Research

Oikos 118: 1732–1742, 2009
doi: 10.1111/j.1600-0706.2009.17367.x,

Subject Editor: Tadashi Fukami. Accepted 11 May 2009

Feedbacks of consumer nutrient recycling on producer biomass and stoichiometry: separating direct and indirect effects

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Herbivores can have both direct (consumptive) and indirect (nutrient-mediated) effects on primary producer biomass and nutrient stoichiometry. Ecological stoichiometry theory predicts that herbivores of contrasting body stoichiometry will differentially remineralize nutrients, resulting in feedbacks on producer stoichiometry. We experimentally separated direct and indirect effects of aquatic vertebrate grazers on periphyton by manipulating grazer abundance and identity in mesocosms, and using grazer exclusion cages to expose periphyton to recycled nutrients in the absence of direct grazing. In experiment 1, we used a catfish with high body phosphorus (low body N:P), *Ancistrus triradiatus*, to assess consumptive versus nutrient-mediated effects of grazer density on periphyton. In experiment 2, we compared the nutrient-mediated effects of grazing by *Ancistrus triradiatus* and *Rana palmipes*, a tadpole with low body phosphorus and high body N:P. In experiment 1, we found that increasing catfish density led to lower biomass and particulate nutrients in periphyton through direct consumptive effects, but that nutrient-mediated indirect effects enhanced periphyton biomass when grazers were experimentally separated from direct contact with periphyton. As predicted by stoichiometry theory, nutrient recycling by this P-rich grazer tended to increase algal C:P and N:P (although effects were not statistically significant), while their consumptive effects reduced algal C:P and N:P. In experiment 2, grazer identity had strong effects on dissolved water nutrient concentrations, N recycling (measured with a ^{15}N tracer), and periphyton stoichiometry. In accordance with stoichiometry theory, catfish increased N concentrations and recycling rates leading to higher periphyton N:P, while tadpoles had greater effects on P availability leading to lower periphyton N:P. Our experiments elucidate the importance of both the density and identity of grazers in controlling periphyton biomass and stoichiometry through consumptive and nutrient-mediated effects, and support the power of ecological stoichiometry theory to predict feedbacks on producer stroichiometry arising from consumer stoichiometry through nutrient recycling.

Herbivores affect primary producers via multiple pathways (Huntly 1991, Andersen 1997, Strauss and Irwin 2004, Wardle and Bardgett 2004, Carline et al. 2005, Hillebrand et al. 2008). Herbivores can lower plant biomass, selectively reduce particular species, and alter competitive interactions among plants. In addition, herbivores can affect primary producers through a variety of nutrient cycling pathways that can result in higher producer particulate nutrients and growth rates (Hillebrand et al. 2008).

It can be difficult to disentangle the mechanisms by which herbivores affect plants because consumptive and nutrient-mediated effects occur simultaneously and may involve several feedbacks. In the case of plant particulate nutrients, herbivores can affect the availability of nutrients such as nitrogen (N) and phosphorus (P) via many pathways (Liess and Hillebrand 2004, Hillebrand et al. 2008). By lowering plant biomass, herbivores can reduce overall plant nutrient demand and increase nutrient uptake per unit plant biomass; this can alleviate plant nutrient limitation and increase particulate nutrients in plant tissues (McNaughton 1985, Hillebrand et al. 2008). In addition, excretion and egestion of nutrients by herbivores can further reduce the severity of nutrient limitation and thereby affect plant species composition (Sterner 1986, Mulholland et al. 1991, McNaughton et al. 1997, Wardle and Bardgett 2004). In benthic aquatic habitats where algae grow within biofilms, grazers can affect algal particulate nutrients by removing senescent algal cells and detritus. Grazing on senescent algae and detritus can increase nutrient uptake by algae, and thus increase the particulate nutrients in algal tissues (McCormick and Stevenson 1991, Hillebrand et al. 2008). Alternatively, removal of sediment detritus by grazers may increase light availability to algae, rendering algae more nutrient-limited by alleviating light limitation (Power 1990, Flecker 1992, Flecker et al. 2002).

Ecological stoichiometry theory (Sterner and Elser 2002) provides a useful framework for understanding plant-herbivore interactions mediated by nutrient cycling (Hillebrand et al. 2008). Herbivores can be nutrient-limited because they have higher body particulate nutrients than plant tissues (Elser et al. 2000), and they exhibit homeostatic regulation of body nutrient ratios. In contrast,

producers have flexible stoichiometry that reflects the relative availability of nutrients in the environment (Sterner and Elser 2002). Ecological stoichiometry theory predicts that the relative imbalance between herbivore and plant particulate nutrients determines both nutrient limitation of herbivore growth and the rates at which herbivores recycle nutrients (Sterner and Elser 2002). For example, herbivores with high body particulate P (i.e. a low body N:P ratio) are more vulnerable to P-limitation than herbivores with lower body P (i.e. a higher body N:P ratio) (Frost and Elser 2002, Sterner and Elser 2002, Hood et al. 2005, Ferrão-Filho et al. 2007). Because herbivores with high body P need to sequester relatively more dietary P than herbivores with low body P, they may also recycle relatively little P and therefore release nutrients at a high N:P ratio (Elser et al. 1988, Vanni et al. 2002, Evans-White and Lamberti 2005, 2006). Thus, both the consumptive and nutrient-mediated effects of herbivores on plants depend greatly on the identity of consumer and resource (Hillebrand et al. 2008).

To better understand the pathways by which herbivores affect the biomass and nutrient stoichiometry of primary producers, it is necessary to isolate and quantify consumptive and nutrient-mediated effects (Vanni et al. 1997, Liess and Haglund 2007), and to compare these effects among herbivore species. In this study, we manipulated two vertebrate herbivores common in Neotropical streams, and assessed their effects on periphyton biomass and nutrient stoichiometry. We selected herbivore species that differ greatly in both body particulate nutrients and rates and ratios of N and P excretion (Vanni et al. 2002). *Ancistrus triradiatus* is an armored catfish that has high body P (low body N:P), while tadpoles of the frog *Rana palmipes* have low body P and thus a high body N:P (Vanni et al. 2002). In accordance with predictions of stoichiometry theory, *Ancistrus* shows evidence of P-limitation (Hood et al. 2005) and excretes nutrients at a much higher N:P ratio than *Rana* (Vanni et al. 2002, Hood et al. 2005). At our study site in Rio Las Marias, Venezuela, these and other grazer species significantly reduce algal biomass, alter algal community composition (Flecker et al. 2002), and contribute substantially to whole-stream nutrient cycling (McIntyre et al. 2007, 2008). The goal of the present study was to separate the direct and indirect mechanisms underlying these effects, and test whether consumer stoichiometry predicts feedbacks on producer stoichiometry through nutrient recycling.

We used mesocosm experiments to test the prediction from ecological stoichiometry theory that differences in body stoichiometry of grazers results in differential nutrient recycling that subsequently drives divergence in periphyton stoichiometry. We hypothesized that *Ancistrus'* high body P would result in decreased N limitation and increased P limitation due to a high excreted N:P ratio. Conversely, we hypothesized that *Rana* (low body P) would increase N limitation and decrease P limitation. In addition to measuring grazer effects on periphyton stoichiometry, we used a ^{15}N tracer to directly compare the effects of these two grazers on N mineralization and uptake. These experiments were designed to promote a more mechanistic understanding of the direct and indirect means by which grazers affect algal communities. Overall, our results support the notion that nutrient recycling by herbivores has diverse effects on producers, and that consumer stoichiometry gives rise to predictable feedbacks on producer stoichiometry.

Methods

Study site

Our mesocosm experiments were conducted near Rio Las Marías (RLM), a small, clear-water river in the Andean piedmont region of Venezuela (9°10′N, 69°44′W). RLM has distinct dry (December–April) and wet (May–November) seasons, and our experiments were conducted near the stream during the dry season. The fish assemblage at our study site is species-rich and includes a high diversity (11 species) and biomass of armored catfish (Loricariidae). The catfish species we used in our experiments, *Ancistrus triradiatus,* is one of the most common loricariids in this stream (McIntyre et al. 2008). Tadpoles of *Rana palmipes* are frequently observed during the dry season within RLM and nearby pools, and they often reach high densities (Flecker et al. 1999, McIntyre et al. 2004). Both *Rana palmipes* and *Ancistrus triradiatus* are often found in static peripheral pools and slow-moving habitats in the river channel, therefore flow conditions in the mesocosms likely mimic those sometimes experienced by these species. Periphyton productivity in RLM is N-limited (Flecker et al. 2002). Detailed descriptions of RLM are found in Flecker (1996) and Flecker et al. (2002). All animals, periphyton and water used in both of our experiments were collected from RLM.

Experiment 1: direct and indirect effects of grazing catfish

This experiment was designed to tease apart the consumptive and nutrient-mediated effects of the high body P armored catfish, *Ancistrus triradiatus*, on periphyton communities. Within mesocosms, we manipulated catfish density and examined grazing and nutrient recycling effects with the use of grazer exclusion cages nested within the mesocosms (Fig. 1). Exclusion cages (1 cm^2 mesh Vexar netting, 0.096 m^2 surface area) in the center of each mesocosm allowed movement of water between grazed and ungrazed areas, while fish were restricted to the outer area. Thus, periphyton outside the cage was exposed to direct grazing and nutrient recycling by catfish, whereas periphyton inside the cage was exposed only to nutrient recycling by catfish. We refer to the periphyton in the exclusion cages as 'protected' and periphyton outside the exclusion cages as 'grazed'.

The experiment was conducted over 21 days in 12 circular plastic basins (0.5 m^2 surface area) each holding 136 l of stream water from RLM. A similar density of periphyton-covered rocks were placed inside and outside the exclusion cages, while catfish were only added outside the cages (Fig. 1). We used a randomized complete block design with three replicates of each of four catfish density treatments: control (no fish), low (one fish, wet mass = 6.57 ± 0.59 g (SD)), medium (three fish, group wet mass = 25.6 ± 0.75 g (SD)), and high (six fish, group wet

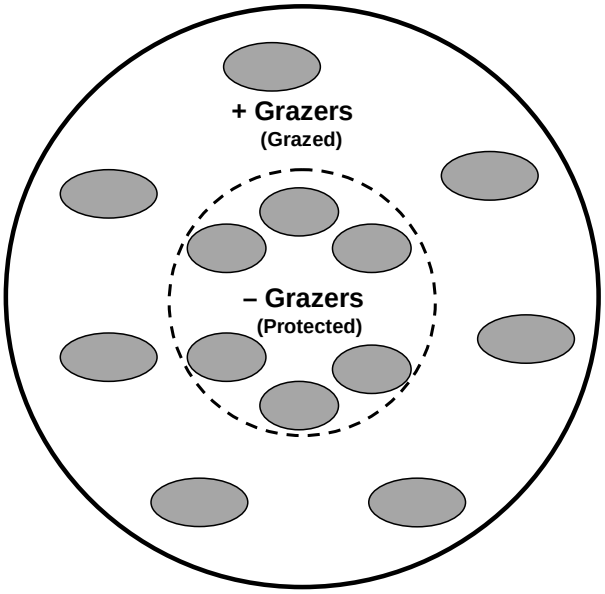

Figure 1. Schematic representation of mesocosm design used in experiment 1 and experiment 2. Dashed line represents grazer exclusion ring that allowed movement of water, but excluded grazers. Gray ovals represent rocks with natural periphyton assemblages.

mass = 51.97 ± 3.91 g (SD)). Natural densities and biomass of *Ancistrus* range from 0–16 fish m^{-2} and 0–26.7 g m^{-2} respectively, among RLM stream segments. In addition, a large-scale fish community survey in RLM revealed that algivore biomass can reach 65.5 g m^{-2}. Although some of our experimental densities exceeded those in the community survey, our use of the upper end of the body size range of catfish led to modest densities of individual fish. Furthermore, we chose these densities because we wanted to elicit a response in our first experiment of this kind. Temperature and dissolved oxygen concentrations in mesocosms were similar to those in RLM.

Samples of periphyton chlorophyll a and particulate nutrients (C, N and P) were collected weekly. Three rocks inside and three rocks outside the exclusions were sampled for chlorophyll a, while one rock inside and one rock outside were sampled for particulate carbon (PC), nitrogen (PN), and phosphorus (PP) samples. Periphyton was sampled by scrubbing all material from a rock surface of known area, and filtering a subsample of the resulting slurry onto a precombusted Pall-Gelman A/E glass fiber filter. Samples for chlorophyll a analysis were kept on ice in the dark, extracted in 90% ethanol for ~24 h, and quantified by fluorometry. PC, PN and PP samples were dried and kept frozen until analysis. PC and PN were analyzed by the standard Dumas method using an elemental analyzer, and PP samples were analyzed by HCl digestion and spectrophotometry (Stainton et al. 1977). Prior to analysis, some samples of chlorophyll a (first week of the experiment) and particulate nutrients (fourth week) were lost.

To quantify fish growth and nutrient gain or loss, wet mass of each catfish was measured at the beginning and end of the experiment. At the end of the experiment, fish were frozen until analysis. In the laboratory, we dried fish to a constant mass at 60°C, measured dry mass, ground them to a fine powder with a mortar and pestle followed by a dental amalgamator, and then analyzed subsamples for PC, PN and PP by methods described above for periphyton.

Experiment 2: effects of grazer identity on nutrient dynamics

This experiment was designed to test whether differential nutrient recycling arising from the differences in body stoichiometry among vertebrate grazers feeds back upon periphyton stoichiometry, as predicted by theory. We selected armored catfish (*Ancistrus triradiatus*) and tadpoles (*Rana palmipes*) as the focal grazer species because they represent extremes of body stoichiometry within the diverse assemblage of grazing vertebrates in RLM. *Ancistrus* has high body particulate P that results in high excreted N:P (mean = 49.9 (molar), SE = 9.5, n = 32), while *Rana* has low body P that results in low excreted N:P (mean = 6.5, SE = 0.8, n = 33) (data from Vanni et al. 2002).

Using the same exclusion design as in experiment 1, we compared three treatments: control (no grazers), catfish (five fish, group wet mass mean ± SD = 15.59 ± 0.55 g), and tadpoles (five tadpoles, group wet mass mean ± SD = 14.67 ± 0.60 g). Treatments were arranged in 6 randomized complete blocks of circular plastic basins (0.14 m^2 surface area; 25 l volume). As in experiment 1, grazers were placed outside the exclusion cages, and flat rocks with a natural periphyton assemblage were used to cover the bottom of the mesocosm both inside and outside the ring. The experiment lasted 24 days, and temperature and dissolved oxygen concentrations were similar in the mesocosms and in RLM.

To focus on the indirect effects of grazers through nutrient cycling, we sampled periphyton only from inside the exclusion cages (protected periphyton). To do so, we haphazardly selected one rock from each mesocosm and measured chlorophyll a and particulate nutrients (PC, PN and PP) as described earlier. To assess treatment effects on nutrient dynamics, we measured dissolved water nutrients on days 6 and 24 of the experiment. Water samples were filtered and analyzed immediately for ammonium (NH_4-N) by fluorometry (following Holmes et al. 1999) or frozen for later analysis of soluble reactive phosphorus (SRP) and nitrate (NO_3-N) by standard colorimetric methods.

We also used a ^{15}N label to trace the movement of N from periphyton in the outer ring (where grazers were present) into the exclusion cage (where grazers were absent). Midway through the experiment, we incubated additional periphyton-covered rocks from RLM in a separate basin containing highly ^{15}N-enriched RLM water (~0.37 g ^{15}N as NH_4Cl in 47 l) for two days. These rocks were rinsed thoroughly, and two rocks were placed in the outer ring of each experimental basin where they could be fed upon by grazers. The upward surface area of the ^{15}N-labeled rocks was calculated from digital photos, and did not differ between treatments. After two and nine days, periphyton was scrubbed from one rock in the exclusion cages of each basin, dried, and analyzed for ^{15}N using a mass spectrometer. Results for each date and basin were converted from the standard $\delta^{15}N$ notation to atomic fractions ($^{15}N/[^{14}N + ^{15}N]$) and multiplied by N stocks (g N m^{-2}) to yield g ^{15}N m^{-2}. From this value, we subtracted the

expected g $^{15}N\ m^{-2}$ based on periphyton $\delta^{15}N$ of 1.0 ($n = 4$), as observed before addition of the ^{15}N label. Thus, these calculations account for variation in both isotope ratios and N stocks to estimate the amount of tracer ^{15}N mineralized from the grazed areas and taken up by periphyton in protected areas.

Statistical analyses

For experiment 1, in which we assessed the direct consumptive and indirect nutrient-mediated effects of catfish density on periphyton (chlorophyll a, PC, PN, PP, C:P, C:N, N:P), we employed MANOVA (using Roy's greatest root as a test statistic) followed by one-way ANOVAs with Tukey HSD post hoc comparisons. We incorporated blocking into all MANOVAs and ANOVAs. In the interest of brevity, for block effect tests we explicitly report only significant effects. Thus, if block effect statistics are not reported for a particular analysis, the block effect was not significant. Initial analyses indicated no strong temporal patterns, so data were averaged across sampling dates (excluding the initial date, before treatments were imposed) to yield a single estimate from each mesocosm. For experiment 2, we utilized the same statistical approach to analyze the nutrient recycling effects associated with grazer identity on periphyton (chlorophyll a, C:P, C:N, N:P) and dissolved water nutrients (SRP, NH_4-N, NO_3-N).

In experiment 1, to explicitly compare grazing and nutrient recycling effects, we calculated effect sizes for three response variables: chlorophyll, C:P ratio and N:P ratio. These analyses were designed to explicitly compare the magnitude of grazing versus nutrient recycling effects rather than solely examining the effect of catfish density. For all three response variables, we used the mean of all sampling dates and calculated log effect ratios (Osenberg et al. 1997). To estimate nutrient recycling effect sizes, results from inside the exclusion cages (where nutrient recycling but not grazing occurred) were compared between mesocosms with and without catfish. For example, the nutrient recycling effect size in each Low treatment pool was obtained as $ES_{NC} = \ln(R_{Low}/R_{Control})$, where R is the response variable (chlorophyll, C:P or N:P) inside the exclusion cages. Effect sizes were calculated separately for each experimental block. Grazing effect size was calculated for each pool by comparing the response variable outside exclusion cages to the response variable inside the cage. Thus, $ES_G = \ln(R_{Outside}/R_{Inside})$. We compared grazing and nutrient recycling effect sizes using 2-way ANOVA with treatment (low, medium, high) and effect type (grazing vs nutrient recycling) as factors. We also examined the treatment × effect type interaction. However, in all three ANOVAs this interaction was not significant ($p > 0.58$ in all cases), so we deleted the interaction terms and re-ran ANOVAs. Each ANOVA model had 18 observations (three treatments [low, medium, high] × three replicate pools × 2 effect types [grazing, nutrient cycling]). Because grazing effects are negative while nutrient cycling effects are positive, we used absolute values to compare the relative magnitude of grazing and nutrient recycling effects.

In experiment 2, we tested treatments effects on movement of the ^{15}N label within each mesocosm. Due to strong time effects, we used repeated-measures ANOVA to compare treatments and temporal trajectories. To further interpret the significance of temporal patterns, we used Tukey HSD comparisons among treatments on each sampling date.

All data were log-transformed prior to analysis. Analyses were performed using JMP (ver. 6, SAS Institute Inc. 2005).

Results

Experiment 1: direct and indirect effects of grazing catfish

Overall, periphyton biomass (chlorophyll) and particulate nutrients decreased with increasing catfish density on grazed rocks, while periphyton biomass increased with increasing catfish density on protected rocks (Fig. 2). Using MANOVA, we found a significant effect of fish density and block on periphyton parameters (chlorophyll a, PC, PN, PP, C:P, C:N, N:P) whether periphyton was protected (density, $p = 0.002$, $F_{6,3} = 74.87$; block, $p = 0.048$, $F_{6,2} = 20.14$) or grazed (density, $p = 0.0001$, $F_{6,3} = 9028$ block, $p = 0.0003$, $F_{6,2} = 3530$). Subsequent one-way ANOVA showed that grazed periphyton biomass was highest in the Control treatment ($p = 0.008$, $F_{3,6} = 10.51$), and all grazer densities reduced chlorophyll to similar low levels (Fig. 2A). Protected periphyton chlorophyll was significantly higher in the high treatment than in the low and control treatments ($p = 0.006$, $F_{3,6} = 11.81$, Fig. 2A).

Periphyton particulate nutrients responded in a manner similar to chlorophyll (Fig. 2B–D). Particulate C on protected rocks (Fig. 2B) was significantly higher in the medium and high treatments than the control ($p = 0.019$, $F_{3,6} = 7.37$), while in grazed areas C was higher in the control treatment than all other treatments ($p = 0.015$, $F_{3,6} = 8.20$). Trends were similar for N, but no significant differences among treatments were found for protected or grazed areas ($p = 0.094$, $F_{3,6} = 3.42$, $p = 0.087$, $F_{3,6} = 3.57$, respectively, Fig. 2C). Particulate P in protected areas (Fig. 2D) was higher in the medium and high treatments than the control and low treatments ($p < 0.0002$, $F_{3,6} = 41.32$), while no significant differences were found for P on grazed rocks ($p = 0.103$, $F_{3,6} = 3.23$).

Nutrient ratios showed a variety of responses (Fig. 2E–G). In grazed areas, catfish significantly reduced C:P ratios relative to the control ($p = 0.013$, $F_{3,6} = 8.75$, Fig. 2E), while in protected areas C:P ratios were not significantly different among treatments ($p = 0.192$, $F_{3,6} = 2.17$, Fig. 2E). No significant differences among treatments were observed for C:N in protected or grazed areas ($p = 0.383$, $F_{3,6} = 1.21$; density, $p = 0.067$, $F_{3,6} = 4.10$, block, $p = 0.006$, $F_{2,6} = 13.60$ respectively, Fig. 2F). Similarly, no significant differences were observed for N:P in protected ($p = 0.424$, $F_{3,6} = 1.09$) areas. However, in grazed areas (Fig. 2G), N:P was higher in the control than the medium treatment (density, $p = 0.04$, $F_{3,6} = 5.33$, block $p = 0.03$, $F_{2.6} = 7.14$).

On average, catfish gained wet mass in the low treatment, but lost wet mass in the medium and high treatments. However, changes in mass were not significantly

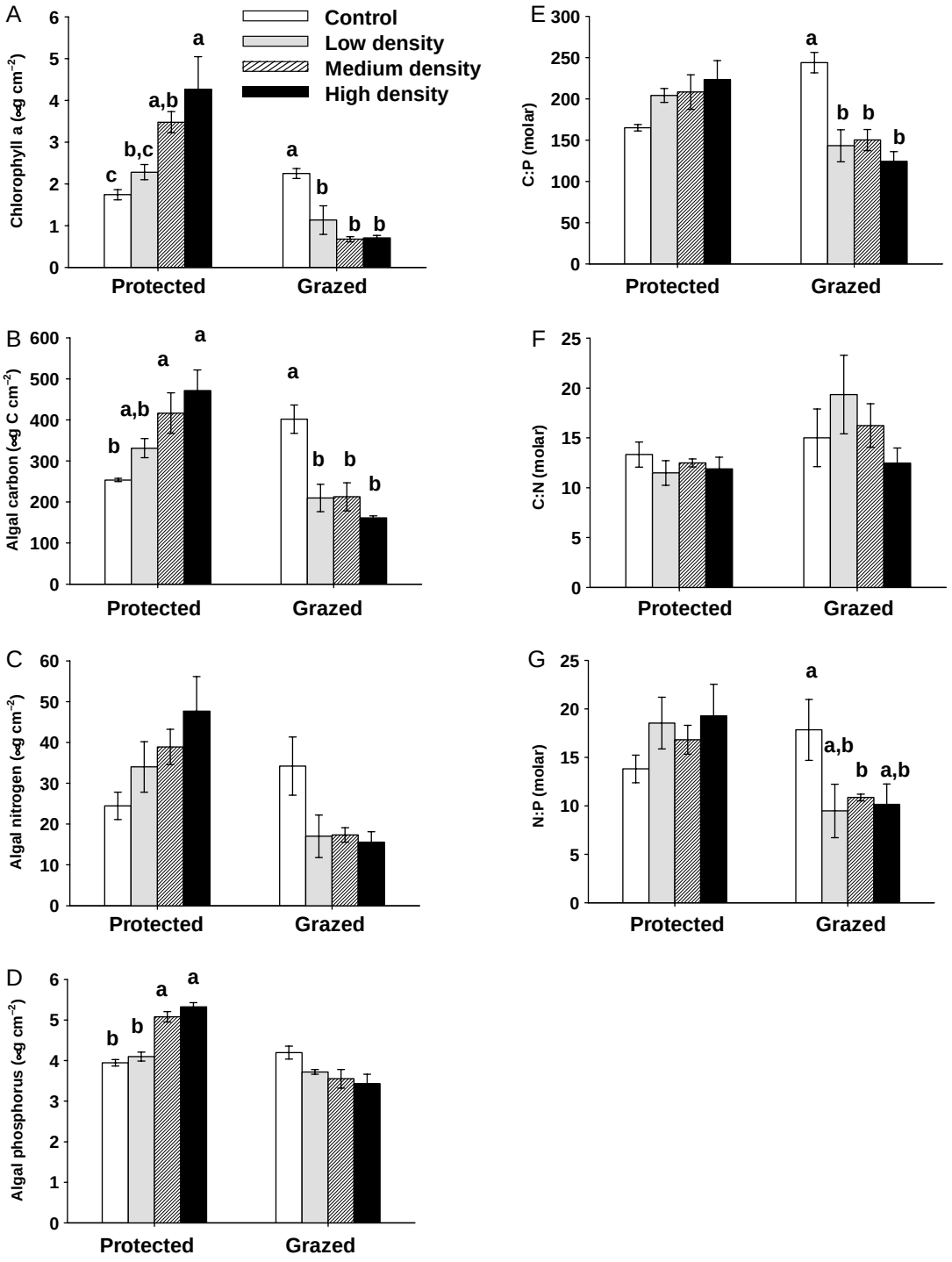

Figure 2. Catfish density effects on mean (±SE) periphyton chlorophyll a (A), particulate carbon (B), particulate nitrogen (C), particulate phosphorus (D), C:P ratios (E), C:N ratios (F), N:P ratios (G) found inside (protected) and outside (grazed) grazer exclusion rings in experiment 1. Bar colors denote different catfish densities. Different letters indicate significant differences among treatments (Tukey HSD, $p < 0.05$).

different from zero in any treatment due to high variation among replicates (Table 1). Dry mass change was near zero, on average, in the low treatment while catfish lost dry mass in the other two treatments. Trends in C mass balance were identical to those for dry mass (Table 1). Fish N mass change was positive in the low treatment, roughly zero in the medium treatment and negative (but not significantly different from zero) in the high treatment. P mass change was on average positive, but not significantly different from zero, in all treatments. Overall, changes in mass were inversely related to catfish abundance. The contrasting response of dry mass vs. element content in some cases can be explained by changes in the particulate nutrients of catfish bodies. For example, particulate P of fish bodies (P g

Table 1. Mass balances in the pools. Values are means (±SE) for each treatment. SEs represent variation among pools, not among individual fish. Positive values mean there was an increase during the experiment, while negative values mean there was a decrease during the experiment.

	Pool mass balances (change in mass, g per pool)		
Wet mass	0.433 (0.406)	−0.500 (0.306)	−0.633 (0.470)
Dry mass	−0.001 (0.099)	−1.450 (0.184)	−1.807 (0.103)
Carbon	0.019 (0.065)	−0.564 (0.100)	−0.624 (0.012)
Nitrogen	0.020 (0.008)	0.004 (0.026)	−0.041 (0.053)
Phosphorus	0.010 (0.007)	0.005 (0.031)	0.040 (0.063)

dry mass^{-1}) tended to increase with catfish density, offsetting the decrease in dry mass with increasing density.

Effect sizes of grazing exceeded those of nutrient recycling for all response variables (Fig. 3A–C). Grazing effect sizes were always negative, reflecting lower values of the response variables in the presence of grazers, while recycling effect sizes were always positive. For chlorophyll, there was a significant effect of both fish density treatment ($p = 0.008$) and effect type (grazing vs nutrient cycling; $p < 0.001$, $F_{3,14} = 23.05$, Fig. 3A). Thus, grazing reduced chlorophyll more than nutrient cycling enhanced chlorophyll, and the effects of both grazing and nutrient cycling increased with catfish density. C:P and N:P effect size results (Fig. 3B–C) were similar to each other in that catfish density had no significant influence, and the negative effects of grazing exceeded the positive effects of nutrient cycling (C:P, $p = 0.024$, $F_{3,14} = 3.52$; N:P, $p = 0.006$, $F_{3,14} = 4.12$).

Experiment 2: effects of grazer identity on nutrient dynamics

Both grazers tended to increase the concentrations of dissolved water N and P relative to the control, and grazer identity had a significant effect on dissolved water nutrient concentrations (MANOVA, $p < 0.0001$, $F_{2,10} = 240.60$). As predicted, catfish caused a greater increase in N than did tadpoles, while tadpoles had a stronger effect than catfish on P (Fig. 4). One-way ANOVA showed that SRP concentration was significantly higher in the tadpole treatment than in the grazer-free control and that the catfish treatment was intermediate ($p = 0.006$, $F_{2,15} = 7.30$, Fig. 4A). In contrast, NH_4-N concentration was higher in the tadpole and catfish treatments than in the control and the two grazer treatments did not differ from each other ($p < 0.001$, $F_{2,15} = 250.08$, Fig. 4B). NO_3-N concentrations and DIN:SRP ratios, were highest in the catfish treatment, significantly lower in the tadpole treatment, and still lower in the control ($p < 0.001$, $F_{2,15} = 250.22$, $p < 0.001$, $F_{2,15} = 293.52$, respectively, Fig. 4C).

Grazer identity also had significant effects on periphyton parameters (chlorophyll, C:N, C:P and N:P) on protected rocks (MANOVA, $p = 0.016$, $F_{3,8} = 6.46$; Fig. 5). Chlorophyll was significantly higher in the catfish treatment than in the tadpole or control treatments ($p = 0.021$, $F_{2,15} = 5.20$, Fig. 5A). Periphyton C:N ratios were lower in the catfish treatment than in the grazer-free control, with the tadpole treatment intermediate ($p = 0.013$, $F_{2,15} = 5.91$, Fig. 5B). No significant differences among treatments were found for C:P ratios ($p = 0.091$, $F_{2,15} = 2.83$, Fig. 5C). As

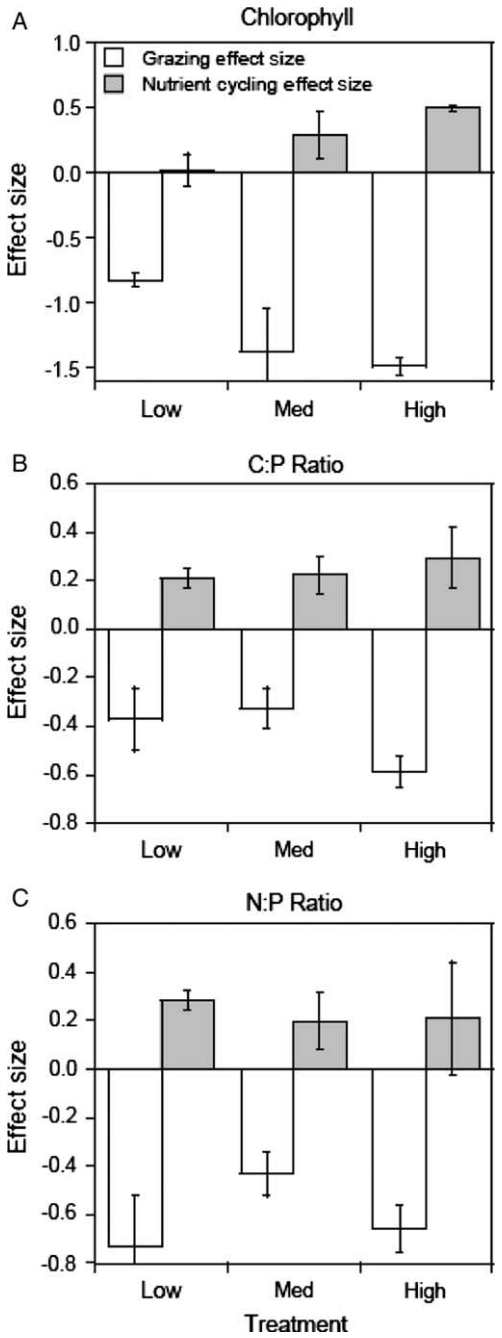

Figure 3. Effect sizes of grazing (open bars) and nutrient cycling (gray bars) for periphyton chlorophyll a (A), C:P ratios (B), and N:P ratios (C) in experiment 1.

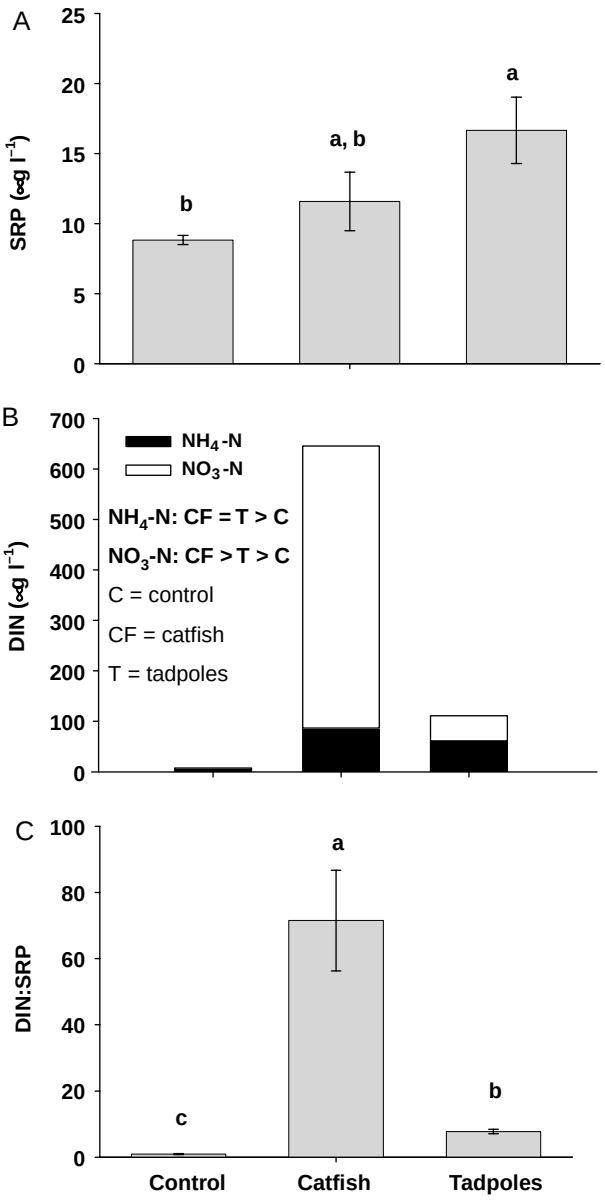

Figure 4. Mean (±SE) dissolved water nutrient concentrations of SRP (A), DIN (B), and DIN:SRP (C) ratios in experiment 2 treatments. DIN includes NH_4-N and NO_3-N. Different letters indicate significant differences among treatments (Tukey HSD, $p < 0.05$).

predicted, N:P ratios were higher in the catfish treatment than the tadpole treatment ($p = 0.041$, $F_{2,15} = 3.99$, Fig. 5D), though neither consumer treatment differed significantly from the control.

Catfish and tadpole final wet masses were lower than initial masses in all mesocosms. Catfish lost an average of 2.97 ± 0.32 g (SD) per mesocosm, while tadpoles lost 0.75 ± 0.30 g (SD) per mesocosm. We did not measure dry mass or particulate nutrients on catfish or tadpoles in this experiment.

We observed significant time and treatment (catfish vs tadpoles) effects on the amount of ^{15}N transferred from grazed periphyton to protected periphyton (RM-ANOVA, $p = 0.001$, $F_{1,15} = 16.22$, $p = 0.034$, $F_{2,15} = 4.26$, respectively, Fig. 6). Concentrations of the ^{15}N label in periphyton on protected rocks were not significantly different among treatments after two days ($p = 0.187$, $F_{2,15} = 1.88$). However, after nine days, ^{15}N concentrations were higher in the catfish treatment than the control, with the tadpole treatment intermediate ($p = 0.036$, $F_{2,15} = 4.21$).

Discussion

Our study is among the first to experimentally separate the consumptive versus nutrient recycling effects of grazers on periphyton biomass and stoichiometry. We provide experimental evidence that vertebrate grazers have both direct effects on periphyton biomass and indirect effects on periphyton stoichiometry through nutrient recycling. Moreover, grazer effects were regulated both by density and species identity, and our comparison of consumer species supported predictions from ecological stoichiometry theory. Specifically, the differential effects of grazer species on periphyton nutrient stoichiometry are consistent with differences in grazer body nutrient stoichiometry and nutrient excretion ratios.

Over 100 experiments have experimentally examined the effects of grazers on periphyton biomass and stoichiometry, as detailed in a recent meta-analysis (Hillebrand et al. 2008). These studies manipulated grazer abundance but did not experimentally separate grazing and nutrient recycling effects. Thus, periphyton responses in these studies represent the combined effects of grazing and nutrient recycling by grazers. Our results support several aspects of Hillebrand et al.'s (2008) analysis, and provide experimental evidence that nutrient recycling effects on periphyton are predictable from ecological stoichiometry theory.

Experiment 1

This experiment clearly showed that catfish can greatly reduce periphyton biomass (chlorophyll a) via grazing, and can stimulate periphyton growth via nutrient recycling. In some instances, grazer density appeared to regulate effect intensity. For example, growth of periphyton that was protected from grazing increased with catfish density, as did chlorophyll a effect size (Fig. 2A, 3A, respectively). In contrast, the presence of grazers appeared to affect some periphyton parameters independent of grazer density (C:P, Fig. 2E; C:P and N:P effect size, 3B–C). Some, but not all, of our results are consistent with the findings of Hillebrand et al. (2008). Their meta-analysis showed that the net effect of grazers by consumptive and indirect pathways is generally to increase the particulate N and P (i.e. decrease C:N and C:P) of periphyton. In the grazed areas in experiment 1, we found that catfish decreased periphyton C:P, while they had no significant effect on C:N (although the trend is for lowest C:N at highest catfish density). Hillebrand et al's analysis showed that, overall, grazers increased periphyton particulate N more than particulate P, such that periphyton N:P increased with grazer density, although this effect on N:P was variable and not statistically significant. In contrast, in experiment 1 (in grazed areas), we found periphyton N:P to decrease in our Medium treatment

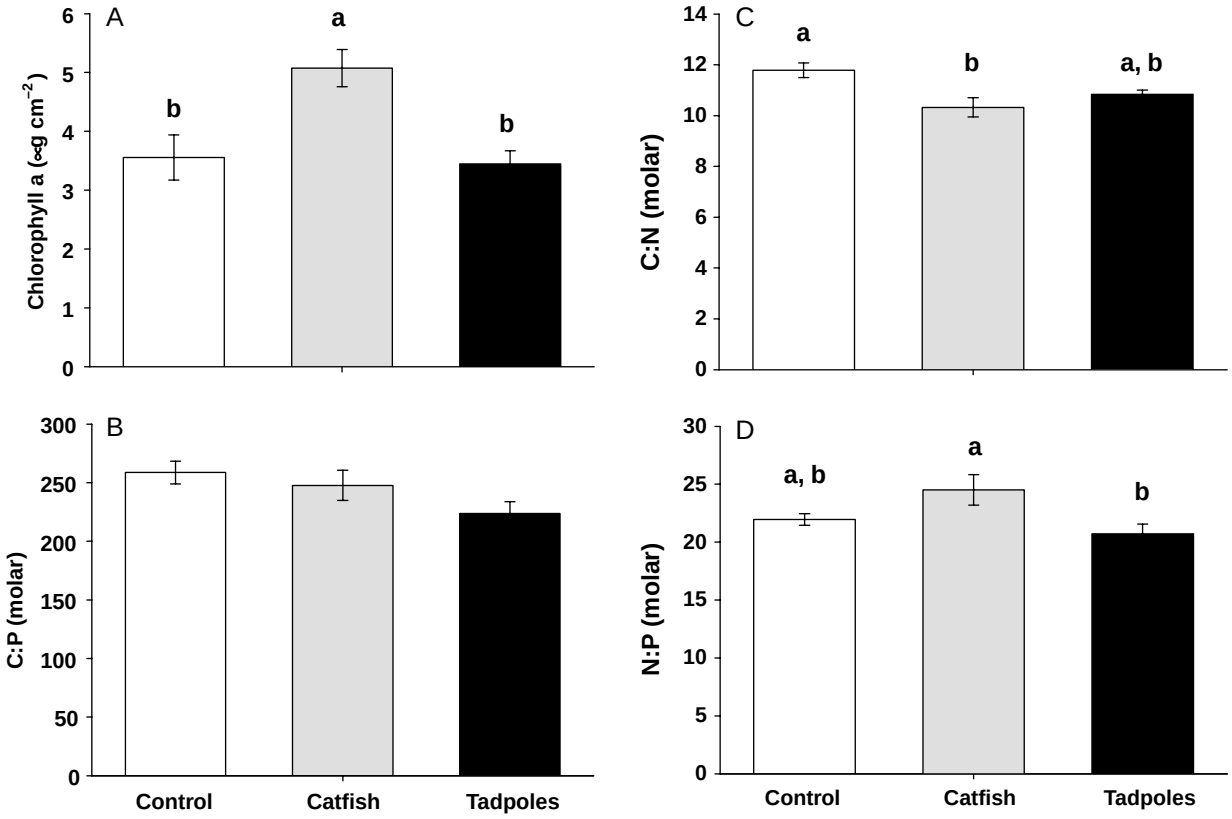

Figure 5. Mean (±SE) periphyton chlorophyll a (A), C:P ratios (B), C:N ratios (C), and N:P ratios (D) in experiment 2 treatments. Different letters indicate significant differences among treatments (Tukey HSD, $p < 0.05$).

relative to the grazer-free control. Clearly, effects on nutrient ratios vary widely and will depend on grazer feeding selectivity, nutrient cycling rates and ratios, and other traits. It is worth mentioning here that most studies have considered effects of invertebrate grazers (Hillebrand et al. 2008) while we focused on vertebrate grazers. Vertebrate grazers usually have higher body particulate P than invertebrates, which could lead to higher P sequestration and thus differential nutrient recycling ratios (compared to invertebrates).

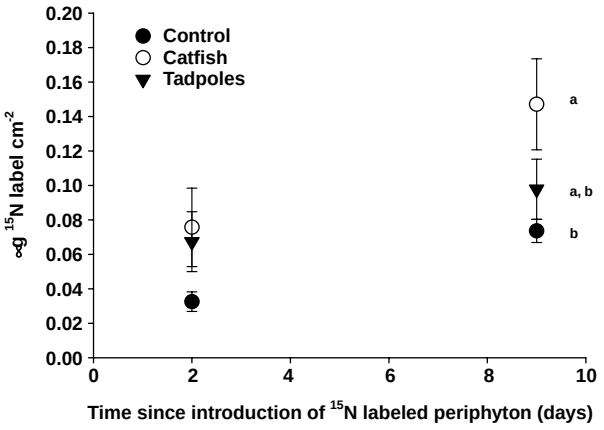

Figure 6. The effect of grazer identity on movement of periphyton labeled ^{15}N. Different letters next to day 9 indicate significant differences among treatments for that day (Tukey HSD, $p < 0.05$).

We predicted that catfish would increase P limitation (i.e. increase C:P and N:P) and decrease N limitation (i.e. decrease C:N) of protected periphyton, because this grazer excretes nutrients at a high N:P ratio (Vanni et al. 2002, McIntyre et al. 2008). Trends were in these directions for all three ratios (Fig. 2), but treatment effects were not significant. The lack of strong nutrient recycling effects may have resulted from the lack of grazer growth and consequently low P sequestration in catfish bodies. Because loricariids have high body P, we predicted that the combined processes of consumption, growth and nutrient recycling would lead to sequestration of P in fish bodies and thus a reduced P supply (relative to N supply) for periphyton. In general, well-fed fish sequester and release greater amounts of nutrients than non-fed fish (Mather et al. 1995, Roy and Lall 2003, Glaholt and Vanni 2005). Thus it is possible that this species may, under natural conditions of slow growth (Hood et al. 2005), have stronger effects on periphyton stoichiometry than we observed in the experiment since we did not observe catfish growth. Despite losing mass, catfish in the low and medium treatments gained N and P and catfish in the high treatment gained P (Table 1). We expected that catfish would gain mass at a low N:P, and this trend emerged in the low and medium treatments (Table 1).

The direct consumptive and indirect recycling effects of grazing catfish tended to affect periphyton C:P and N:P in opposite directions. The combination of recycling and consumption (i.e. in grazed areas) led to significant decreases in periphyton C:P and N:P. Previous work

suggests that reductions in algal biomass alleviates periphyton P-limitation (Mulholland et al. 1991, Hillebrand et al. 2004, 2008, Liess and Hillebrand 2004), and our results agree with this pattern. In contrast, nutrient cycling alone (i.e. in protected areas) tended to increase periphyton N:P and C:P (although not significantly). If direct and indirect effects of grazers influence periphyton stoichiometry in opposing directions, a key implication is that net effects of grazers on periphyton stoichiometry may be difficult to discern in natural ecosystems or experiments that do not separate grazing and nutrient cycling. Thus, grazing may mask the influence of nutrient cycling, such that the net effects documented in previous studies (Hillebrand et al. 2008) underestimate nutrient recycling effects of grazers on periphyton stoichiometry.

Experiment 2

This experiment provides several lines of evidence that grazer identity has strong and predictable effects on periphyton growth and nutrient stoichiometry mediated by differences in grazer body stoichiometry. Previous studies have shown that grazer identity can affect periphyton nutrient stoichiometry (Frost et al. 2002, Hillebrand et al. 2004, Evans-White and Lamberti 2005, 2006), but these studies did not isolate consumptive (direct) and nutrient-recycling (indirect) effects. Furthermore, ours is the first experimental study to use a ^{15}N label to track the movement of recycled nutrients, thereby demonstrating that changes in producer stoichiometry can arise from differential recycling of nutrients by grazers. Such applications of isotopic tracers offer a powerful tool for hypothesis testing in ecological stoichiometry research.

Treatment effects on dissolved water nutrient concentrations matched predictions from ecological stoichiometry theory; SRP concentrations were highest in the tadpole treatment, while NO_3-N concentrations and DIN:SRP ratios were highest in the catfish treatment (Fig. 4). The ^{15}N tracer also shows that nutrient cycling by catfish results in greater transfer of N from grazed to ungrazed periphyton (Fig. 6). Though we did not measure excretion rates by these consumers directly, we have previously shown that catfish excrete at high N:P ratios while tadpoles excrete at low N:P ratios (Vanni et al. 2002). Note that both catfish and tadpoles lost mass during this experiment. As discussed above, we might expect to see stronger effects of nutrient recycling if the consumers had gained mass because well-fed animals should be able to increase the amount of nutrients sequestered and released relative to non-fed individuals (Mather et al. 1995, Roy and Lall 2003, Glaholt and Vanni 2005). In addition, if nutrient release from these consumers was primarily due to lost body mass (i.e. burning body nutrients for fuel), we would expect that tadpoles (high body N:P) would excrete high N relative to P and catfish (low body N:P) would excrete low N relative to P. Our dissolved water nutrient results show the opposite pattern in that we found high DIN:SRP in the catfish treatment with much lower DIN:SRP in the tadpole treatment (Fig. 4). These results suggest that nutrient mineralization by the catfish and tadpoles arose primarily from differential recycling of dietary nutrients rather than release of body nutrients, despite their lack of growth. Thus, our results support a causal chain linking consumer body stoichiometry to individual nutrient recycling stoichiometry to the stoichiometry of nutrients available in ecosystems.

The shifts in ambient nutrient availability associated with each grazer species led to opposing patterns of periphyton N and P (Fig. 5). As predicted, the low body N:P of catfish resulted in increased periphyton N:P (i.e. increased relative P limitation), while the high body N:P of tadpoles yielded decreased periphyton N:P (i.e. increased relative N limitation). Hillebrand et al's (2008) meta-analysis suggested that such effects of grazers on periphyton stoichiometry depend upon the degree of stoichiometric mismatch between grazers and periphyton, hence the differential effects of catfish and tadpoles were expected due to their sharp contrasts in body N:P. Though the absolute magnitude of the shift in periphyton N:P might seem modest, it represents a much larger difference in N:P of nutrients assimilated during the experiment because these nutrients were diluted into relatively large standing stocks of N and P held in ungrazed periphyton.

Our experimental isolation of nutrient recycling and tracking of N movement with isotopic tracers provide particularly strong mechanistic evidence that differential nutrient recycling arising from grazer body stoichiometry indeed accounts for effects on periphyton stoichiometry. Moreover, the isotopic tracer shows that metazoan consumers can substantially increase nutrient mineralization above the background rates supported by microbial processes in sediment-free habitats, thereby affecting ambient nutrient availability and periphyton stoichiometry (Fig. 6). This inference must be tempered by the fact that experimental pools did not contain stream sediments, thereby eliminating nutrient cycling by sediment-dwelling microbes. However, our design provided ample surface area for microbial growth within biofilms on rocks and mesocosm walls as well as in the water column. In addition, we found considerable ^{15}N fluxes from the outer ring to the inner ring in our control treatment, suggesting active microbial mineralization in the absence of animals (Fig. 6). In the presence of animal consumers, we found enhanced rates over the control treatment. Thus, our results show that nutrient recycling by metazoan consumers may be important when compared to microbial activities (Vanni et al. 2002).

The fact that catfish, but not tadpoles, stimulated growth of protected periphyton is consistent with the N-limited status of periphyton in RLM (Flecker et al. 2002). We observed both higher DIN concentrations and more uptake of recycled ^{15}N in the catfish treatment; therefore a stronger growth response to nutrient recycling by catfish was expected. However, by the end of the experiment the mesocosms with catfish showed DIN:SRP and periphyton N:P considerably greater than the Redfield ratio. This suggests that N-limitation could not have been maintained in this treatment, although we recognize that this cannot be inferred with certainty given the prevalence of N and P co-limitation displayed by algae over a wide range of conditions (Elser et al. 2007). Thus, the observed increase in periphyton biomass could have arisen from an initial burst of production in response to enhanced N availability early in the experiment. In any case, the periphyton growth

responses to recycled nutrients in both of our experiments have interesting implications under field conditions. N recycling by the fish assemblage in RLM varies 47-fold among stream channel units, and excreted N:P ranges from 11–66 (McIntyre et al. 2008). If periphyton in the field responds to recycled nutrients in the ways observed in mesocosms, spatial hotspots of nutrient recycling by fish may also show higher periphyton primary productivity and particulate nutrients.

Conclusions

Taken together, our experiments demonstrate the importance of grazing, nutrient recycling, and grazer identity in controlling periphyton biomass and stoichiometry. Our results corroborate the emerging pattern that grazing enhances periphyton particulate nutrients (Hillebrand et al. 2008). In addition, we experimentally separated and quantified the relative effect sizes of grazing and nutrient recycling, showing that direct consumptive effects generally have primacy. We have also demonstrated that grazer identity, mediated by differences in body nutrient stoichiometry, has strong effects on N recycling fluxes, dissolved water nutrient availability, and periphyton nutrient stoichiometry. This pattern of results supports the power of ecological stoichiometry theory to predict feedbacks between consumers and producers based on imbalance in their nutrient ratios. We join Hillebrand et al. (2008) in advocating for further experiments that quantify the complex pathways by which grazers affect nutrient availability and how this translates into effects on periphyton stoichiometry.

Acknowledgements – We thank L. Braitman, T. Bruce, A. Galford, K. Gordon, J. Hood, P. Levi, D. Lowry and C. Rockwood for assistance in the field or lab. We are also grateful to R. Hall for providing the ^{15}N label and critical advice, and to A. Kasson for conducting isotope analyses at the Cornell Isotope Laboratory. D. Taphorn and the Figueredo and Perez families provided critical assistance in Venezuela. Support for our research was provided by grants from the National Science Foundation (DEB-9615349, DEB-9615620, DEB-0321471, INT-0321443).

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