PRIMARY RESEARCH PAPER

The importance of dissolved N:P ratios on mayfly (*Baetis* spp.) growth in high-nutrient detritus-based streams

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Abstract The concept of ecological stoichiometry has been useful for understanding nutrient dynamics in aquatic food webs; however, the majority of studies have focused on autotrophic systems, leaving detritus-based food webs largely understudied. In addition, most detritus-based studies have explored enrichment in high-gradient, low-nutrient systems, despite the fact that many of the streams most likely to face enrichment (those surrounded by agriculture) are low-gradient and contain inherently higher dissolved nutrient concentrations due to differences in soil type, geomorphology, and atmospheric deposition. Constraints on consumer growth due to consumer-resource imbalances have been documented in these low-nutrient streams, but the extent to which consumer growth may be limited in higher-nutrient, detritus-based streams is unknown. We investigated the impact of dissolved nutrients (N and P) on mayfly growth, using artificial streams simulating a high-nutrient detritus-based system. Mayflies were reared and sampled under two total nutrient concentrations, one meant to mimic a more natural undisturbed (ambient) watershed and one to mimic a disturbed (enriched) watershed. Under each of these conditions two N:P ratios (low and high) were tested. The low N:P treatments produced higher mayfly growth under both ambient and enriched conditions, showing that nutrient limitation can occur even in high-nutrient streams.

Keywords Ecological stoichiometry · Lotic · Aquatic invertebrates · Detritus · Enrichment

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Introduction

Increased urbanization and industrial and agricultural practices have caused alterations in nutrient cycling patterns around the globe (Ptacnik et al., 2005; Elser et al., 2007). Changes in nutrient cycling, particularly in regards to nitrogen (N) and phosphorus (P), can precipitate the influx of excess amounts of nutrients into aquatic systems where they can have significant impacts on ecosystem processes (Paul & Meyer, 2001; Walsh, 2005). Eutrophication, toxic algal blooms, fish kills, and losses in biodiversity have all been documented in aquatic systems as a consequence of nutrient loading (Carpenter et al., 1998). Outside of toxic effects, these nutrients can impact aquatic food

webs by enriching resource quality and altering consumer growth rates (Sterner & Elser, 2002).

Community alterations and trophic cascades resulting from changes in dissolved N and P concentrations have been well documented in lake ecosystems (Carpenter et al., 1985; Carpenter et al., 2001; Jeppesen et al., 2005). However, the majority of studies done on nutrient enrichment have focused on autotrophic systems rather than heterotrophic ones, leaving detritus-based food webs largely understudied (Gulis & Suberkropp, 2002; Sterner & Elser, 2002; Cross et al., 2003). Despite this, studies in forested headwater streams have shown that enrichment in low-nutrient streams can increase consumer growth and potentially lead to seasonal resource limitations due to increased microbial processing and invertebrate consumption (Cross et al., 2003; Cross et al., 2006; Cross et al., 2007). Evidence also suggests that increases in consumer growth are primarily due to changes in resource quality, particularly P content (e.g. Cross et al., 2003; Hladyz et al., 2009). In some regions, however, even relatively undisturbed streams can show ambient dissolved nutrient concentrations that are much higher than those found in high-gradient forested headwater streams. In these systems, moderate to high nutrient levels are primarily due to differences in soil type, geomorphology, and atmospheric deposition (Clark et al., 2000), rather than human-induced enrichment. However, this distinction is often overlooked as many of these high-nutrient streams, particularly in the United States, occur where agricultural land use is intense, and as a result are actually more likely to face enrichment (Johnson et al., 1997; Clark et al., 2000). Despite the cause, very little has been done assessing consumer growth alteration in high nutrient detritus-based systems (Griffiths et al., 2009).

Ecological stoichiometry studies have repeatedly shown that nutrient ratios can have strong effects on consumer growth mediated by consumer-resource imbalances (Elser et al., 2000a, b; Sterner & Elser, 2002; Elser et al., 2003). Cross et al. (2003) showed a propensity for nutrient imbalances to exist in low-nutrient detritus-based streams, and also that enrichment reduced many of these imbalances, especially in regards to P. However, it is not known whether similar consumer-resource imbalances have the same effects in low- and high-nutrient detritus-based systems. Even at high total nutrient concentrations, it is possible that dissolved nutrient ratios may affect growth due to nutritional imbalances, but benthic invertebrates may employ compensatory feeding and utilization mechanisms to address these nutrient imbalances (Behmer, 2009). Understanding these processes in lotic systems hinges on exploring the interactions between dissolved nutrient ratios and total dissolved nutrient concentrations, as well as their impacts on consumer-resource dynamics (Francoeur, 2001; Stelzer & Lamberti, 2001).

In this study, artificial streams were used to simulate the dissolved nutrient conditions of a high-nutrient detritus-based stream. An experiment was performed to explore the effect of dissolved N:P ratios on consumer growth (*Baetis* spp. mayflies) under two different total nutrient concentrations. Our objectives were to determine the effect of dissolved nutrient ratios on consumer growth in a high-nutrient detritus-based food web, and to assess whether the relationship between nutrient ratio and consumer growth would change between ambient nutrient conditions and enrichment in a high-nutrient detritus-based system. We also explored the effect that nutrient ratio had on the quality of the resource base in these streams by measuring the elemental content of stream leaf material, as the predicted resource, and consumer gut contents, as the resource that was actually ingested. Elemental body composition was also measured to determine whether mayflies were homeostatic across nutritional environments.

Given the evidence that consumer-resource elemental imbalances can have strong impacts on consumer growth (e.g. Elser et al., 2003), we hypothesized that (1) dissolved N:P ratio would have a significant effect on mayfly growth regardless of total nutrient concentration, and (2) that differences in mayfly growth would be facilitated by changes in the quality of the resource base which would impact consumer-resource imbalance. Lastly, (3) we predict that the mayflies would maintain a similar body composition across treatments, as many insect species have been shown to be homeostatic across variable nutritional environments (Sterner & Elser, 2002; Vrede et al., 2004; Frost et al., 2005).

Methods

Reference stream

A 20-meter reach of the Snake River, located 3.2 km west of Grasston, MN, U.S.A. (Latitude: 45.79365,

Longitude: −93.1811), was selected as a reference stream for this study. Stream microbes and invertebrates were collected from this site and used in the experiments described below. Bio-monitoring data were available for this site through the Minnesota Pollution Control Agency (MPCA) and indicated a high density of *Baetis* species.

To determine variability in dissolved N ([N]) and P ([P]) concentrations in the watersheds near the reference stream, water quality data collected by the MPCA (MPCA Website: http://www.pca.state.mn.us) were examined from stream monitoring sites occurring within an 80 km^2 radius from the reference stream. It is likely that this data set included streams with varying levels of disturbance, which can have strong effects on water quality; however, identifying the range of concentrations provides a more accurate view of what constitutes enrichment in this area. Because of this variation in disturbance, concentrations documented at the low end of this range were assumed to result from abiotic factors and were used to inform the "Ambient" treatment, while concentrations at the high end of this range were assumed to be a consequence of enrichment and were used to inform the "Enriched" treatment (see Treatments section below). Dissolved inorganic N values were reported as nitrate and nitrite concentrations; however, ammonia was not included, suggesting that there may be a slight underestimation of total [N]. Phosphorus was reported as total P, which includes organically bound P; this likely gives a slight overestimate of total [P] in the streams.

Mayflies (*Baetis* spp.) and isopods (*Asellus* spp.) were collected from the reference stream in mid-February 2010 and again in mid-March 2010, respectively, (see Artificial Steams section below), using a kick net equipped with 1 mm mesh. Mayfly species from the *Baetis* genus (Family: Baetidae) were the focal consumer in this study. The artificial streams also included isopods, as shredding invertebrates. They facilitated the breakdown of the leaves into fine particulate matter, which can then be consumed by the mayflies (Cummins et al., 1989).

Treatments

A factorial design was used to test the effect of N:P ratio (all ratios are molar), and total nutrient concentration on mayfly growth, although the total nutrient concentrations were tested at separate times (see Data analysis section). Two N:P ratios, henceforth referred to as Low and High, and two total nutrient levels, referred to as Ambient and Enriched were selected using the MPCA data. Nitrogen and P concentrations were highly variable for streams surrounding the reference stream ([N]: 0.010–32.47 mg/l, median = 0.64 [P]: 0.010-4.65 mg/l, median = 0.143) and values were also much higher than those reported in the low-nutrient forested streams studied by Cross et al. (2006) ([N] = 0.027 mg/l; [P] = 0.0037 mg/l). The lowest N:P recorded within the reference area was 0.0166 and the maximum was 30.44. The average N:P across streams was 31.11 (median = 9.93). We selected our treatment N:P ratios to encompass this range, with the Low N:P treatments approximating a ratio of 3:1 and the High N:P a ratio of 15:1.

The concentrations of total nutrients ([N + P]) also varied substantially among streams. The average [N + P] was 2.26 mg/l (median = 0.984 mg/l), with a range of 0.043–37.11 mg/l. At the low end of the range, 0.8 mg/l was selected for the Ambient treatment, and at a higher end of the range, 3.0 mg/l was selected as the Enriched treatment as these values are good representations of the range of total dissolved nutrients.

Each Low and High N:P ratio was tested under Ambient and Enriched total nutrient levels and there were three replicates per treatment. Well water served as the water source for the streams and treatments were created by adding additional N, as ammonium nitrate (NH_4NO_3), and P, as monopotassium phosphate (KH_2PO_4) in their appropriate proportions. Dissolved nutrient concentrations were quantified at least once a week. When necessary, additional N and P were added to the streams to ensure that the ratios stayed as close as possible to the treatment N:P. Distilled water was added when necessary to keep the volume and nutrient concentrations stable.

Water quality testing was done using a Hach spectrophotometer (DR/4000U) to determine the concentrations of N and P throughout each experiment. Nitrite was quantified using a diazotization method (NitraVer 3 reagent powder pillows). Nitrate was measured using a cadmium reduction method (NitraVer 5 powder pillows), and ammonia was measured using the Nessler Reagent Method. Reactive phosphorus concentrations were quantified by measuring the presence of orthophosphate using an ascorbic acid method (PhosVer3 powder pillows).

Artificial streams

In total we had space to run six artificial streams at a given time, so the Ambient and Enriched treatments were ran at different times. All of the streams were housed in a greenhouse outfitted with 80% light-restriction shade cloth. The shade cloth was used to create a light-limited environment, common in headwater streams, and to ensure that a heterotrophic detritus-based system with limited algal production was simulated. The greenhouse was not temperature controlled, so stream temperature conditions inside the greenhouse were only slightly warmer than the temperature outside.

The re-circulating flumes consisted of a plywood trough lined with polyvinyl chloride (PVC) pond liner (20 mil, Beckett brand). Each trough was 2.44 m in length, 0.61 m wide, and 0.28 m deep. PVC pipe was used for all of the plumbing, and an inline Cal PLP 3000 waterfall pump (3000 gph, 115v) was used to circulate the water throughout the flume. A T-shaped diffuser and a block of corrugated plastic were placed in front of the inlet pipe to break up the flow. One-millimeter nylon mesh was installed inside the trough over the opening of the outlet pipes to prevent invertebrates from circulating through the system. This design resulted in a completely closed system.

Two nylon mesh bags were filled with approximately 4 grams of leached and dried sugar maple (*Acer saccharum*) leaves taken from a single tree providing substrate for microbial colonization and ultimately serving as the resource base in each stream. Four terracotta saucers (0.10 m in diameter) were added to each stream to both hold the leaf packs in place and to provide shelter for the invertebrates. The saucers were leached in well water for over 48 h before being added to the streams. The placement of the saucers within each stream was standardized.

The streams were filled with well water to a depth of 0.13 m. A 3 l aliquot of stream water was taken from the reference stream and added to each artificial stream after being filtered through a 250 μm sieve to remove any particulate matter. The reference stream water served to inoculate the artificial streams with microbes. At this time, nutrients were added to each stream in the proportions dictated by the respective treatment assignment. The leaf packs were then given a seven-day conditioning period after the microbial inoculation before the mayflies were introduced to the

system. Each stream was initially stocked with 150 mayflies and 20 isopods. The size structure of both the mayfly and isopod populations was standardized for each stream by distributing the same number of similarly sized individuals into each stream.

Stream physical parameters

Both air and water temperature were measured at 4 h intervals using data loggers (Onset HOBO® Pendant Loggers). Conductivity, pH, and velocity were measured for each stream biweekly. Conductivity and pH were measured using a YSI 620-D with a Multi-Parameter Water Quality Monitor 6820 (Environmental Monitoring Systems), and velocity was measured with a FLO-Mate portable flowmeter (Marsh-McBirney Inc., model 2000). The location of each measurement was standardized across streams. Table 1 shows the physical properties of each artificial stream during the experiment, as well as the MPCA reported values for the reference stream. Temperature, conductivity, pH, and velocity were similar across all artificial streams. It is important to note, however, that average water temperature at the time the Enriched treatment was tested was on average about 7° warmer than that of the Ambient treatment, constituting a significant difference ($P < 0.0001$). This temperature difference was due to variability in weather conditions and the inability to control temperatures conditions inside the greenhouse. Table 2 shows the average nutrient concentrations and ratios for each treatment.

Because flow characteristics can impact the colonization of microbes, the spatial distribution of invertebrates, and the cycling of nutrients within a stream (Nowell & Jumars, 1984; Statzner et al., 1988; Allan, 1995), the hydrodynamic properties (Froude number, Reynolds number, boundary layer thickness, and the roughness Reynolds number) of the artificial streams were measured and compared to those reported for natural streams, as recommended by Craig (1993). All values (data not reported) fell within a range comparable to natural lotic systems.

Growth

Mayflies were sampled four times throughout the experiment at 5-day intervals. Each treatment was tested for a total of 27 days, including the 7-day microbe conditioning period. At each sampling period,

Table 1 Reference (R) and artificial stream physical parameters for each treatment, ambient (A) and enriched (E)

Treatments		Water temperature [°C]			Conductivity (S/cm)	pH	Velocity (m/s)
		Min	Max	Mean (±SE)			
R	Snake River	–	–	–	0.261	7.61	1.25
A	High N:P	18.35	25.49	21.51 ± 0.45	0.52 ± 0.02	8.22 ± 0.03	0.12 ± 0.003
	Low N:P	18.15	24.91	21.39 ± 0.45	0.52 ± 0.02	8.23 ± 0.02	0.12 ± 0.002
E	High N:P	10.65	17.82	14.29 ± 0.58	0.52 ± 0.02	8.29 ± 0.03	0.11 ± 0.002
	Low N:P	10.81	17.32	14.26 ± 0.57	0.53 ± 0.01	8.29 ± 0.03	0.11 ± 0.003

Reference stream measures are taken from MPCA monitoring data

Table 2 Average nutrient concentrations and ratios for the reference stream (R) and each treatment, ambient (A) and enriched (E), as well as the dates for the experiment and the initial mayfly mass

Treatments		Date	Initial Mass	DIN (mg/l)	SRP (mg/l)	[N+P] (mg/l)	N:P
R	Snake River	–	–	0.28	0.073	0.353	8.49
A	High N:P	2- 17-09 to	0.603 (n = 50)	0.58 ± 0.02	0.20 ± 0.01	0.78 ± 0.02	7.60 ± 0.40
	Low N:P	3-09-09		0.25 ± 0.01	0.17 ± 0.003	0.41 ± 0.02	3.28 ± 0.20
E	High N:P	3-26-09 to	0.978 (n = 97)	2.36 ± 0.05	0.51 ± 0.03	2.87 ± 0.06	11.42 ± 0.5
	Low N:P	4-15-09		1.70 ± 0.03	1.00 ± 0.02	2.71 ± 0.04	3.85 ± 0.01

Reference stream parameters were taken from MPCA data collected in July 2006. Concentrations are given in mg/l and all ratios are molar. DIN refers to dissolved inorganic N but excludes ammonia, and SRP refers to soluble reactive phosphorus except for the reference stream which is measured as total phosphorus. Initial mass is in milligrams

as many mayflies as possible were collected and placed in a clear plastic container. From this group, ten mayflies were randomly selected by placing a numbered grid underneath the container and collecting individuals positioned within randomly selected numbered squares. The remaining mayflies were then returned to the artificial streams. After sampling, the mayflies were euthanized via freezing and their body length was measured to the nearest 0.1 mm using a compound microscope and ruler. Dry mass was determined using a length-mass regression derived for Baetidae from Benke et al. (1999). A preliminary set of mayflies collected from the reference stream and not included in the experiment was used to estimate initial mass for the first sampling period.

Growth rate for the mayflies was calculated over the entire experiment, using the formula employed by Frost & Elser (2002): Growth Rate = [ln(M_{final}) − ln($M_{initial}$)]/20 days.

Food quality

To understand how the dissolved nutrient treatments were affecting the resource base, submerged leaves, as well as a set of reference leaves that were not submerged, were analyzed for C, N, and P. It was prohibitively difficult to measure the nutrient content of the microbial resources fed upon by the mayflies, so leaf measurements were instead used as a proxy for resource quality. Leaves from each mesh bag were collected from every stream at the end of the experiment, as well as a reference sample of leaves that were leached but not used in either experiment. They were dried in a drying oven at 60°C, ground using a Wiley® Mill (Model 3383-L10, 115v, ¼ HP), and pulverized with a SPEX SamplePrep Mixer/Mill (Model 8000D). Nitrogen and carbon were measured simultaneously using a CHN analyzer. The samples were processed using either a FlashEA® 1112 NC Soil Analyzer (Thermo Electron Corporation) or an Elementar CN Max analyzer (Elementar Analysensysteme GmbH). Phosphorus was measured using an automated (Flow Solution IV®, O.I. Analytical) ascorbic acid method with a persulfate digestion (APHA, 1998). Carbon, N, and P content were compared between treatment leaves to determine nutrient contributions from microbial biomass. Because all leaves were leached before being placed

in the streams, any difference in C, N, and P content is likely derived from microbial colonization. Differences in C content between the reference leaves and the submerged leaves were used to indicate increases in microbial colonization on the leaf tissue.

Mayfly gut contents were analyzed for N and P as a more specific indicator of food quality. Gut contents were collected from every mayfly sampled from the artificial streams. A sample was taken from the anterior ¼ of the alimentary canal. Due to the limited amount of material and the quantities needed for elemental analysis, samples had to be pooled across individuals and sampling periods within treatments. This resulted in a single anterior sample for all the mayflies taken during the last two sampling dates for each treatment. Elemental analyses gut contents were done using the same methods outlined for leaves (see above).

Elemental body composition

The concentration of carbon (C), nitrogen (N), and phosphorus (P) in mayfly tissue was quantified to determine the extent to which these elements are regulated in mayfly tissue. Of the ten mayflies collected from each stream during each sampling period, two individuals were analyzed for nitrogen and carbon and two were analyzed for phosphorus. Samples were processed in the same manner as the leaf and gut content samples (Food quality section) Individuals were randomly selected for each analysis. If the randomly selected individual did not meet minimum sample requirements, it was pooled with another individual of similar size to standardize for allometric effects. Body composition C:N, C:P, and N:P ratios were also calculated. Body N:P and leaf N:P were then used to calculate consumer-resource imbalance as done in Liess et al. (2009): $N:P_{im} = N:P_{leaf} - N:P_{mayfly}$. Because we only had leaf nutrient data at the end of the experiment, we only used the body composition values from the fourth sampling period to compare with leaf N:P.

Data analysis

Because the Ambient and Enriched treatments were tested at different times, and there were significant differences in initial mayfly mass and temperature, these treatments were analyzed separately. An
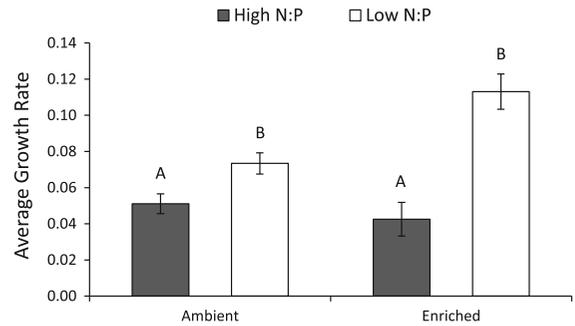
Fig. 1 Average growth rate for the mayflies in each treatment (± SE). *Letters* indicate statistical differences between high and low N:P within each treatment but not across Ambient and Enriched treatments, as they were analyzed separately

ANCOVA was performed to determine significant differences in dry mass over time and treatment effects on growth rate. Temperature can have strong effects on mayfly growth rate (Brittain, 1975; Humpesch, 1979; Benke & Jacobi, 1986) and was used as a covariate in the ANCOVA. A one-way ANOVA was used to determine differences in stream parameters, such as temperature, pH, conductivity, and velocity (see Stream physical parameters section above), and to determine differences in leaf nutrient ratios, mayfly body composition ratios, and consumer-resource imbalance between treatments. A Tukey's HSD post hoc test was used to determine differences between groups when necessary. A MANOVA was done to determine differences in mayfly body and leaf nutrient concentrations. Univariate ANOVAs were conducted as the post hoc test when MANOVA results were significant. All analyses were performed using SPSS Statistics 21 Software (Chicago, IL, USA).

Results

Mayfly growth

Ambient treatment

There was a significant treatment effect on mayfly growth rate ($F_{1,238} = 27.30, P < 0.0001$); growth rate was higher in the low N:P treatment compared to the high N:P treatment (Fig. 1). Mayflies in the low N:P gained an average of 0.609 mg (±0.056 SE) over the entire experiment, while mayflies in the high N:P

treatment only gained an average of 0.248 mg ($\pm$0.042 SE). Analysis of dry mass showed a significant time-by-treatment effect ($F_{3,232} = 4.34$, $P < 0.0001$). Dry mass, which was recorded at 4 different time points, increased slightly then leveled off by the second sampling period in the high N:P treatment, but increased steadily across all sampling periods in the low N:P treatment.
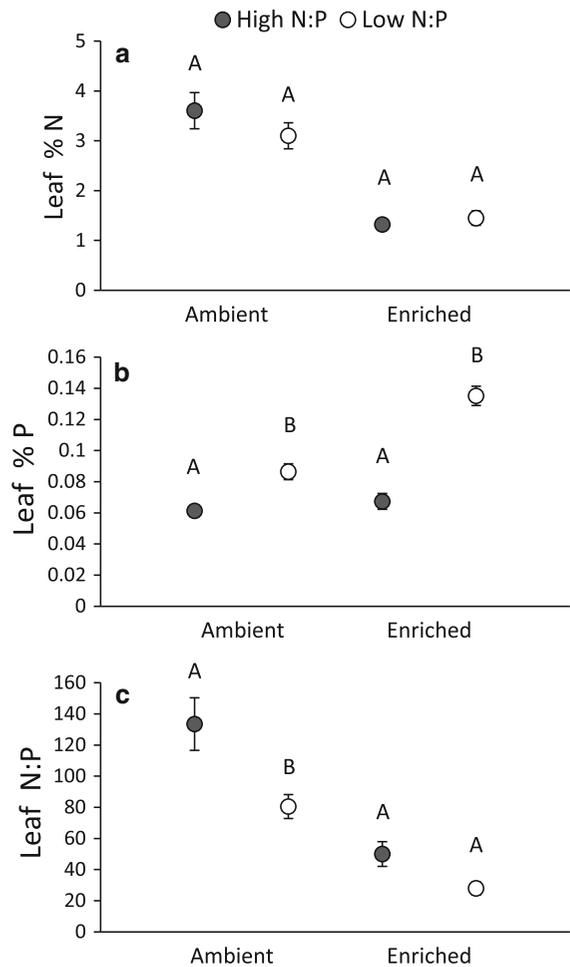
Enriched treatment

Growth rate was significantly different between the low and high N:P treatments ($F_{1,238} = 7.74$, $P = 0.006$), and was again higher in the low N:P treatment (Fig. 1). Mayflies in the low N:P gained an average of 0.507 mg ($\pm$0.044 SE), while mayflies in the high N:P gained 0.344 ($\pm$0.037 SE) throughout the experiment. For dry mass, a marginally significant time-by-treatment effect was detected ($F_{3,232} = 2.34$, $P < 0.074$). Mayflies in the high N:P treatment showed increases in dry mass initially but these gains leveled off by the second sampling period. Mayflies in the low N:P treatment, however, showed increases throughout the entire experiment.

Food quality

Ambient treatment

The dissolved N:P treatments had a significant impact on the N and P content of the microbial community colonizing the submerged leaves (Fig. 2). The MANOVA showed significant differences in leaf nutrient content between treatments ($P = 0.010$); however, this was a result of differences in P only ($P = 0.002$), as leaf N did not differ between treatments (Fig. 2a, b). There were no treatment effects on leaf C:N ($F_{1,10} = 0.694$, $P = 0.424$); however, there were significant differences in leaf C:P ($F_{1,10} = 17.46$, $P = 0.002$) and N:P ($F_{1,10} = 8.14$, $P = 0.017$), with the low N:P treatment showing lower C:P and N:P ratios (Fig. 2c). Carbon content in the reference leaves was significantly lower compared to the two N:P treatments ($F_{1,12} = 13.78$, $P = 0.001$), but there was no difference in C content between treatments (Tukey HSD, $P = 0.99$). These results indicate that leaves in both treatments accumulated microbial biomass, but that the microbes in the low N:P treatment contained more P. Gut nutrient content showed higher


Fig. 2 The **a** %N, **b** %P, and **c** N:P ratio of the leaves in each treatment at the end of the experiment ($\pm$ SE). All ratios are molar. Letters indicate statistical differences between high and low N:P within each treatment but not across Ambient and Enriched treatments, as they were analyzed separately

concentrations of P than N for both treatments, but despite the leaf nutrient data, there was no difference in gut N or P between treatments (Fig. 3).

Enriched treatment

In the Enriched treatment, the microbial community on the leaves in the low N:P treatment was more P-rich than the high N:P treatment (Fig. 2). The MANOVA showed a significant treatment effect ($P < 0.0001$) resulting from significant changes in P content only ($P < 0.0001$), as leaf N content was not significantly different between treatments ($P = 0.246$) (Fig. 2a, b). There was not a significant treatment effect on leaf C:N
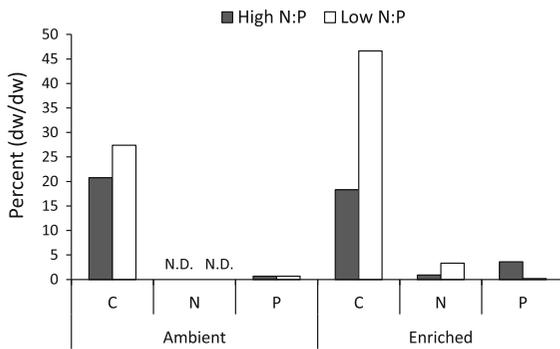
Fig. 3 The percentage of C, N, and P in mayfly gut contents. Bars labeled N.D. represent nutrient levels below detection (<1 ppm). Small sample size precluded statistical analysis

$(F_{1,13} = 0.223, P = 0.645)$ or leaf N:P $(F_{1,13} = 1.36, P = 0.264)$; however, there was a significant treatment effect on leaf C:P $(F_{1,16} = 4.92, P = 0.041)$, with the low N:P treatment showing a lower C:P ratio (Fig. 2c). Leaf carbon content did not differ between treatments and was not significantly different from the reference leaves $(F_{1,18} = 0.107, P = 0.90)$, suggesting that microbial biomass did not increase. Despite the differences in P content seen in the leaf microbes, N and P in mayfly gut contents did not differ substantially between treatments (Fig. 3).

Body composition and consumer-resource imbalance

Ambient treatment

There was also no significant difference between body N:P $(F_{1,46} = 0.013, P = 0.911)$, C:N $(F_{1,43} = 0.218, P = 0.643)$, or C:P $(F_{1,43} = 0.009, P = 0.924)$ between treatments. Table 3 shows the percentage and ratio of each element in mayfly body tissue. There was a significant difference in consumer-resource imbalance between N:P treatments $(F_{1,10} = 9.50, P = 0.012)$, with the low N:P treatment showing less of a discrepancy between mayfly body N:P and leaf N:P (Fig. 4).

Enriched treatment

There was no significant difference between mayfly body N:P $(F_{1,41} = 0.356, P = 0.554)$, C:N $(F_{1,41} = 0.035, P = 0.825)$, or C:P $(F_{1,39} = 0.085, P = 0.772)$

between treatments. Table 3 shows the percentage and ratio of each element in mayfly body tissue. There was also no significant difference in consumer-resource imbalance between N:P treatments $(F_{1,10} = 9.50, P = 0.122)$.

Discussion

We hypothesized that dissolved nutrient ratios would have strong effects on mayfly growth regardless of total nutrient levels. The low N:P treatment showed significantly higher mayfly growth in both the Ambient and Enriched treatments, which suggests that P was more limiting to mayflies than N. Frost & Elser (2002) also found P to be growth-limiting for mayflies in a lentic system, and Cross et al. (2003) documented significant consumer-resource P imbalances in a detritus-based stream that were reduced after experimental enrichment. The fact that N:P ratio had similar and significant effects under both total nutrient concentrations suggests that the proportion of dissolved nutrients plays a primary role in benthic consumer-resource interactions, and that growth limitations due to nutrient ratios also occur in high-nutrient streams.

We also predicted that differences in mayfly growth across N:P treatments would be, at least partially, mediated by changes in resource quality impacting consumer-resource imbalance. Our data support the claim that dissolved N:P impacted resource nutrient concentrations, as leaf P increased significantly in both the Ambient and Enriched treatments. However, the connection between resource quality and consumer-resource imbalance is less clear. Although leaf P was higher in the low N:P treatment (at both nutrient levels), this only impacted leaf N:P at Ambient nutrient levels, as there was no difference in leaf N:P between treatments at Enriched nutrient concentrations. We also only saw a treatment effect on imbalance at Ambient nutrient levels, as little to no discrepancy was seen between mayfly N:P and leaf N:P in the Enriched treatments. Despite significant differences in mayfly growth rate and leaf P content between N:P treatments at both Ambient and Enriched nutrient levels, the calculated imbalance suggests that our dissolved N:P treatments should have produced similar mayfly growth in the Enriched treatment.

Table 3 Mayfly elemental body composition (±SE) for each treatment, ambient (A) and enriched (E)

Treatments		%C	%N	%P	C:N	C:P	N:P
A	High N:P	48.6 ± 1.1	10.4 ± 0.04	0.73 ± 0.01	5.0 ± 0.02	193 ± 2.9	38.7 ± 0.62
	Low N:P	44.5 ± 0.13	10.0 ± 0.04	0.76 ± 0.02	5.4 ± 0.03	194 ± 9.1	35.8 ± 1.6
E	High N:P	48.6 ± 1.1	10.7 ± 0.07	1.1 ± 0.02	5.4 ± 0.23	157 ± 8.8	30.0 ± 0.65
	Low N:P	49.4 ± 0.23	11.0 ± 0.06	1.1 ± 0.02	5.3 ± 0.03	158 ± 3.5	30.2 ± 1.0

Values represent the average percentage across both treatments over the course of the entire experiment
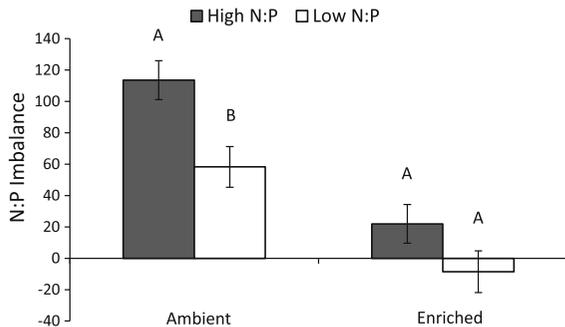
Fig. 4 The consumer-resource imbalance between mayfly body N:P and leaf N:P, calculated as: $N:P_{im} = N:P_{leaf} - :P_{mayfly}$ as in Liess et al. (2009). *Letters* indicate statistical differences between high and low N:P within each treatment but not across Ambient and Enriched treatments, as they were analyzed separately

The most likely explanation is that we were not able to accurately identify, collect, and characterize the resource(s) eaten by the mayflies. Small sample size precluded us from statistically analyzing the gut nutrient content, but some differences between N:P treatments were apparent. These differences, however, were not congruent to the leaf nutrient data. For instance, in the Enriched treatments, N content was higher in the low N:P treatment, and P content was higher in the high N:P treatment. This inconsistency suggests that the mayflies may have been consuming other resources beyond leaf microbes. While most *Baetis* species are thought to be collector-gatherers and not scrapers (Cummins & Klug, 1979; Brittain, 1982; Hershey & Lamberti, 1998), they may also be feeding on algae. If this was the case, the leaf nutrient data, which included contributions from leaves, microbes (bacteria and fungi), and algae collectively, would not have had the resolution to detect elemental difference between each of these resources. It is also likely that egested material from other organisms, which was not analyzed, was ingested to some degree, as feces have been documented as a common resource for

some benthic invertebrates (Brittain, 1982; Shepard & Minshall, 2006). As such, it is possible that the nutrient ratios of the resource(s) were not accurately described, or measured, thus impacting the consumer-resource imbalance calculations. Despite these ambiguities, the strong connection observed between dissolved N:P ratio, leaf P concentrations, and mayfly growth suggests that dissolved N:P ratios do have significant effects on how these elements move through food webs.

Our final hypothesis was that mayfly body composition would remain stable across treatments, and this was the case. Mayfly body composition did not change significantly between N:P treatments at either Ambient or Enriched nutrient concentrations. This perhaps is not surprising considering that most metazoans tend to be homeostatic (Sterner & Elser, 2002; Vrede et al., 2004; Frost et al., 2005), although exceptions have been documented in zooplankton (Walve & Larsson, 1999; Plath & Boersma, 2001) and fish (Sterner & George, 2000).

The interactions between resource quantity and quality have been more thoroughly examined in autotrophic systems, and several of these studies found that resource quality has a stronger effect on consumer growth at high resource abundance because when resources are scarce, energy limits growth regardless of nutrient balance (Urabe & Watanabe, 1993; Sterner & Robinson, 1994; Sterner, 1997). Frost & Elser, (2002) found support for this hypothesis in lab-reared mayflies, as increased P only increased growth at high food concentrations. This implies that dissolved nutrient ratios are more likely to constrain consumer growth in high nutrient systems (as long as there is a positive relationship between total nutrient concentration and productivity). Although we did not test mayfly growth at extremely low nutrient levels, the dissolved N:P treatments had similar effects on mayfly growth across two high nutrient concentrations, showing that our results are in line with this hypothesis.

When nutritional imbalances do occur there are several ways for consumers to mitigate disparities between what their nutritional requirements are and what is available in a given resource. First, when resource quantity is high, but the quality of the resource is low, consumers may compensate through increased consumption (Behmer, 2009). While this process increases the ingestion of limiting nutrients, there can be energetic cost associated with collecting, processing, and digesting greater quantities of food (Anderson et al., 2005; Frost et al., 2005). Compensatory feeding has been observed in a range of terrestrial (Behmer, 2009) and aquatic organisms, including benthic grazers (Cruz-Rivera & Hay, 2000; Fink & Von Elert, 2006), but the ability of compensatory feeding to successfully mitigate nutritional deficiencies is not well understood in aquatic invertebrates. Second, consumers can utilize post-ingestive processes to alter the extraction of limiting nutrients from their food by increasing nutrient absorption, assimilation efficiency, or selectively excreting excess nutrients (Boersma & Kreutzer, 2002; Sterner & Elser, 2002; Behmer, 2009). This process also likely incurs an energetic cost. Lastly, consumers can deal with imbalances by changing the nutrient composition of their tissues, thereby changing their growth requirements (Walve & Larsson, 1999; Sterner & George, 2000; Plath & Boersma, 2001; Sterner & Elser, 2002; Behmer, 2009).

Our growth data show that *Baetis* was not able to maintain similar growth across the dissolved N:P ratios tested. Given the mechanisms discussed above, pre- and post-ingestive mechanisms may have been employed in the high N:P treatments, but were either insufficient or too energetically costly to maintain the growth rate seen in the low N:P treatments. Compensatory feeding may have been constrained by primary production, preventing the mayflies from reaching their P requirements in the high N:P treatments. Although we did not measure mayfly consumption, we did use the C content of the leaves in the streams as a proxy for microbial growth. Leaf C content in the Ambient treatment did exceed that of the reference leaves, indicating more microbial biomass. This, however, does not mean that the preferred mayfly resources were accumulating, as both microbial and algal growth were apparent. In fact, the increase in C content could indicate that the mayflies were preferentially ingesting one resource over the other at a faster rate, allowing the less preferred resource to

accumulate. No C accumulation was documented in the Enriched treatment. However, because C content was only measured at the end of the study, we do not know if the lack of accumulation was a function of increased consumption by the mayflies, or low microbial growth.

Mayflies may have also been constrained by post-ingestive mechanisms. Many detritivores, including mayflies, have evolved a suite of physiological characteristics to survive on extremely nutrient-poor resources; however, this does not make them resistant to imbalances. Rothlisberger et al. (2008) showed mayflies are very efficient at retaining P, with P assimilation efficiencies documented as high as 92% under normal conditions. This shows that while mayflies are efficient at utilizing P in their diet, they may not have the post-ingestive flexibility to deal with severe nutritional imbalances, because they may already be operating at maximum efficiency. Additionally, if P assimilation was somehow restricted, it may reduce the efficacy of compensatory feeding, as the only way for dietary P retention to increase with a static P assimilation rate would be through increasing N excretion (but at a metabolic cost). If this is the case, compensatory feeding can become energetically prohibitive, limiting growth.

The interaction between dissolved nutrient ratios and total concentrations, as well as their impact on food-web dynamics, is a topic that has yet to be thoroughly explored in aquatic ecosystems. In auto-trophic systems, interactions between nutrient ratios and concentrations have been explored in relation to food-web dynamics (Hesson et al., 2002; Acharya et al., 2004; Fink & Von Elert, 2006), but in heterotrophic systems, which have very different nutrient-energy dynamics, similar studies are lacking (Sterner & Elser, 2002; Hesson et al., 2004). Instead, the work in detritus-based streams has focused on enrichment, with little attention given to the elemental proportions, or ratio, of the nutrient addition. Furthermore, most of this work has focused on high gradient, forested, low-nutrient streams that are less likely to face enrichment than lower gradient streams (given the land use associated with these areas). Such studies provide insights into consumer-resource dynamics and nutrient cycling in detritus-based systems, but whether these relationships exist in higher-nutrient systems remains unknown.

In conclusion, our study suggests that nutrient imbalance can exist in high-nutrient detritus-based

streams, and that the proportion of dissolved nutrients plays a primary role in these limitations for stream invertebrates. It is important to note that the N:P ratios and concentrations tested in this study were well within the range reported for the reference area (MPCA Website: http://www.pca.state.mn.us), and these nutrient conditions commonly occur in streams in this area. Given that we found a strong effect of dissolved N:P for both the Ambient and Enrichment treatments, these results have important implications for water quality monitoring protocols and the implementation of water quality standards. Our results suggest that nutrient limitations more likely mediated by imbalances in the proportion of multiple nutrients, rather than the total concentrations of single nutrients. In light of this, regulatory agencies should focus less on single nutrient levels, such as TMDLs (total maximum daily loads), and place more emphasis on interactions among nutrient proportions and total concentrations.

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