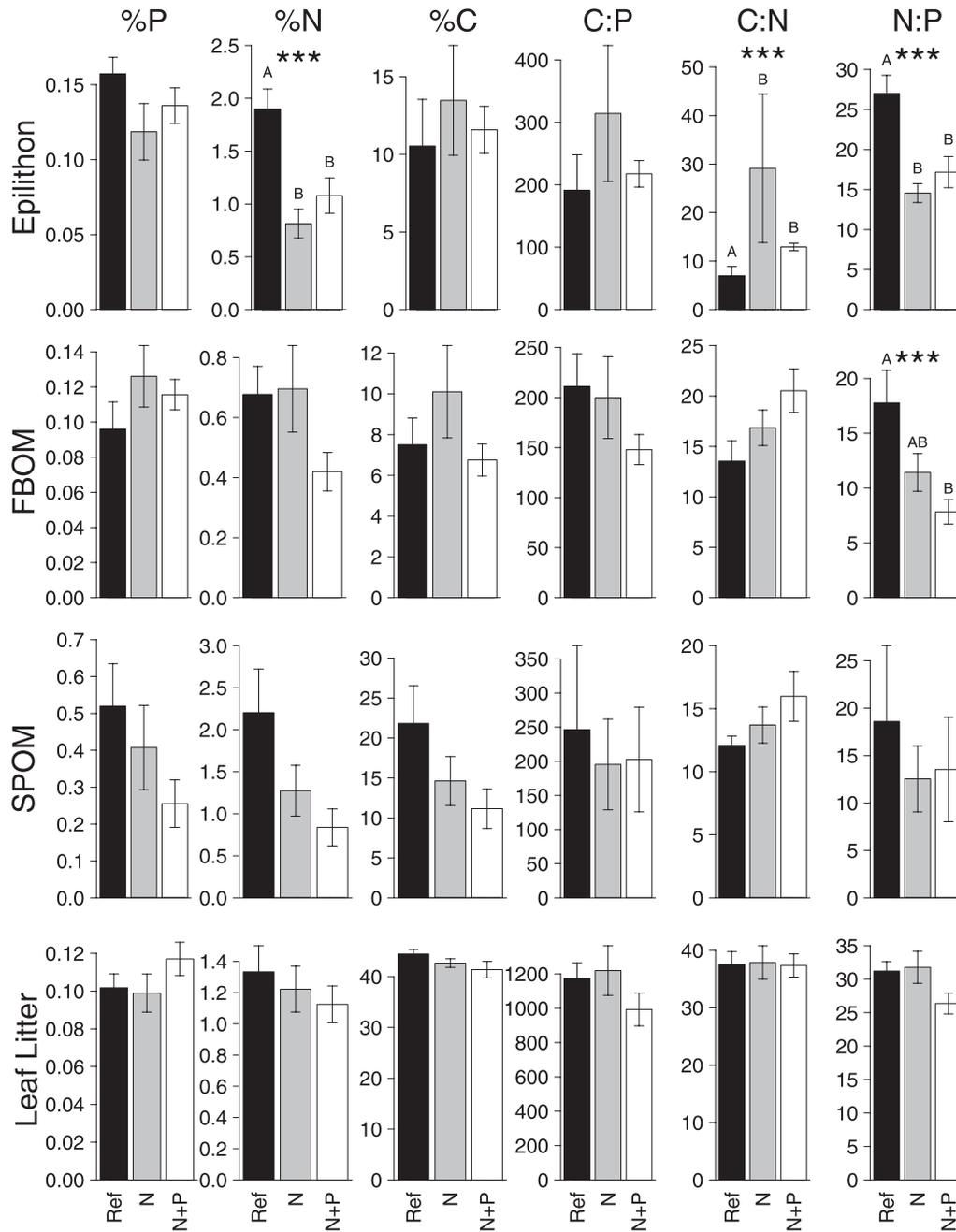


FIG. 1. Mean ( $\pm 1$  SE;  $n = 3$ ) %P, %N, %C, C:P, C:N, and N:P ratios of epilithon, fine benthic organic matter (FBOM), stream-water particulate organic matter (SPOM), and leaves from leaf-litter bags in reference (Ref), N-enriched, and N+P-enriched streams. Bars with the same letter are not significantly different. \*\*\* =  $p < 0.01$ .

variable in N streams than in Ref streams. Variation in C:P and N:P ratios of the macroinvertebrates was driven by variation in %P. C:P and N:P varied most in N streams and least in Ref streams. Percent C and C:N varied little among stream groups.

Stream group affected the stoichiometry of only some macroinvertebrate orders, and patterns of variation were not consistent among these orders

(Fig. 3A–E). Amphipoda, a taxon abundant in suburban streams, had higher mean %P and %N in N streams than in Ref streams (both  $p < 0.05$ ; Fig. 3A, B). In contrast, Diptera, also common in suburban streams, had higher %P in Ref than in N streams (Fig. 3A), higher %N in Ref streams than in N or N+P streams (Fig. 3B), lower C:P and C:N in Ref than in N+P streams (Fig. 3C, D) ( $p < 0.05$  for %P, %N, and

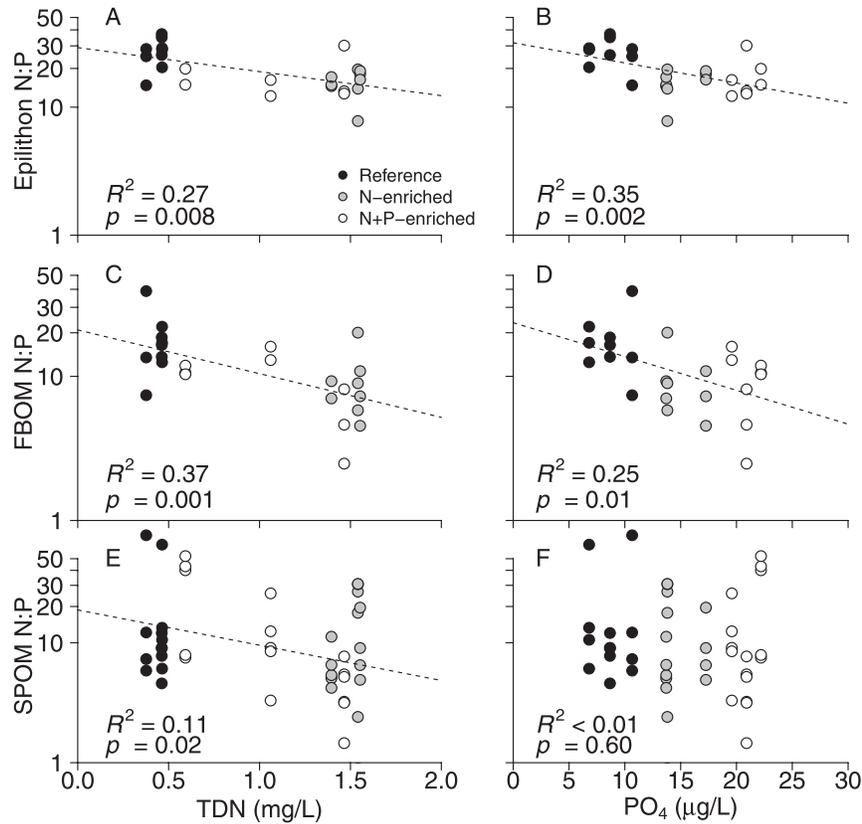


FIG. 2. N:P ratios of epilithon (A, B), fine benthic organic matter (FBOM) (C, D), and suspended particulate organic matter (SPOM) (E, F) samples plotted against mean total dissolved N (TDN) (A, C, E) and mean PO<sub>4</sub><sup>3-</sup> (B, D, F) stream-water concentrations. Dashed lines represent the linear regression with R<sup>2</sup> and p-values in the lower left corner of each graph. Note log scale for N:P.

C:P; *p* < 0.1 for C:N). Coleoptera had higher C:P in Ref than in N streams (Fig. 3C) (*p* < 0.05). N:P did not differ among stream groups for any order (Fig. 3E).

The nutrient content of most macroinvertebrate taxa was unaffected by higher nutrient concentrations in suburban streams. Some taxa did deviate from strict homeostasis in streams with different nutrient

concentrations (Fig. 4A, B), but no clear pattern emerged in which individuals had consistently higher %N or %P in suburban (N and N+P) or Ref stream groups. However, the taxa that did exhibit a deviation from homeostasis were those with higher abundance in suburban streams. Percent N of *Ceraclea* spp., Chironomidae, *Gammarus*, leeches, and Tipulidae differed among stream groups (*p* < 0.1; Fig. 4B).

TABLE 4. Results of multivariate analysis of variance (MANOVA) for effects of stream group (reference, N enriched, N+P enriched), macroinvertebrate order and genus, and month on macroinvertebrate nutrient and univariate analysis of variance (ANOVA) results for significant MANOVAs. Significant differences are in bold.

Independent variable	ANOVA													
	MANOVA		%C		%N		%P		C:N		C:P		N:P	
	Wilk's	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Stream group	0.74	<b>0.009</b>	3.32	<b>0.040</b>	10.24	<b>0.0001</b>	0.10	0.91	2.70	0.072	0.14	0.87	0.48	0.62
Order	0.10	<b>&lt;0.0001</b>	8.01	<b>&lt;0.0001</b>	3.14	<b>0.0012</b>	3.18	<b>0.0011</b>	3.39	<b>0.0006</b>	7.63	<b>&lt;0.0001</b>	7.23	<b>&lt;0.0001</b>
Genus	0.33	<b>&lt;0.0001</b>	2.42	<b>0.016</b>	2.76	<b>0.0067</b>	1.31	0.24	1.56	0.14	2.91	<b>0.0045</b>	2.72	<b>0.0075</b>
Month	134.00	<b>0.0008</b>	3.01	0.056	9.19	<b>0.0003</b>	1.61	0.21	2.31	0.11	3.05	0.054	3.41	<b>0.039</b>

TABLE 5. Coefficients of variation for elemental content variables measured in macroinvertebrate communities from reference (Ref), N-enriched, and N+P-enriched stream groups.

Elemental content variable	Stream group		
	N	N+P	Ref
%P	37%	35%	29%
%C	15%	15%	16%
%N	20%	16%	18%
C:N	18%	18%	17%
C:P	50%	45%	31%
N:P	46%	39%	29%

Tipulidae and Chironomidae had higher %N in Ref streams, whereas *Gammarus* and leeches had higher %N in N streams, and *Ceraclea* spp had higher %N in the N+P streams (Fig. 4B).

## Discussion

### *Effect of suburban nutrient enrichment on basal food-resource stoichiometry*

Contrary to our original hypothesis, higher nutrient concentrations in suburban streams were not associated with consistent increases in nutrient content of basal food resources as seen in studies of point-source inputs (Cross et al. 2003, Bowman et al. 2005, Singer and Battin 2007). Increases in nutrient concentrations in suburban streams did shift the stoichiometric quality of some basal food resources by reducing their N:P ratio. However, these shifts were not uniform, and the most abundant food resource (leaf litter) was the least affected. The negative linear relationships between FBOM and epilithon N:P and in-stream  $\text{PO}_4^{3-}$  concentrations suggest increased direct uptake of  $\text{PO}_4^{3-}$  from the water column in

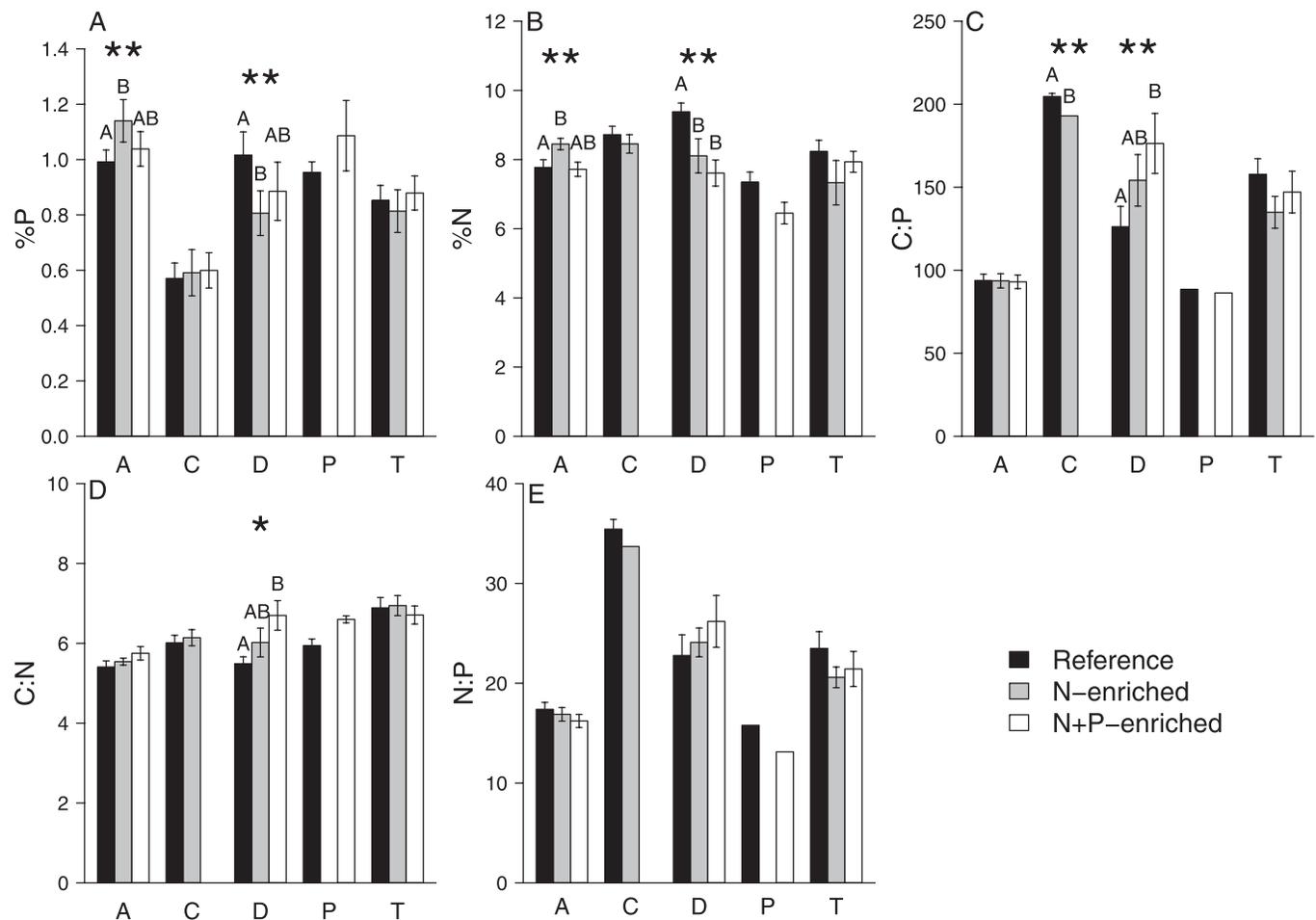


FIG. 3. Mean ( $\pm 1$  SE;  $n = 3$ ) %P (A) and %N (B), and C:P (C), C:N (D), and N:P (E) ratios for stream benthic macroinvertebrate orders among 3 stream groups (reference, N-enriched, and N+P-enriched). Letters along the x-axis represent the major orders sampled in the study streams: A = Amphipoda, C = Coleoptera, D = Diptera, P = Plecoptera, and T = Trichoptera. Bars with the same letter are not significantly different. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ .

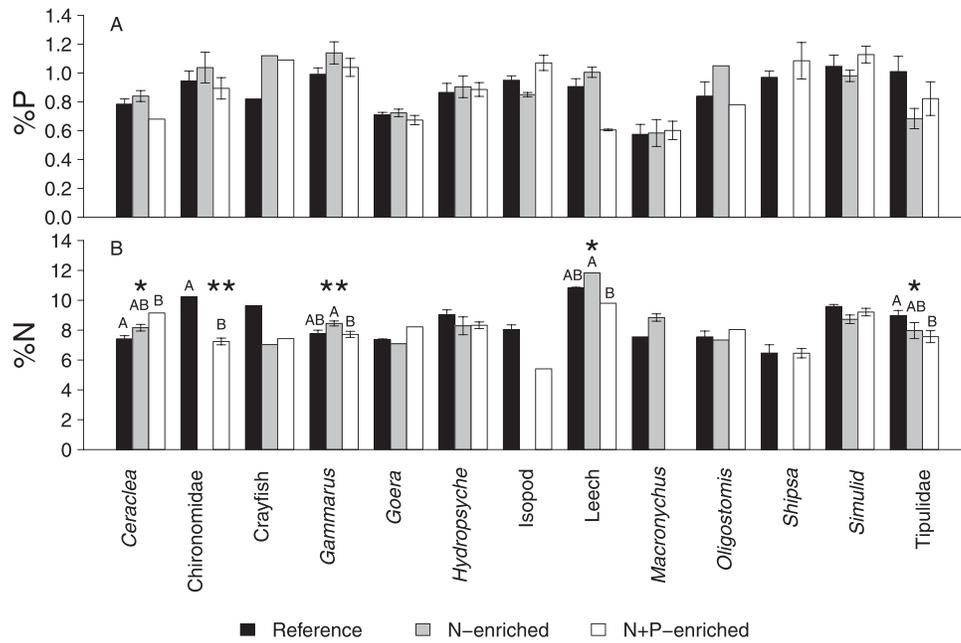


FIG. 4. Mean ( $\pm 1$  SE;  $n = 3$ ) %P (A) and %N (B) of macroinvertebrate taxa present in all stream groups (reference, N-enriched, N+P-enriched). Bars with the same letter are not significantly different. \* =  $p < 0.1$ , \*\* =  $p < 0.05$ .

suburban streams with higher  $PO_4^{3-}$  concentrations. Direct P uptake would result in lower N:P of basal resources, an outcome that has been observed in other nonsuburban systems (Cross et al. 2003, Bowman et al. 2005).

Potential mechanisms underlying the negative relationship between DIN and basal-resource N:P were not as apparent. One potential explanation would be that the relationship was driven by increasing  $PO_4^{3-}$  (i.e., increases in DIN concentration were lower in magnitude than those of  $PO_4^{3-}$ ), but DIN and  $PO_4^{3-}$  concentrations were not linked in our study streams. The negative relationship between DIN and basal resource N:P that we observed could represent a shift in how epilithon and FBOM in suburban streams responded to N addition. It is likely that DIN was present in such excess in our suburban stream sites that changes in its concentration did not affect epilithon and FBOM stoichiometry. N saturation and declining efficiency of N uptake has been demonstrated at the reach scale (Earl et al. 2006, Mulholland et al. 2008), but saturation of primary producers specifically has been observed experimentally only in shallow lakes and not in streams (Nydick et al. 2004).

Periphyton community structure is more responsive than periphyton biomass to the effects of nutrient enrichment (Stelzer and Lamberti 2001). Another explanation for the decrease in epilithon N:P with

increased DIN and  $PO_4^{3-}$  concentrations is a change in the relative abundance of algal species in the epilithon. Moreover, periphyton stoichiometry can vary widely and is influenced by a number of environmental conditions, such as hydrology, conductivity, light, and temperature (Godwin et al. 2009, Tsoi et al. 2011). Thus, higher nutrient concentrations in conjunction with additional changes in pollutants, road salt, temperature, and hydrology that are associated with the urban stream syndrome probably affected the primary producer communities at our suburban sites (Walsh et al. 2005), and changes in epilithon and FBOM stoichiometry may have been driven by factors other than or in addition to nutrients. Higher nutrient inputs in conjunction with other environmental impacts (e.g., flashier hydrology, temperature fluctuations, pollutants) could have affected N uptake differently than P uptake.

Leaf litter showed the weakest response among basal food resources to in-stream nutrients. Leaf-litter stoichiometry may not respond strongly to nutrient enrichment if other physical, chemical, and biological impacts of suburbanization override its effects. Our study streams had heavily forested riparian zones, so the stream ecosystems probably were strongly heterotrophic (Bernot et al. 2010), with leaf litter as the dominant source of C. Effects of nutrient enrichment on litter stoichiometry probably are greatest immediately after litter enters the stream, with lower-

magnitude changes after longer periods in the stream (Webster et al. 2009). We collected natural litter from the stream in spring and summer, long after autumn leaf fall, when the effect of stream nutrients on litter would have been greatest. Moreover, although urbanization affects stream (Paul and Meyer 2001, Walsh et al. 2005) and soil (Groffman et al. 2002) nutrient content, its effect on initial litter stoichiometry of naturally senesced leaves has not been reported. We have no way to know whether the natural litter collected in reference and suburban streams had different initial nutrient stoichiometries, but higher in-stream production of microbes and greater P uptake would be expected to increase the nutrient content of leaf litter in streams affected by suburbanization.

#### *Consumer response to suburban P enrichment*

We found no support for our hypothesis that P content of consumer taxa would increase with P enrichment of stream water. Taxonomic order and taxon influenced body stoichiometry more strongly than did stream group (consistent with Cross et al. 2003, Evans-White et al. 2005). This result indicates that changes in community composition and foodweb structure are more likely than physiological changes in individual taxa within the community to influence the stoichiometry of the food web.

Macroinvertebrate %P was more variable than %C or %N, a result suggesting that macroinvertebrate %P is more responsive than %C or %N to environmental factors. Other investigators also have reported that macroinvertebrate %P is more variable than %C or %N (Cross et al. 2003, Evans-White and Lamberti 2005). In a study of effects of urbanization on macroinvertebrate stoichiometry, Tsoi et al. (2011) found higher variation in C:P and N:P than C:N, indicating that P content was also more variable than N content.

The nutrient content of most macroinvertebrate taxa was not affected by stream group, but stoichiometry of some taxa did deviate from strict homeostasis. We predicted that only macroinvertebrate %P would differ among stream groups, but %N of some taxa differed among stream groups, with no clear pattern or direction. Moreover, macroinvertebrates did not have uniformly higher %P in N+P than in Ref or N streams. These results suggest that a shift in body %N, rather than a shift in body %P, might be a potential mechanism that can alter body stoichiometry. Changes in P content have been observed by other investigators (e.g., DeMott et al. 1998, Cross et al. 2003, Evans-White and Lamberti 2005, Shimizu and

Urabe 2008), but ours is the first study in which differences in %N among consumers appear to be associated with shifts in dissolved nutrient inputs.

Crustaceans were particularly responsive to the higher nutrient concentrations of the suburban streams. Amphipods (*Gammarus* spp.), the dominant crustacean at our study sites, are tolerant of the nutrient enrichment and high salinity typical of suburban runoff in the northeastern USA, and this taxon is often abundant in suburban, headwater streams (Meidel 2005). *Gammarus* spp. had higher %P, lower N:P and C:P, and similar %N compared to other invertebrate orders. This pattern has been observed in other crustacean taxa and has been attributed to higher ribosomal ribonucleic acid (rRNA) content and P associated with elevated Ca levels (Vrede et al. 1999, Evans-White and Lamberti 2005, but see Tsoi et al. 2011).

Our results confirm that some stream benthic macroinvertebrates, often taxa found at relatively higher abundance in suburban systems, do not exhibit strict homeostasis, and that their stoichiometry can be affected in unpredictable ways by nutrient concentrations typical of many suburban watersheds. Overall, variation in body stoichiometry in response to stream nutrient concentrations was less than the differences among different taxonomic groups. Moreover, macroinvertebrate elemental content changed less than did the elemental content of their food resources. The changes we observed did not follow a general pattern and could not be attributed to simple mechanisms, such as luxury storage of P.

#### *Foodweb stoichiometry and the urban stream syndrome*

Our results are consistent with those of other studies of ecosystem properties or functions in urban streams, in that effects of urbanization are complex and often do not yield general patterns seen in less-impacted systems (e.g., Paul 1999, Meyer et al. 2005, Small and Pringle 2010, Tsoi et al. 2011). Complex interactions among the physical, chemical, and biological factors associated with the urban stream syndrome can result in multiple controls on ecosystem processes that occur at different time scales and that vary among streams. Further research to identify the factors that control specific mechanisms is necessary to understand better the implications of nutrient enrichment for foodweb dynamics in streams affected by urbanization.

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