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Nutrient loading and grazing by the minnow *Phoxinus erythrogaster* shift periphyton abundance and stoichiometry in mesocosms

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SUMMARY

1. Anthropogenic activities in prairie streams are increasing nutrient inputs and altering stream communities. Understanding the role of large consumers such as fish in regulating periphyton structure and nutritional content is necessary to predict how changing diversity will interact with nutrient enrichment to regulate stream nutrient processing and retention.
2. We characterised the importance of grazing fish on stream nutrient storage and cycling following a simulated flood under different nutrient regimes by crossing six nutrient concentrations with six densities of a grazing minnow (southern redbelly dace, *Phoxinus erythrogaster*) in large outdoor mesocosms. We measured the biomass and stoichiometry of overstory and understory periphyton layers, the stoichiometry of fish tissue and excretion, and compared fish diet composition with available algal assemblages in pools and riffles to evaluate whether fish were selectively foraging within or among habitats.
3. Model selection indicated nutrient loading and fish density were important to algal composition and periphyton carbon (C): nitrogen (N). Nutrient loading increased algal biomass, favoured diatom growth over green algae and decreased periphyton C : N. Increasing grazer density did not affect biomass and reduced the C : N of overstory, but not understory periphyton. Algal composition of dace diet was correlated with available algae, but there were proportionately more diatoms present in dace guts. We found no correlation between fish egestion/excretion nutrient ratios and nutrient loading or fish density despite varying N content of periphyton.
4. Large grazers and nutrient availability can have a spatially distinct influence at a microhabitat scale on the nutrient status of primary producers in streams.

Keywords: algae, ecological stoichiometry, fish, nutrient cycling, prairie streams

Introduction

Intermittent streams are characterised by flood and drought, and subsequent recovery of ecosystem structure and function is influenced by colonisation dynamics of consumers (Bertrand *et al.*, 2009; Murdock *et al.*, 2010). Grasslands and wooded grasslands contain large numbers and linear distances of small intermittent streams (Nadeau & Rains, 2007) and drain approximately a third of the run-off from the

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Earth's terrestrial surface (Dodds, 1997). Grassland streams worldwide are among the most endangered habitats (Dodds *et al.*, 2004), and nutrient pollution is common in prairie catchments (Dodds *et al.*, 2004; Bernot *et al.*, 2006). Increased nutrient loading can lead to reduced water quality (Dodds & Welch, 2000), altered algal composition (Pringle & Bowers, 1984; Kelly, 1998) and ultimately decreased biodiversity (Vitousek *et al.*, 1997; Seehausen, van Alphen & Witte, 2007; Evans-White *et al.*, 2009). Concurrent decreases in biodiversity related to nutrient loading and other anthropogenic factors (e.g. Cross & Moss, 1987; Fausch & Bestgen, 1997) could interact with nutrient pollution to alter stream ecosystem function. Understanding the interactive effects of changing consumer communities and increased nutrient loading on intermittent stream nutrient cycling is needed to better realise the ecological consequences of species loss in these streams and the mechanisms behind nutrient retention.

Stream grazers are generally recognised as having a strong influence on algal communities (McCormick & Stevenson, 1989; Mulholland *et al.*, 1991; Pringle & Hamazaki, 1997; Bengtson, Evans-White & Gido, 2008). Diverse responses by algae to consumers (positive, negative or neutral) can be attributed to selective foraging as a result of algal location, morphology (Lubchenco & Gaines, 1981; Geddes & Trexler, 2003) or nutritional content (van Dam *et al.*, 2002). Selective foraging and nutrient excretion by grazers can change periphyton nutrient ratios, specifically with regard to carbon (C), nitrogen (N) and phosphorus (P) (Evans-White & Lamberti, 2005, 2006; Hillebrand, Frost & Liess, 2008).

Grazers can decrease periphyton C : N and C : P ratios (Hillebrand *et al.*, 2008), which may correlate with changes to algal communities (Frost, Hillebrand & Kahlert, 2005b). Grazer activity also indirectly removes senescent algae, which alters light and nutrient levels to the underlying cells (Rosemond, Mulholland & Elwood, 1993; Hillebrand & Kahlert, 2001; van Dam *et al.*, 2002) and can increase the overall nutrient concentration of periphyton by increasing the proportion of live material. Differences in light penetration may promote microscale differences in nutrient demand (Dodds, 1989; Dodds *et al.*, 1996) and could have differential consequences for nutrient ratios in 'understory' and 'overstory' layers of algae. Additionally, grazing can alter periphyton community structure and architecture, and this can modify flow patterns and advective transport rates (Biggs, Goring & Nikora, 1998; Dodds & Biggs, 2002). The interrelationships among periphyton architecture, flow and nutrient dynamics coupled with patchy distribution of grazers should make riffle and pool habitats, as well as overstory and understory algal communities, exhibit differential stoichiometric responses to a changing environment.

Variation in algal stoichiometry is linked to resource supplies (Sterner & Elser, 2002), and C : N and C : P ratios of periphyton generally decrease with increasing nutrient availability (Hillebrand & Kahlert, 2001; Frost, Cross & Benstead, 2005). Alternatively, the stoichiometry of most consumers, including grazing fish, is relatively homeostatic (Sterner & Elser, 2002), but can vary across taxa and body sizes (Vanni *et al.*, 2002; Cross *et al.*, 2005; Torres & Vanni, 2007). Ecological stoichiometry theory (Sterner & Elser, 2002) predicts that limiting nutrients are retained by consumers and abundant nutrients are excreted in higher proportions. These excreted nutrients can have a large effect on nutrient cycling and availability (Vanni & Layne, 1997; Vanni, Layne & Arnott, 1997; Gido, 2002; McIntyre *et al.*, 2008), and a better understanding of the impacts of fish excretion on nutrient cycling is critical for understanding system dynamics, yet it has not been studied extensively (Frost, Cross & Benstead, 2005), especially in lotic systems (McIntyre *et al.*, 2008).

The goal of our study was to examine how varying nutrient availability and the abundance of southern redbelly dace [*Phoxinus erythrogaster* (Rafinesque)], a grazing minnow, influence the retention of nutrients in periphyton biomass in large outdoor mesocosms. We had three main questions: 1) how does grazer abundance and nutrient availability influence the biomass and stoichiometry (C : N : P ratios) of periphyton in different habitats (i.e. pools versus riffles and overstory versus understory) and 2) the stoichiometry of grazing fish tissue and excretion? Additionally, 3) are shifts in algal community structure with nutrient loading and grazer density observed in grazer diets? We predicted that increasing nutrient loading would increase algal biomass and decrease periphyton C : N and C : P ratios as a result of changes in algal species composition and alleviation in nutrient limitation (Dodds & Priscu, 1990; Dodds & Whiles, 2010). Increased grazer density was also hypothesised to increase algal productivity and

decrease C : N and C : P ratios because inadvertent foraging on senescent cells and subsequent excretion makes nutrients readily available to periphyton (see Hillebrand *et al*., 2008). We predicted that the diet of dace would closely resemble overstory algal assemblages because the overstory should be more easily exploited given dace mouthpart morphology, which is adapted to biting edges of algal mats that extend from the attachment surface (Forbes & Richardson, 1920), and that the selective foraging might have spatially explicit effects on stoichiometry of primary producers. Finally, we expected negligible changes in elemental ratios of grazer tissue, but hypothesised that the N and P content of their excreted and egested material should increase with nutrient loading and subsequent increases in N and P quality of diet items.

Methods

Study site

Our study was conducted in 36 large, outdoor stream mesocosms located at the Konza Prairie Biological Station (KPBS) near Manhattan, Kansas (39°05′N, 96°35′W) during May–June of 2007. Each mesocosm consisted of a 2.54-m^2 pool and 0.84-m^2 riffle as described in Matthews *et al*. (2006). Water was continuously supplied by a spring (about 1000 L day^{-1} to each mesocosm) and recirculated with an electric trolling motor creating a discharge of approximately 10 L s^{-1} and a current velocity of 6–8 cm s^{-1} in the riffle. Spring water nutrient concentrations were low [average total nitrogen (TN) 130 μg L^{-1}, total phosphorus (TP) 2.8 μg L^{-1}, NO_3^- 30 μg L^{-1}, soluble reactive phosphorus (SRP) 1 μg L^{-1}]. A shade canopy reduced ambient sunlight by 60%, and water temperature ranged diurnally from 18.6 to 23.7 °C with a mean of 21.1 °C. Mesocosm characteristics were similar to nearby headwater prairie streams in terms of substrata size and type (a mixture of gravel and pebble from a local quarry), pool and riffle size, hyporheic conditions (subsurface area) and water velocities. Mesocosms were filled on 20 April 2007, left for one month and then scoured with a high pressure hose to simulate flooding. Suspended organic materials were rapidly drained from each mesocosm, and they were refilled within 24 h. Bertrand *et al*. (2009) showed this greatly reduced algal biomass (chlorophyll *a* <10 mg m^{-2}), invertebrate densities and amounts of total particulate organic material. Flooding resulted in no macroscopically detectable filamentous algae and led to barely detectable rates of gross primary production, thus homogenising all streams at the beginning of the experiment.

Experimental design

We used an experimental approach that crossed gradients of nutrient availability and grazer densities that was designed to detect the functional responses and relative intensities of both independent variables (Cottingham, Lennon & Brown, 2005). Nutrients were continuously added to each mesocosm by mixing a concentrated nutrient solution with the inflowing spring water using FMI metering pumps (model QBG, Fluid Metering Inc., Syosset, NY, U.S.A.) pumping at a rate of 5 mL min^{-1} from 18-L sealed buckets. Nitrogen (as dissolved KNO_3^-) was added at target inflow concentrations of 30 (ambient nutrients), 60, 120, 240, 500 or 1000 μg TN L^{-1}, and phosphorus (as dissolved $KHPO_4^-$) was added simultaneously at the Redfield ratio (with molar N : P = 16 : 1) to six streams per treatment. Incoming water was sampled weekly for TN, TP NO_3^- and SRP and analysed with standard methods (Murphy & Riley, 1962; Solorzano & Sharp, 1980; Dodds, 2003). Dace were stocked at six densities (0, 10, 20, 30, 40 and 50 individuals per stream, average of about 1-g wet mass $fish^{-1}$, 0- to 15-g wet mass m^{-2}), creating 36 grazer–nutrient combinations. Densities were determined from the range of fish densities that typically occur in nearby Kings Creek (Franssen *et al*., 2006; Bertrand & Gido, 2007), which are similar to other prairie stream *P. erythrogaster* densities (Stasiak, 2007). The experiment ran for 23 days. Dead fish were replaced (death rate was <1%) and inflow discharge was adjusted each day to maintain consistent grazer abundance and nutrient input.

Periphyton

On 15 June 2007, we collected periphyton from three randomly selected rocks from each pool and riffle. Long filamentous algal mats (up to 40-cm long in some treatments) developed on substrata, and samples from each rock were partitioned by layer as 'overstory' and 'understory' growth. Overstory was defined as the loosely attached periphyton that could easily be

removed by gently scraping with the back of a toothbrush, and the understory was the remaining attached material that was removed by vigorous brushing. In the laboratory, each sample was homogenised with a hand blender, and deionised water was added to bring the volume to 50 mL. The sample was then partitioned into four subsamples for algal community composition, chlorophyll *a*, C : N and %P analyses.

Ten millilitres of the periphyton sample was filtered onto a GF/F filter and frozen until analysed for chlorophyll *a*. Chlorophyll was extracted within 30 days of collection by adding 25 mL of 95% ethyl alcohol and the filter to a centrifuge tube. The tube was placed into a water bath at 78 °C for 5 min and extracted for 24 h in the dark at 4 °C (Sartory & Grobbelaar, 1984). Chlorophyll *a* concentrations were determined using a Turner Fluorometer (model 112) with a filter set and lamp that avoids interference from phaeophytin (Welschmeyer, 1995). Projectional rock surface areas were measured by tracing the perimeter of each rock and scanning the outlines into a digital image. The area within each outline was determined using SigmaScan version 5 (Systat Software Inc. San Jose, CA, U.S.A.).

For algal assemblage composition, 10 mL of the periphyton slurry was poured into plastic scintillation vials and preserved with a final concentration of approximately 3% formalin for subsequent algal identification. A 50-μL subsample was added to a glass slide, and ten fields of view were counted per slide (minimum of 250 cells) at 400× magnification. Dense samples were diluted with a known volume prior to microscopic analysis. Cells were classified as green algae, diatoms or cyanobacteria. The lengths and widths of 10 cells of each cell category were measured to estimate biovolume, and per cent biovolume of each major group was calculated.

The remaining periphyton sample was split into two 15-mL aliquots, dried in an oven at 50–55 °C and ground to a fine powder. One sample was used to measure C and N content using a CHN-2400 Elemental Analyzer. The other sample was ashed in a muffle furnace at 500 °C for 2 h, digested and analysed for SRP to estimate total P content (Murphy & Riley, 1962).

Grazer diet analysis

One or two fish from each stream (58 total) were collected with a backpack electroshocker and immediately placed in a plastic bag with 10% formalin for diet analysis. Gut contents were removed from the foregut according to Bowen (1996) and placed on a slide with 50 μL of water. Diet algal composition was analysed using the same method as for algal community composition. The presence of invertebrate parts was also noted.

Grazer excretion, egestion and stoichiometry

Fish were collected with a backpack electroshocker by exerting a short pulse of electricity and rapidly netting stunned fish to minimise stress. Fish were then placed in spring water for 15–30 min to recover from shock. Excretion was measured by placing three fish (average length of 51 mm) in 1-L bottles filled with 800 mL of filtered spring water. A background nutrient sample was collected prior to adding fish to the bottle, and afterwards water samples were taken every 15 min for 1 h. Samples were immediately placed on ice and frozen within 4 h. NH_4^+ and SRP concentrations were analysed using the indo-phenol blue and ascorbic acid methods, respectively, using an O-I Analytical Flow Solution IV autoanalyzer (APHA, 1998). Excretion rates were calculated as the slope of the increase in NH_4^+ or SRP over time and converted to mg N or P g wet mass^{-1} day^{-1}, as this rate was assumed to be constant through time. Solid egested material was collected from the bottles used for the excretion analysis on ashed GF/F filters and immediately frozen. Fish were frozen for subsequent tissue C : N : P analysis after water samples were collected. Intestines and gonads were removed prior to drying the fish at 55 °C and were then ground to a fine powder with a coffee grinder. C, N and P content for both solid egestion and fish tissue was measured using the same methods as described for periphyton.

Data analysis

Water chemistry data from all dates preceding periphyton sampling were averaged according to treatment. Estimates from incoming water indicated that P (as SRP) was at relatively constant loading rates in all treatments throughout the experiment (Table 1). Therefore, the molar N : P ratio of incoming water was below Redfield ratio (16 : 1) on average in the lower three nutrient treatments and above Redfield ratio in the highest three treatments.

Table 1 Average observed nutrient concentrations of incoming water for the duration of the experiment and the maximum estimated N cycling by fish (assuming 50-g wet mass per stream)

Target NO_3^- loading (mg day^{-1})	Average observed NO_3^- loading (mg day^{-1})	Average observed PO_4^{3-} loading (mg day^{-1})	Dissolved molar N : P of incoming water	Maximum % of N loading excreted by grazers per day
30 (ambient)	69	23	7	45
60	116	23	11	27
120	124	22	13	25
240	181	21	20	17
500	372	28	29	8
1000	659	33	41	5

Prior to statistical analyses, nutrient and algal assemblage composition data were $\log_{10}$ transformed to normalise data and reduced the influence of extreme points. We created linear models (Zuur, Ieno & Smith, 2007) using the combinations of explanatory variables [dace, nutrients, habitat (pool or riffle) and periphyton layer (overstory or understory)] to describe variation in periphyton response variables, with global models including all explanatory variables. After candidate models were selected, we used Akaike's Information Criterion corrected for small sample sizes (AIC*c*) to rank results of various possible regression model sets by assigning an AIC*c* value to each model, with the lowest value indicating the highest rank. The difference between individual AIC*c* values and the lowest AIC*c* value was calculated for each model, as well as the weight, which is the probability that an individual model is the best approximating model for the data (Burnham & Anderson, 2002). In addition to the AIC*c* comparison, ranked models and individual variables were compared with linear regression to obtain adjusted R-squared values (Table 2). AIC controls for the statistical effect of adding independent variables to multiple regression models (i.e. helps guard against over fitting data which can happen if only R-squared values are compared).

Algal functional composition and diet samples were dominated by diatoms and green algae, which together comprised between 85 and 100% of the total material observed. Therefore, we calculated percentage of biovolume ratios of green algae to diatoms for each diet and benthic algal sample to identify major assemblage shifts. We compared the green algae : diatom ratios in fish guts with algal ratios on rocks in different habitats and layers (pool versus riffle and overstory versus understory) using simple linear regression to test for selective feeding and how it varied by habitat. Slopes from regressions were compared to 1 : 1 lines using the slope.test function in the 'smatr' R package (Warton *et al*., 2006). Models with slopes significantly deviating from '1' were further analysed by calculating the difference between green algae : diatom ratios from dace gut contents and the corresponding ratios from rocks for respective habitats and layers. Resulting values were compared with zero to test for algal group foraging preference, with significance indicated by *t*-test ($a = 0.05$). All statistics were performed using the R console, version 2.9.2 (R Development Core Team, 2008).

Results

Periphyton

Algal community diversity was low and was dominated by filamentous green algae (*Spirogyra sp.*, *Oedogonium sp.* and *Microspora sp.*) and pennate diatoms (*Synedra sp.* and *Fragilaria sp.*). Nutrient loading and dace density explained between 32 and 50% of the variation in green algae to diatom ratios in overstory and understory communities, respectively. Increased nutrient supply increased the proportion of diatoms (i.e. decreased the green algae : diatom ratio) in all locations (r^2 from regression ranged from 0.25 to 0.50, Fig. 1), while grazers alone had less influence (Table 2). The best AIC*c* model explaining variation in algal structure included both nutrients and dace ($w_i = 48\%$; Table 2). Nutrient loading increased overstory and understory algal biomass in both riffles and pools (Fig. 2), and the 'nutrients + layer + habitat' model for chlorophyll *a* captured 62% of the weight, followed by the inclusion of dace in the global model with 33% (Table 2).

Table 2 AIC*c* output and adjusted R^2 values for models describing periphyton response variables in mesocosms

Model	*k*	AIC*c*	ΔAIC*c*	AIC*c*Wt.	Cum.Wt	Adj. R^2
Green algae : diatoms						
nutrients + dace	4	1753.456	0.0000	0.4837	0.4837	0.2501
nutrients + dace + habitat	5	1754.871	1.4148	0.2384	0.7222	0.2486
nutrients + dace + layer	5	1755.376	1.9202	0.1852	0.9074	0.2459
nutrients + dace + layer + habitat	6	1756.810	3.3536	0.0904	0.9978	0.2444
nutrients	3	1764.601	11.1447	0.0018	0.9997	0.1826
nutrients + layer + habitat	5	1767.979	14.5232	0.0003	1.0000	0.1759
dace	3	1783.402	29.9459	0.0000	1.0000	0.0669
dace + layer + habitat	5	1786.723	33.2666	0.0000	1.0000	0.0597
null	2	1792.156	38.6997	0.0000	1.0000	n.a.
Chlorophyll *a*						
nutrients + layer + habitat	5	1432.590	0.0000	0.6224	0.6224	0.3890
nutrients + dace + layer + habitat	6	1433.864	1.2746	0.3291	0.9515	0.3884
nutrients + dace + layer	5	1437.696	5.1060	0.0485	1.0000	0.3668
dace + layer + habitat	5	1457.590	25.0003	0.0000	1.0000	0.2722
nutrients + dace + habitat	5	1483.294	50.7039	0.0000	1.0000	0.1289
nutrients	3	1484.005	51.4151	0.0000	1.0000	0.1109
nutrients + dace	4	1485.552	52.9620	0.0000	1.0000	0.1081
null	2	1499.735	67.1451	0.0000	1.0000	n.a.
dace	3	1501.335	68.7452	0.0000	1.0000	−0.0037
Periphyton C : N						
nutrients + dace + layer + habitat	6	911.1171	0.0000	0.9984	0.9984	0.4637
nutrients + layer + habitat	5	924.0694	12.9478	0.0015	0.9999	0.4085
nutrients + dace + layer	5	930.2630	19.1460	0.0001	1.0000	0.3825
nutrients + dace + habitat	5	944.7867	33.6697	0.0000	1.0000	0.3170
dace + layer + habitat	5	953.7606	42.6435	0.0000	1.0000	0.2731
nutrients + dace	4	959.5284	48.4114	0.0000	1.0000	0.2374
nutrients	3	968.0663	56.9492	0.0000	1.0000	0.1847
dace	3	989.9099	78.7928	0.0000	1.0000	0.0511
null	2	996.3880	85.2709	0.0000	1.0000	n.a.
Periphyton C : P						
nutrients + layer + habitat	5	2039.453	0.0000	0.5832	0.5832	0.1378
nutrients + dace + layer + habitat	6	2041.069	1.6157	0.2600	0.8432	0.1349
dace + layer + habitat	5	2042.338	2.8847	0.1379	0.9811	0.1197
nutrients + dace + layer	5	2046.461	7.0080	0.0175	0.9986	0.0932
nutrients + dace + habitat	5	2052.186	12.7328	0.0010	0.9996	0.0551
nutrients	3	2055.974	16.5209	0.0002	0.9998	0.0935
null	2	2056.749	17.2959	0.0001	0.9999	n.a.
nutrients + dace	4	2057.643	18.1900	0.0001	1.0000	0.0092
dace	3	2058.458	19.0048	0.0000	1.0000	−0.0045
Periphyton N : P						
null	2	1102.165	0.0000	0.3469	0.3469	n.a.
nutrients	3	1103.337	1.1721	0.1930	0.5399	−0.0007
dace	3	1103.392	1.2270	0.1878	0.7277	−0.0011
nutrients + dace	4	1104.537	2.3720	0.1060	0.8337	−0.0014
nutrients + layer + habitat	5	1106.107	3.9419	0.0483	0.8820	−0.0107
dace + layer + habitat	5	1106.570	4.4044	0.0384	0.9204	−0.0110
nutrients + dace + habitat	5	1106.956	4.7910	0.0316	0.9520	−0.0046
nutrients + dace + region	5	1106.998	4.8326	0.0310	0.9829	−0.0079
nutrients + dace + layer + habitat	6	1108.191	6.0255	0.0171	1.0000	−0.0113

AIC, Akaike's Information Criterion.

k indicates the number of parameters, ΔAIC*c* is the difference between the AIC*c* value and the lowest AIC*c* value, AIC*c*Wt is the relative likelihood that the model is the best approximating model, and Cum.Wt is the cumulative weight. Habitat = pool or riffle, layer = overstory or understory, nutrients = $\log_{10}$ average nitrogen loading (mg $N - NO_3^-$ day^{-1}), dace = g wet mass, and n.a. is not applicable.

Periphyton C : N decreased with increased nutrient loading and grazer density (Figs 3 & 4). C : N ratios at all locations were a function of nutrient loading, explaining 11–37% of the variation in periphyton communities with simple regression (Fig. 3). Grazer density decreased C : N content of both riffle and pool periphyton overstory layers (Fig. 4), but had less influence on pool (r^2 = 0.000) and riffle (r^2 = 0.027) understory layers. The global model (including nutrients, dace, layer and habitat) for C : N was heavily ranked above all other candidate models with almost 100% of the weight (Table 2). Dace did not strongly affect periphyton C : P ratios, and AIC*c* ranked the 'nutrients + layer + habitat' model as the most explanatory with 58% of the weight, followed by the global model with 26% (Table 2). Responses of periphyton N : P ratios to treatments were more ambiguous, and the null model received 35% of the weight, followed by nutrients (19%) and dace (19%) (Table 2).

Grazer diet

Thirty-three per cent of dace guts contained invertebrate parts, but guts were overwhelmingly dominated (volumetrically) by diatoms and green algae. Nutrients and grazer density explained 42% of the observed variation in the proportion of diatoms and green algae in the diet. Both grazer density and nutrient concentration were negatively associated with green algae : diatom diet ratios (Fig. 5). Regressions of algal ratios in the diet as a function of available periphyton ratios by habitat explained up to 43% of the variation in riffles and up to 21% in pools (Fig. 6). Slopes from all models were significantly lower than a slope of unity, but slopes of diet compared to pool community structure were the most different (0.51 and 0.28 for pool overstory and pool understory). Across all habitats and layers, the difference between diet and periphyton green algae : diatom ratios was significantly less than zero, indicating greater relative diatom biovolumes in diet (*t*-test, $t = -3.93$, $p < 0.01$). However, when comparing gut contents with individual communities, the difference was only significant for the pool overstory (*t*-test, $t = -3.70$, $p < 0.01$).

Grazer tissue and excretion stoichiometry

Stoichiometries of dace tissue, egestion and excretion were not related to fish density or nutrient loading.

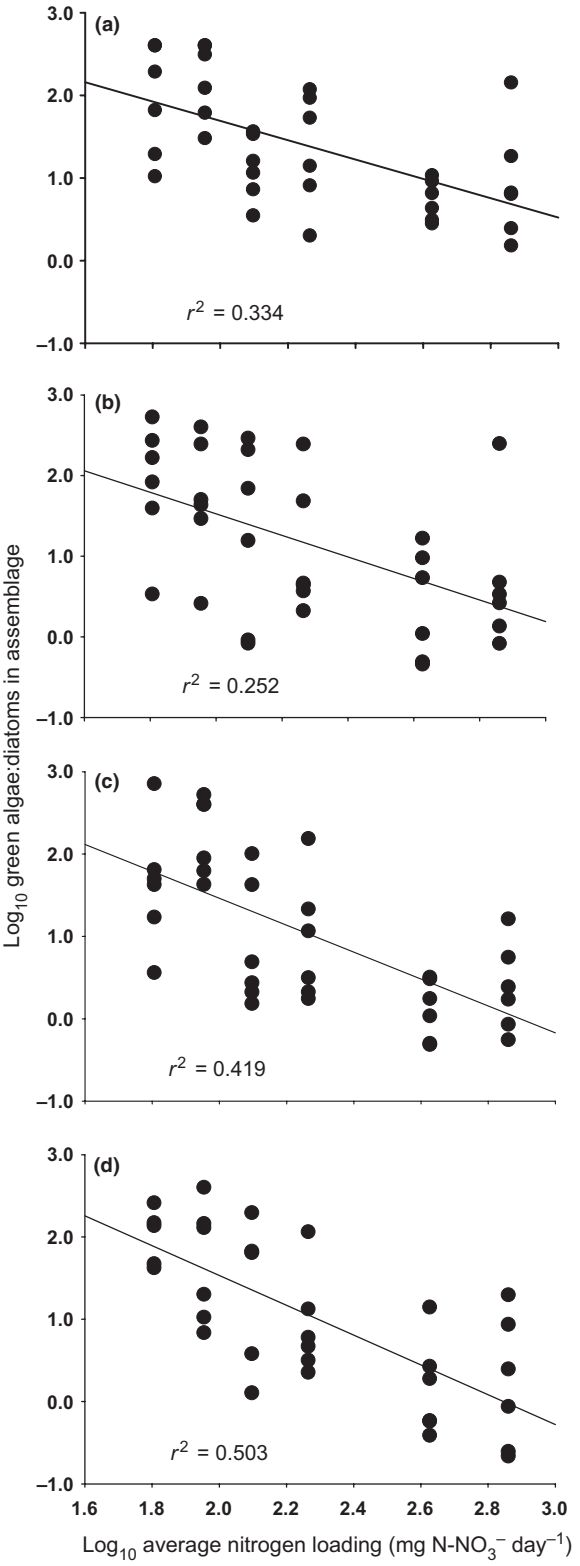

Fig. 1 The ratio of green algae to diatom biovolumes in the (a) pool overstory, (b) pool understory, (c) riffle overstory and (d) riffle understory habitats plotted against nutrient loading.

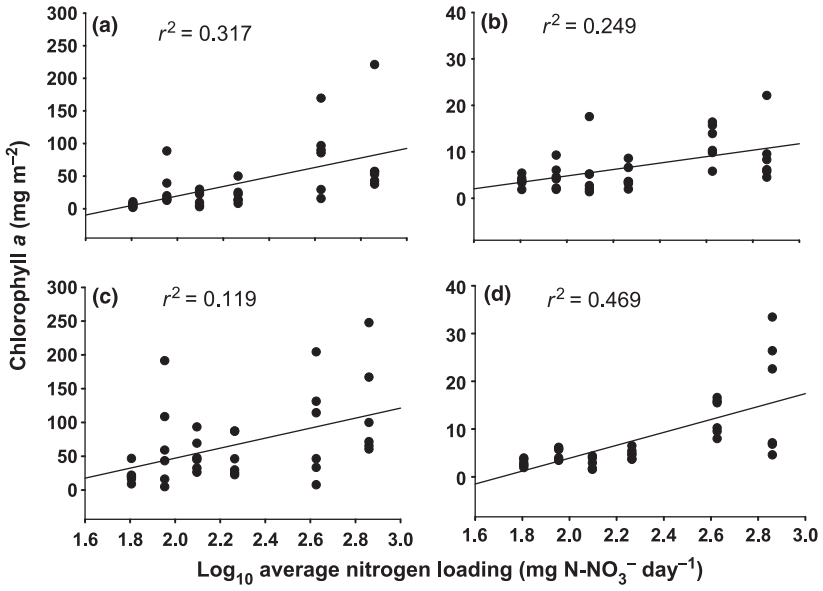

Fig. 2 Algal biomass as estimated by chlorophyll *a* plotted against nutrient loading for (a) pool overstory, (b) pool understory, (c) riffle overstory and (d) riffle understory.

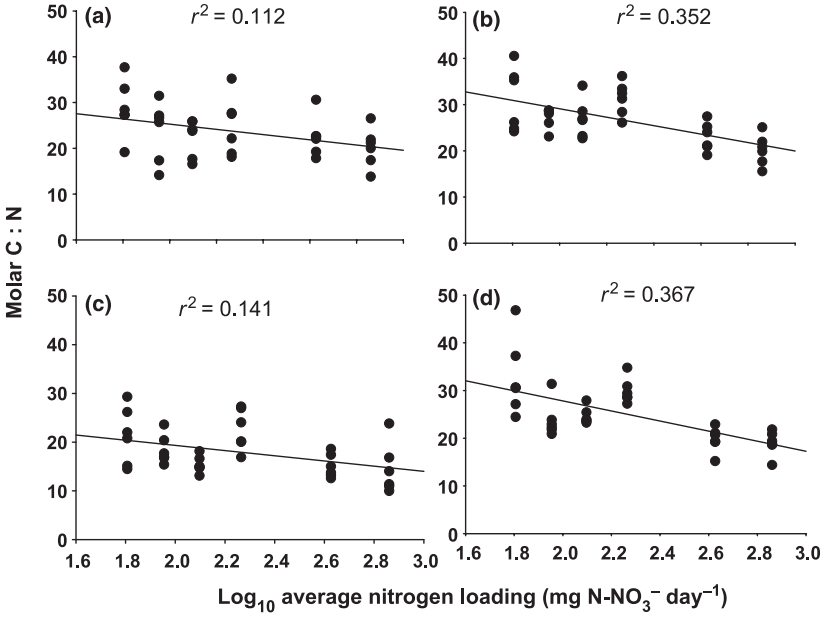

Fig. 3 Molar C : N of periphyton plotted against nutrient loading for (a) pool overstory, (b) pool understory, (c) riffle overstory and (d) riffle understory.

Dace tissue had an average C : N ratio of 5.72 ± 0.12 (mean ± SE) and C : P ratio of 158.75 ± 10.90, while solid excretion material had higher values of 13.15 ± 0.39 and 216.49 ± 21.16 for C : N and C : P, respectively. Dace tissue N : P ratios were significantly higher (27.94 ± 1.93) than solid excretion N : P (16.37 ± 1.44, *t*-test, $t = 4.76$, $p < 0.01$). Estimates of nitrogen excretion by dace averaged 0.62 ± 0.05 mg N g wet mass^{-1} d^{-1} and ranged from a relatively small fraction of total loading rates (<1% with lowest fish density and highest nutrient loading) up to 45% of nitrogen loading with maximum fish density and ambient nutrient loading (Table 1). SRP excretion from dace was below the limit of detection for all treatments (0.004 μg P g wet mass^{-1} min^{-1}).

Discussion

We investigated how gradients of grazing fish densities and nutrient supply affect nutrient cycling in a

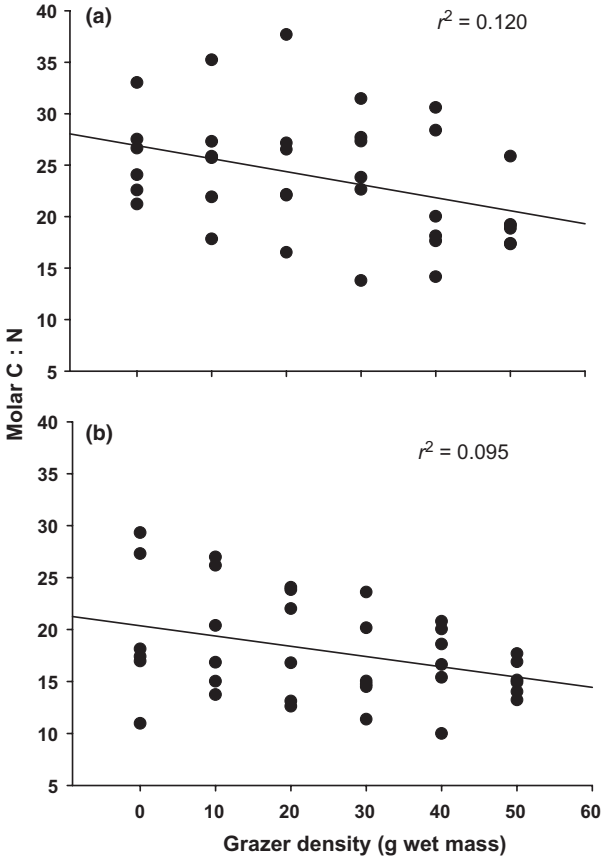

Fig. 4 Molar C : N of periphyton plotted against grazer density for (a) pool overstory and (b) riffle overstory.

simplified system. We found both nutrients and grazers to have an effect on periphyton elemental composition and community structure. Dissolved incoming water nutrients deviated from Redfield ratio, with phosphorus levels generally higher than the targeted 16 : 1 N : P concentrations, leading to little overall treatment effects on periphyton C : P and N : P ratios. The loading ratios also created N-limited conditions, so C : N ratios were more responsive to experimental manipulation. As a result, the grazers increased the N quality of overstory periphyton layers, presumably because of remineralisation increasing N supply and consumption removing C-rich materials. Our experiment further suggests that N loading by grazer excretion was comparable to N loading rates in the lower ranges of nutrient loading treatments. These results add to the growing body of literature that suggest organisms are capable of both directly and indirectly modifying their environment under nutrient-limited conditions.

Periphyton

Periphyton C : N decreased from an average of approximately 33 in mesocosms with ambient nutrient loading to 21 at the highest rate of nutrient loading (habitats and layers combined). This decline in C : N is consistent with previous studies (reviewed by Frost, Cross & Benstead, 2005) and indicates that periphyton C : N moved towards the predicted Redfield ratio (~7) as N became more available. The relationship between the C : P ratios of periphyton and nutrient influx was much weaker and was consistently above Redfield ratio (averaged about 718 for ambient, 797 for highest nutrient loading). Primary producer C : N and C : P ratios are typically correlated with nutrient supply when those nutrients are limiting (Sterner & Elser, 2002). Therefore, our observation that C : N ratios were better explained by target nutrient concentrations than C : P ratios is consistent with the N : P ratios that were observed in the incoming water.

Algal biomass increased at all locations at higher nutrient concentrations, which is consistent with previous experiments with nutrient additions (Rosemond *et al.*, 1993; Flecker *et al.*, 2002). Algal assemblage composition was also influenced by the nutrient additions. The decreased C : N ratios of periphyton at

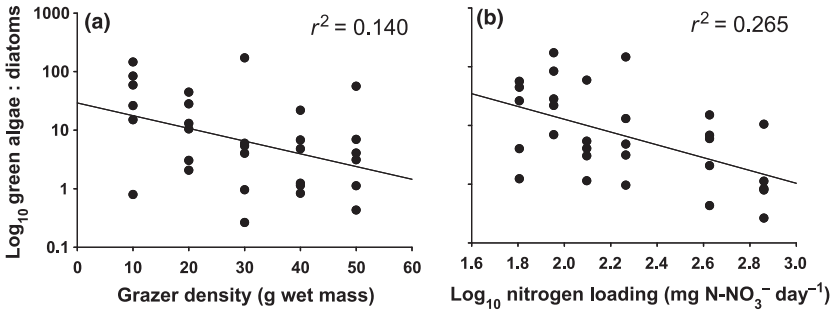

Fig. 5 The ratio of green algae to diatom biovolumes from dace guts plotted against (a) grazer density and (b) nutrient loading of mesocosms.

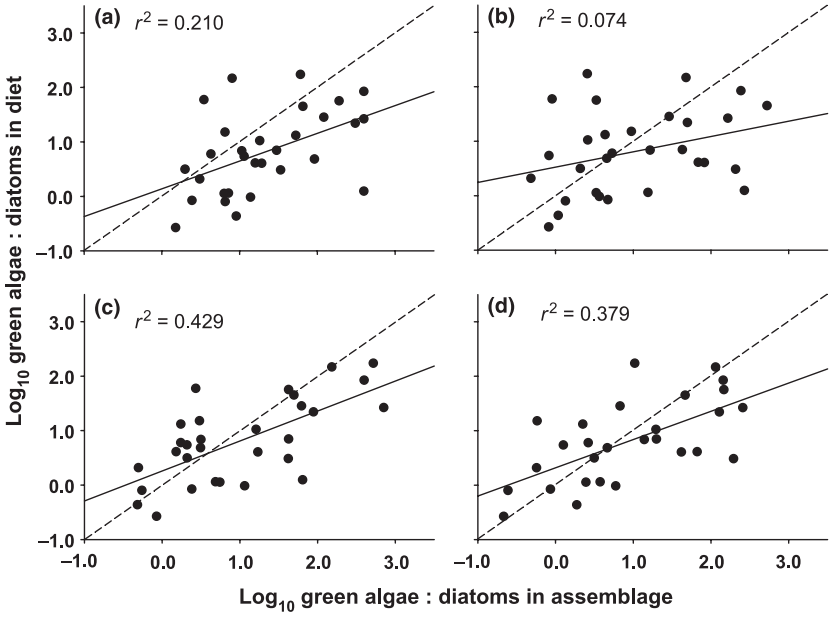

Fig. 6 The ratio of green algae to diatom biovolumes from dace diet plotted against the ratio of green algae to diatoms from (a) pool overstory, (b) pool understory, (c) riffle overstory and (d) riffle understory habitats. Dashed lines indicate 1 : 1 relationship.

all locations in response to increased nutrient loading may have reflected filament-dominated communities shifting to diatom dominance (Hillebrand, 2003). Filamentous green algae have cellulose (C-rich) cell walls whereas diatoms have silica cell walls (Dodds & Whiles, 2010). Increasing nutrient concentrations can change algal communities (Kelly, 1998), and subtle changes in periphyton taxonomic composition may affect community stoichiometry (Hillebrand & Kahlert, 2001; Frost, Hillebrand & Kahlert, 2005).

Grazer effects on periphyton

Fish density had little effect on algal biomass (chl *a*), which is consistent with the findings of Bertrand & Gido (2007) who found that dace altered algal filament lengths but not total algal biomass and suggested that nutrient recycling mediated by dace may offset losses from consumption. The observed reduction in the C : N ratio of overstory periphyton with increased grazer density further suggests nutrients recycled by fish influence periphyton production, as has been seen in other studies of stream consumers (reviewed by Cross *et al.*, 2005). However, there was no strong relationship between grazer density and periphyton C : P ratios for any location, which is consistent with the general lack of P limitation that we observed in our treatments.

Overstory algae may have assimilated the labile N excreted by dace, although the amount excreted was relatively small compared to the amount of NO_3^- added at higher loading rates. However, excreted NH_4^+ may have been preferentially assimilated because of its relative energetic efficiency compared to NO_3^- (Dodds & Whiles, 2010), amplifying the effect of fish excretion relative to experimental NO_3^- addition. The feeding behaviour of dace may have further stimulated or maintained growth by inadvertently foraging on the senescent algal cells on the exterior, providing additional avenues for nutrient delivery and light to underlying communities (see Hillebrand *et al.*, 2008; Holomuzki, Feminella & Power, 2010). The weaker effect of dace on understory periphyton C : N may have occurred because excreted nutrients were rapidly removed by the overstory algae or because the understory algae may have been less vulnerable to the direct or indirect effects of foraging.

Grazer diet

The observed shift towards a diatom-dominated periphyton assemblage with increased nutrient loading was also reflected in the diet of the grazers, as gut contents were correlated with the availability of algal groups under different treatments. Trends between diet and riffle overstory, riffle understory and pool overstory treatments appeared to be similar through regression, while there was more variability when diet was compared with the pool understory community. Stronger associations between diet and habitat-

specific periphyton assemblage composition may indicate preferred feeding locations, and dace may feed more commonly on overstory algae layers in the riffles, or on loose algae that were detached from the riffles and carried into the pool by the current.

However, dace gut contents contained proportionately more diatoms by volume than did the available periphyton communities, and the significantly lower slopes of diet versus available communities when compared to 1 : 1 lines suggest that dace may feed more on diatoms even when they are less available. Diatoms often grow on the exterior of green algae as epiphytes (Marks & Power, 2001), which make them more vulnerable to foraging than underlying filamentous taxa. Therefore, a higher proportion of diatoms in dace guts may reflect the most easily exploitable growth form rather than preference, and removal may have implications for resource acquisition for other periphyton constituents (Dudley, 1992; Kupferberg, 1997; reviewed by Holomuzki *et al*., 2010). However, diatom frustules in the gut may have been more resistant to breakdown as a result of their siliceous cell walls and therefore may have been over represented in the diet counts. While a possibility, effort to minimise this effect was taken by analysing only material in the foregut, which should reduce the level of digestion of diet items.

Grazer stoichiometry and excretion

The stoichiometry of fish tissue was not significantly affected by nutrient addition or grazer density. This result is consistent with previous studies of other fish species and with ecological stoichiometry theory, which assumes individual species of vertebrate consumers to be relatively homeostatic (Sterner & George, 2000; Sterner & Elser, 2002; Vanni *et al*., 2002). However, the duration of our experiment could have been too short to observe stoichiometric feedback for this compartment. While we were unable to find any studies addressing elemental turnover time in dace tissues, studies for other fish species suggest that timescales of months or years may be necessary for tissues to reach equilibrium with diet items (Hesslein, Hallard & Ramlal, 1993; MacAvoy, Macko & Garman, 2001; Miller, 2006).

The stoichiometry of egested (faeces) and excreted materials was also not correlated with different treatments, which contrasts with available literature that suggests the nutrient quality of diet items may be important in calculating nutrient mass balance (Schindler & Eby, 1997; Torres & Vanni, 2007). It may not be surprising that there was no trend in our study with regard to P concentration in excreted material, since periphyton P content was not noticeably altered as a result of our treatments. On the other hand, N content of periphyton at all locations was increased with nutrient additions, as well as by grazer density in the overstory layers of periphyton. We expected feedback through grazer excretion with this change in resource quality (e.g. Pilati & Vanni, 2007; Verant *et al*., 2007), but this was not observed in our mesocosms.

One possible explanation for this outcome is that fish in lower nutrient treatments were enriching their diets with invertebrates or other suspended materials (e.g. Evans-White, Dodds & Whiles, 2003). Although we found invertebrates to be a minor constituent in the guts of these grazers, herbivores may supplement their diets with non-plant material that is assimilated with relatively high efficiency (Evans-White *et al*., 2003). This may cause the stoichiometry of periphyton to fail to exactly reflect stoichiometry of diets even when periphyton dominates gut contents. Moreover, periphyton may include other constituents such as heterotrophic bacteria, fungi and detritus, which were not quantified in the diet analysis. Further experimentation in quantifying the conversion of different food sources to tissue would be necessary to evaluate these hypotheses.

Scaling mesocosm results

While our fish densities were based on the natural average biomass observed in Kings Creek, dace generally form large schools and are spatially distributed in a heterogeneous manner along longitudinal gradients, creating the potential for large effects in some habitats within a reach and negligible effects in others. Nonetheless, schools of dace may promote nutrient hotspots, such as that observed by McIntyre *et al.* (2008), with measurable effects at a pool/riffle scale, as well as within different layers of the algal mat. Although sampling on a single date, as well as employing a simplified grazing regime, cannot provide a holistic representation of grazer–resource interactions in the field (Murdock *et al*., 2010), our study suggests that large grazers and nutrient additions can exert strong influences on the nutrient status

of primary producers in streams. Further elucidation of nutrient flow dynamics with more species-rich animal communities similar to those found in the nearby streams might help further disentangle the importance of these relationships.

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