Gastropod Grazers and Nutrients, but Not Light, Interact in Determining Periphytic Algal Diversity

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PLANT ANIMAL INTERACTIONS

Gastropod grazers and nutrients, but not light, interact in determining periphytic algal diversity

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Abstract The potential interactions of grazing, nutrients and light in influencing autotroph species diversity have not previously been considered. Earlier studies have shown that grazing and nutrients interact in determining autotroph species diversity, since grazing decreases species diversity when nutrients (i.e. N or P) limit autotroph growth, but increases it when nutrients are replete. We hypothesized that increased light intensities would intensify the interactions between grazing and nutrients on algal species diversity, resulting in even stronger reductions in algal species diversity through grazing under nutrient–poor conditions, and to even stronger increases of algal species diversity through grazing under nutrient-rich conditions. We studied the effects of grazing (absent, present), nutrients (ambient, N + P enriched) and light (low light, high light) on benthic algal diversity and periphyton C:nutrient ratios (which can indicate algal nutrient limitation) in a factorial laboratory experiment, using the gastropod grazer *Viviparus viviparus*. Grazing decreased algal biomass and algal diversity, but increased C:P and N:P ratios of periphyton. Grazing also affected periphyton species composition, by decreasing the proportion of *Spirogyra* sp. and increasing the proportion of species in the *Chaetophorales*. Grazing effects on diversity as well as on periphyton N:P ratios were weakened when nutrients were added (interaction between grazing and nutrients). Chlorophyll *a* (Chl *a*) per area increased with nutrient addition and decreased with high light intensities. Light did not increase the strength of the interaction between grazing and nutrients on periphytic algal diversity. This study shows that nutrient addition substantially reduced the negative effects of grazing on periphytic algal diversity, whereas light did not interact with grazing or nutrient enrichment in determining periphytic algal diversity.

Keywords Benthos · Ecological stoichiometry · Nutrient ratios · Periphyton · *Viviparus viviparus*

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Introduction

A fundamental issue in ecology is to understand what determines species richness. The answer remains elusive because of the complexity of interacting factors. Processes like grazing and productivity (related to nutrient availability and irradiance) are thought to regulate the richness of autotroph communities (Fig. 1a, b; based on Worm et al. 2002). Aquatic (Lubchenco and Gaines 1981; Nielsen 2003) and terrestrial studies (Wardle et al. 1999) have shown how herbivory and nutrients can interact in their influence on autotroph species richness. Grazing may reduce richness through the extinction of rare species, but increase richness via grazer-mediated coexistence if preferred food plants are competitively dominant (Lubchenco 1978; Liess and Hillebrand 2004). Conceptual models predict a reversal of grazing impacts on

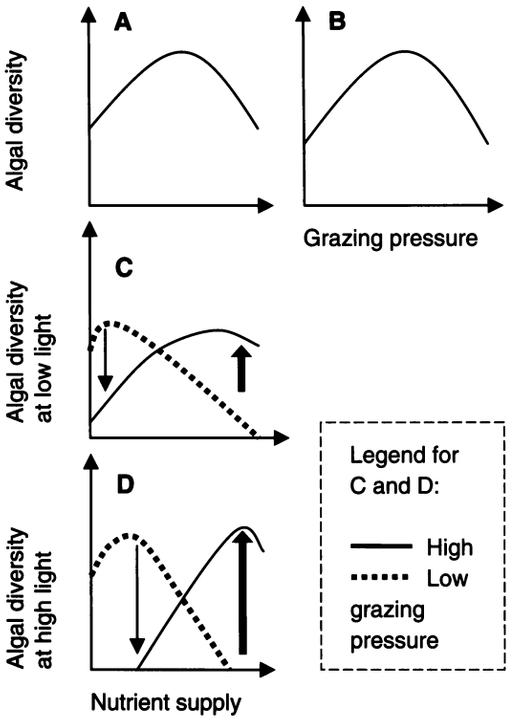


Fig. 1 Conceptual diagram of the relationship between diversity and environmental factors: diversity peaks at intermediate resource supply or productivity (**a**) and at intermediate consumer pressure (**b**). Peak diversity shifts from low to high nutrient supply depending on whether consumer pressure is low (*dotted line*) or high (*solid line*) (**c**, **d**). Consumers decrease richness at low nutrient supply (*thin arrow*), but increase it at high nutrient supply (*thick arrow*). This interaction is weaker under low light (**c**) and stronger under high light (**d**)

plant species richness in nutrient-poor as opposed to nutrient-rich ecosystems (Proulx and Mazumder 1998; Worm et al. 2002), because enhanced nutrient status increases the ability of plants to regrow after grazing, reducing the probability of extinction.

Peak richness is expected to shift from low to high nutrient supply depending on whether consumer pressure is low (Fig. 1c, dotted line) or high (solid line), because consumers decrease richness at low nutrient supply (thin arrow) but increase it at high nutrient supply (thick arrow). However, the strength of the grazing effect (reducing species richness) in nutrient-poor habitats can be offset, at least to some degree, by grazer-mediated nutrient excretion.

In addition to nutrients, light can limit autotroph growth (Hillebrand 2005). Thus, light limitation may be as important as nutrient limitation in determining community composition and richness of primary producers. Light can interact with other factors, such as grazing and nutrients (Rosemond 1993; Rosemond et al. 2000), in shaping autotroph community composition. As with increased nutrients, higher irradiance may help plants counteract grazing pressure. Thus, we predict the interacting effects of consumers and nutrients will be stronger when light intensities are higher (Fig. 1d). In nutrient-poor environments, increased light intensities make nutrient limitation more intense (Frost and Elser 2002a) via a general increase in autotroph C:nutrient ratios (Hessen et al. 2002; Urabe et al. 2002). In nutrient-rich environments, high light intensities increase autotroph growth rates and counteract extinction probabilities.

In benthic habitats, both positive and negative effects on periphytic algal diversity have been observed following nutrient enrichment and grazing (Steinman 1996; Hillebrand et al. 2000). Periphytic algal communities are very suitable model systems to test general ecological mechanisms, and especially food-web interactions, since algae have short generation times and trophic cascades are among the strongest in freshwater benthic communities (Shurin et al. 2002). Additionally, light limitation can be particularly strong for periphytic algae in streams and lake littoral zones. This is due to light absorption in the water column; the asymmetric competition for light with phytoplankton and shading by terrestrial vegetation.

In this study, we estimate benthic algal diversity under varying grazing, nutrient and light conditions as well as employing the C:N, C:P and N:P ratios of periphyton to determine the effects of grazing, nutrients and light on periphyton nutrient conditions to test the following hypotheses: (1) grazing and nutrients interact in determining algal diversity; (2) increased light intensity leads to greater nutrient demand in the periphyton, since increased light intensities facilitate C fixation and increase periphyton C:nutrient ratios; and (3) increased light availability thus increases the strength of the interactions between grazing and nutrients.

Materials and methods

Experimental design

We conducted a laboratory experiment with 1-l aquaria as experimental units. Grazers and periphyton were kept for 3 weeks (summer 2004) under different light and nutrient conditions in a $2 \times 2 \times 2$ factorial design with four replicates. The factors were grazing (snails/no snails), nutrients (ambient/N and P enriched) and light (low light/high light). Treatments were distributed randomly among experimental units. All experimental

units were aerated with aquarium pumps to supply snail grazers with oxygen.

On day 1, each aquarium (n = 32) was filled with 900 ml of GF/C-filtered ambient or nutrient-enriched lake water (ambient nutrient concentrations: N = 45 μmol l^{-1} and P = 1 μmol l^{-1}; enriched nutrient concentrations: N = 180 μmol l^{-1} and P = 4 μmol l^{-1}). Nitrogen was added as NO_3NH_4 and phosphorus as KH_2PO_4 to the enriched treatments. Aquaria were kept at 20°C and illuminated by Osram Biolux and Fluora light tubes (250 μmol photons m^{-2} s^{-1} for the high light and 5 μmol photons m^{-2} s^{-1} for the low light treatment in a 20:4 h light:dark regime). High light intensities were obtained by moving aquaria closer to the light tubes. Six periphyton-covered tiles (each 25 cm^2) were transferred to all experimental units. Prior to the experiment, these tiles had been kept for 2 months in Lake Erken, at circa 50 cm depth.

On day 2, grazers were added to the appropriate aquaria. Grazer densities in the grazed treatments were three juvenile *Viviparus viviparus* per aquarium (200 m^{-2} tile). The snails were collected 1 month earlier in Lake Erken to acclimatize to laboratory conditions, and ranged in length from 6 to 13 mm when experiments were started.

Once every week, evaporation losses were refilled with ca. 200–300 ml water in each aquarium with filtered lake water (more water was lost in the high light treatments than in the low light treatments). On day 22, the experiment was terminated and samples were taken for the estimation of periphytic algal diversity, algal biovolume, chlorophyll *a* (Chl *a*) and periphyton nutrient content.

Choice of experimental parameters

All experimental organisms were taken from Lake Erken, a mesotrophic lake in Sweden (Kahlert et al. 2002). Nutrient enrichment treatments contained four times the ambient N and P concentrations of Lake Erken. High light levels fell into the range of normal light intensities at a depth between 0 and 1 m in Lake Erken (Kahlert et al. 2002). The low light intensities represent the lower end of light intensities experienced by grazer–periphyton communities at a depth of more than 4 m (M. Kahlert, unpublished data). Grazer densities (200 m^{-2} tile) were high compared to natural densities of mature *Viviparum* (30–60 m^{-2}) in Lake Erken. However, the densities of juvenile *Viviparum*, as used in this study, were frequently as high as 200 m^{-2} in summer (A. Liess, unpublished data). We used juveniles, which have the same feeding habits as adults but consume less biomass, so that we could have more individuals per aquarium.

Diversity

We used the Simpson diversity index (D) to estimate diversity of algae. This index is calculated as follows:

$$D = \Sigma\left((n_i(n_i - 1))(N(N-1))^{-1}\right)$$

where N is the total number of species and n_i the number of ith species. The Simpson diversity index (D) embodies evenness as well as species richness, but puts more emphasis on evenness (Magurran 2004). In Fig. 2, we present diversity as D^{-1} so that higher diversity results in higher values on the X-axis. We identified all benthic algae (Clorophyceae, cyanobacteria and the Bacillariophyceae group Centrales) to the species level, with the exception of the Bacillariophyceae group Pennales, where we used size classes. This misinterpretation of size classes as species for the calculation of species diversity was possible only because Pennales constituted such a small fraction of the overall biomass. The Simpson diversity index is influenced strongest by the evenness of the main algal species, and only very little by the number of species that constitute only a small portion of the biomass (Hillebrand and Sommer 2000). Since Pennales constituted only around 10% of the total biovolume, the misrepresentation of species as size classes did not

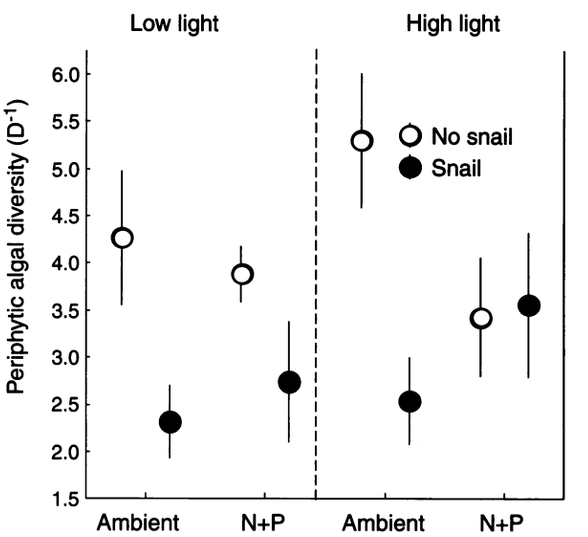


Fig. 2 Diversity index D^{-1} (Simpsons diversity index) in the different experimental treatments. *Bar graphs* represent the mean and *whiskers* the standard error

influence the outcome of the Simpson diversity index significantly.

Laboratory analyses

Periphyton was sampled with razor blades from three tiles per experimental unit and suspended in a defined volume of GF/C-filtered lake water. This suspension was divided into three sub-samples. Each sub-sample was filtered though a precombusted GF/C filter for the analysis of particulate C and N, particulate P and Chl *a*. All filtered samples were stored frozen until analysis. For the C, N and P analyses, filters were stored at –20°C. C and N were measured with a CHN-Analyzer (LECO CHN–932). Particulate P was measured as phosphate after hydrolysis with heating and potassium persulphate (Grasshoff et al. 1983). C, N and P results were transformed into molar C:N, C:P and N:P ratios.

Chlorophyll *a* was measured after acetone extraction according to Strickland and Parsons (1972) and normalized per unit area of the tiles (μg cm^{-2}). Algal cell number was estimated at 400× magnification under an inverted microscope in a 2-ml counting chamber. Algae were identified to species level (if a genus included several species) with the exception of some single celled diatoms, which were partitioned into size groups (see Table 1 for a list of the most common species). Cell numbers were multiplied by the taxon-specific biovolume, which was calculated from the mean of 20 cell measurements of the respective taxa with the equations of Hillebrand et al. (1999).

Table 1 Mean biovolume of the most common algal taxa under ungrazed and grazed condition

Identified taxa	Mean algal biovolume (10 mm^3 m^{-2})	
	Ungrazed conditions	Grazed conditions
Chlorophyceae	2,466	1,838
Chaetophora incrassata[a]	437	939
Chaetophora pisiformis	143	72
Cladophora sp.	343	487
Coleochaete pulvinata	141	91
Coleochaete scutata	27	43
Mougeoutia sp.	34	–
Oedogonium spp. (2 species)	240	52
Spirogyra tenuissima[a]	1,043	134
Other chlorophyceae (14 species)	59	20
Cyanobacteria	60	32
Anabaena sp.	8.2	1.6
Rivulariaceae (1 species)	46	27
Other Cyanophyta (12 species)	7.8	3.4
Bacillariophyceae	225	77
Pennales	171	72
Centrales	54	4.5

Rare taxa (biovolume < 0.01 mm^3 m^{-2}) are not listed separately

[a] Taxa that changed significantly due to grazing

Statistical analyses

All statistical analyses were done with Statistica (Stat Soft, version 6.0). We used three-way univariate analysis of variance (ANOVA) to test effects of grazer, nutrient and light manipulation and their interaction on periphytic algal diversity and other periphyton parameters (Table 2). In the ANOVA, we also tested the effects of grazer, nutrient and light manipulation and their interaction on the proportion of the 13 most common algal taxa (Bonferroni correction for multiple comparisons: adjusted significance level $P = 0.05 \times 13^{-1} = 0.004$), and on the proportion of algal classes (cyanobacteria, green algae and diatoms), to determine if changed experimental conditions led to changes in algal taxonomic structure. We then used Student's *t*-tests on C:N, C:P and N:P ratios to assess nutrient limitation in periphytic algae. To test whether there was a common pattern between diversity and C:N, C:P or N:P ratios we used model II linear regressions. We used this type of regression analyses because both *X* (C:N, C:P and N:P ratios) and *Y* (diversity) values were random variables. Data were $Log_{(x+1)}$ transformed to achieve homogeneity of variances in those cases where raw data did not have homogeneous variances, with the exception of algal species proportions, which were square root arcsine transformed. Significance levels were set at $P = 0.05$ (but see above for Bonferroni corrections).

Results

Periphytic algal diversity and composition

Grazing significantly decreased algal diversity (Fig. 2; Table 2). Light and enrichment showed no significant effect on the algal diversity index (Table 2). However, there was a significant interaction between nutrients and grazing (Table 2). Nutrient addition decreased the negative effects of grazing on algal diversity (Fig. 2).

Grazing, nutrients or light did not significantly change the overall proportion of green algae, diatoms or cyanobacteria (ANOVA: $P > 0.05$). However, there were some marginally significant effects. Grazing had a marginally significant positive effect on the proportion of green algae (Fig. 3, ANOVA: $P = 0.079$) and the

Table 2 ANOVA on the effects of grazing, nutrients and light and their interactions on periphyton biomass, stoichiometry and diversity

Dependent variable	Transf.	Grazing		Nutrients		Light		Grazing × light		Nutrients × light		Grazing × nutrients		Grazing × nut. × light	
		$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24}$	P	$F_{1,24)}$	P
Periphytic carbon	–	**21.5**	**<0.001**↓	3.16	0.088	0.01	0.925	0.44	0.514	0.001	0.968	0.57	0.458	0.00	0.994
Algal biovolume	Log	1.42	0.244	0.44	0.513	2.69	0.114	3.86	0.061	0.01	0.905	0.77	0.389	0.06	0.811
Chl *a*	–	**12.9**	**0.001**↓	**12.8**	**0.002**↑	**21.9**	**<0.001**↓	0.02	0.891	1.73	0.201	2.51	0.126	0.20	0.662
Chl *a*:C	–	1.05	0.315	1.03	0.321	**10.1**	**0.005**↓	0.02	0.889	0.18	0.678	0.15	0.704	0.14	0.711
Particulate C:N	Log	4.03	0.056↑	0.08	0.783	0.10	0.750	2.6	0.123	0.22	0.644	2.64	0.118	1.25	0.275
Particulate C:P	Log	**9.18**	**0.006**↑	0.47	0.498	2.85	0.104	0.56	0.460	0.01	0.931	2.66	0.116	1.04	0.318
Particulate N:P	Log	**7.05**	**0.014**↑	0.47	0.500	2.99	0.097	0.09	0.768	0.05	0.819	**5.32**	**0.030**	0.54	0.470
Algal diversity	–	**11.55**	**0.002**↓	0.23	0.635	0.93	0.344	0.08	0.783	0.29	0.597	**4.82**	**0.038**	1.53	0.229

Arrows indicate the directions of the effects. Significant effects are printed in bold and marginally significant effects are underlined

proportion of cyanobacteria showed a marginally significant interaction between nutrients and light (ANOVA: $P = 0.068$). The proportion of Bacillariophyceae was marginally significantly negative affected by grazing (ANOVA: $P = 0.059$).

Of the 13 most common algal taxa, two taxa showed a significant relative change due to the grazing treatment (see Table 1 for biovolume comparison). The proportion of *Spirogyra* sp. decreased significantly and *Chaetophora incrassata* increased significantly due to grazing after correction for multiple comparisons (Fig. 3; ANOVA: $P = 0.001$ and $P = 0.002$; Bonferroni adjusted: $P_{\text{significant}} < 0.004$). Nutrients or light did not change the relative abundance of these algal taxa (ANOVA: $P > 0.004$)

Periphyton and periphytic algal biomass

Periphyton biomass was measured by three separate methods, i.e., Chl *a*, carbon and biovolume. These measurements showed that Chl *a*, carbon and biovolume were within the natural range of values found in Lake Erken (Kahlert et al. 2002). Carbon ($\mu g\ cm^{-2}$) decreased significantly with grazing, and showed no change due to enrichment or light intensity (Fig. 4a; Table 2). The biovolume ($mm^3\ cm^{-2}$) estimates showed no significant effect of grazing, enrichment or light, or their interactions. However, there was a marginally significant interaction between light and grazing, since negative grazing effects on algal biovolume were more pronounced in the high light treatment (Fig. 4b; Table 2). Chl *a* ($\mu g\ cm^{-2}$) decreased significantly with grazing and light and increased with nutrient addition (Fig. 4c; Table 2). However, there were no significant interactions between any of the factors (Table 2). Biovolume showed a similar pattern to carbon and Chl *a* (Fig. 4). The Chl *a*:C ratio decreased significantly under high light intensities (Fig. 4d; Table 2), but did not change significantly with grazing, enrichment or any of the interactions (Table 2).

Periphyton C:N, C:P and N:P ratios

Overall periphyton C:nutrient ratios were low, with mean C:N ratios = 6:1 and mean C:P ratios = 118 suggesting no strong limitation by N or P. The N:P ratio did not significantly differ from the optimal of 18 for periphyton assemblages (Kahlert 1998) (mean N:P = 19, *t*-test, $P > 0.05$). Thus, there was no strong nutrient limitation in any treatment. The C:N ratio showed a marginally significant increase with grazing

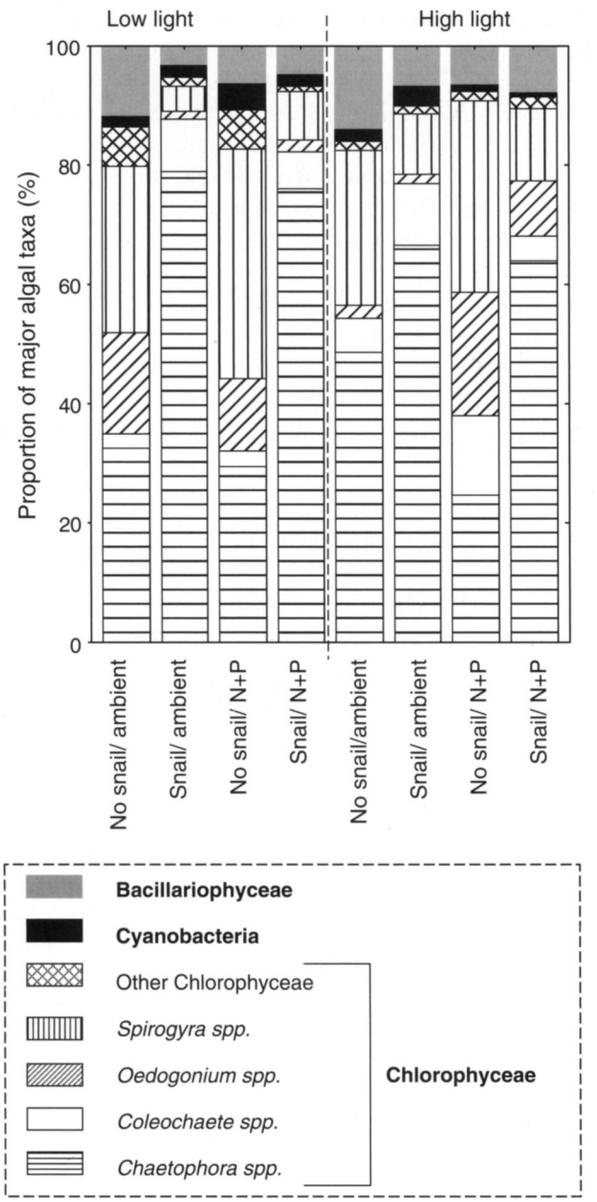
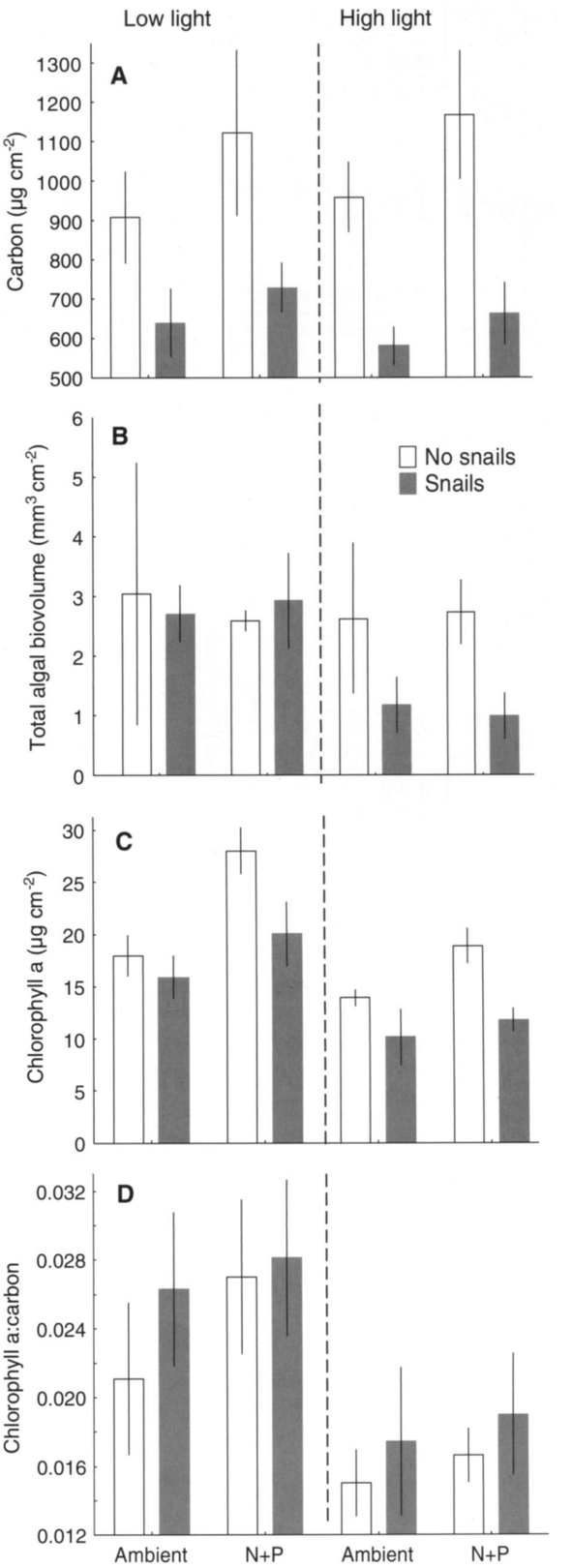


Fig. 3 Proportion of algal taxa to total algae biovolume in the different treatments. For better presentation are some taxa with low contribution presented together. The means of the four replicates are shown

(Table 2) and did not change significantly with enrichment or light or their interactions (Fig. 5a; Table 2). Periphyton C:P and the N:P ratio increased significantly with grazing (Fig. 5b, c; Table 2), but did not change with light, enrichment or their interactions (Table 2). However, there was a significant interaction between grazing and nutrients in determining periphyton N:P ratio (Fig. 5c; Table 2).



◀ **Fig. 4** Biomass of periphyton and algae: **a** carbon in μg cm^{-2}, **b** biovolume of algae mm^3 cm^{-2}, **c** Chlorophyll *a* (Chl *a*) in μg cm^{-2}, **d** Chl *a*:C ratio in the different experimental treatments. *Bar graphs* and *point labels* represent the mean and *whiskers* the standard error

Correlation between stoichiometry and diversity

The effects of snails on algae diversity and on periphyton stoichiometry were similar. Snails had negative effects on algae diversity (Fig. 2; Table 2) and on periphyton nutrient status (Fig. 5; increase in C:P and N:P, Table 2). Snails decreased the diversity index more when no nutrients were added (Fig. 2; interaction grazing × nutrients, Table 2). In the N:P ratio a similar interaction was visible. Snails increased the N:P ratios less when nutrients were added (Fig. 5; grazing × nutrients, Table 2), however the C:N, C:P and N:P ratios were not significantly correlated with diversity ($P > 0.1$).

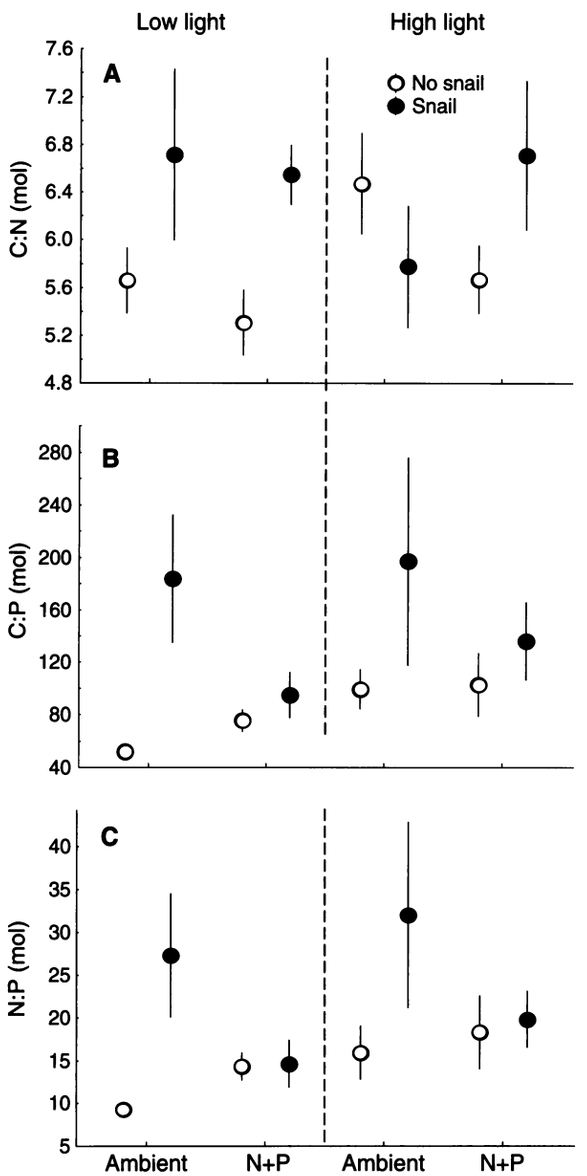


Fig. 5 C:N (**a**), C:P (**b**) and N:P (**c**) ratios of periphyton in the different experimental treatments. *Point labels* represent the mean and *whiskers* the standard error

Discussion

Grazing and nutrients interacted in determining algal diversity (supporting hypothesis 1) in accordance with previous studies (Proulx and Mazumder 1998; Worm et al. 2002). Grazing decreased benthic algal diversity through periphyton biomass removal and disruption of the periphyton matrix, whereas the addition of nutrients weakened this grazing effect, probably by increasing autotroph growth. Increased light can promote growth when nutrients are abundant, although high light intensities might at the same time increase nutrient limitation by increasing the C:nutrient ratio of periphyton (Urabe et al. 2002). Since grazing has been shown to interact with the trophic state of the habitat in determining autotroph diversity (Worm et al. 2002), and since changes in irradiance can change resource limitation depending on the trophic state of the habitat, we expected that higher light intensities would intensify the interaction between nutrient state and grazing. In our study, the increase in light intensity did not change the C:nutrient ratios of the periphyton (rejecting hypothesis 2) and did not intensify the interaction between grazing and nutrients on periphytic algal diversity (rejecting hypothesis 3).

Periphytic algal diversity and taxonomic composition

We found that algal species diversity decreased with grazing (with the exception of the enriched high light treatment) and that this effect was less strong under nutrient enrichment. In a recent meta-analysis, it was found that grazing can affect algal diversity both positively and negatively, but has positive effects on evenness (Liess and Hillebrand 2004). It is also a well-known fact that, as in our study, grazing and nutrients interact to determine diversity (Proulx and Mazumder 1998; Worm et al. 2002). In nutrient-poor environments, nutrient addition helps the grazed algal community to regrow and prevents the decimation of easily

ingested algal species, since these are often the ones that respond strongest to added nutrients. That grazing decreased algal species diversity and nutrient addition alleviated this effect would indicate that the system was relatively nutrient-poor (Proulx and Mazumder 1998; Worm et al. 2002). However, the C:P and C:N values indicated no strong N or P limitation. Possibly, relatively low overall light levels were responsible for negative grazing effects on diversity, since both low nutrient concentration and light limitation lead to lower primary producer biomass and slower recovery from grazing.

We also expected light to influence algal diversity through interactions with grazing, as previously found by Wellnitz and Rader (2003) in a field study. High light intensities can help grazed plants to regrow when nutrients are abundant, but it can also lead to increased nutrient competition within the algal community when nutrients are in short supply. However, we found no light effects on algal diversity. Overall light levels in our laboratory study were very low (low light = 5 μmol m^{-2} s^{-1} and high light = 250 μmol m^{-2} s^{-1}) compared to much higher light levels in Wellnitz and Raders (2003) field study (low light 500 μmol m^{-2} s^{-1} and high light 1,700 μmol m^{-2} s^{-1}), thus possibly leading to light limitation in all treatments. Our results are more consistent with the findings of Rosemond (1993) and Rosemond et al. (2000), where changes in grazing pressure, but not changes in nutrient and light regime (daily averages between ambient = 6 μmol m^{-2} s^{-1} and high light = 137 μmol m^{-2} s^{-1}), lead to rapid taxonomic changes of the benthic algal community. The taxonomic composition of benthic algae in our study only reacted to the grazing treatment, since grazing reduced the relative abundance of the easily ingestible *Spirogyra* sp. and increased the abundance of the grazing resistant gelatinous *C. incrassata.* Similar results of a decrease in easily ingested benthic algae and increase of grazing resistant and prostrate and gelatinous algae have been found previously (Rosemond 1993; reviewed in Steinman 1996; Wellnitz and Rader 2003).

Evidence from periphytic algal biomass

Grazing reduced algal biomass. This means that the reduction in algal diversity with grazing could be due to a relative decrease in the number and abundance of slow growing gleaner species and an increase in the abundance of single fast growing and/or grazing resistant species. We expected that nutrient addition would counteract negative grazing effects on algal biomass and algal diversity by increasing algal growth rates. Nutrient enrichment in our study did not lead to increased algal biomass. However, it did lead to an increase in algal diversity under grazed conditions, probably due to the faster regrowth of some of the grazed species in these treatments. Slightly stronger grazer effects on algal biovolume in the high light than in the low light treatments were possibly due to increased growth of easily ingested algal species (see also meta-analyses, Hillebrand 2005). However, since increased light intensity led to higher values of Chl *a* per area and per carbon, algae partially compensated for decreased photosynthetic rates (Steinman and Lamberti 1996). This reduced the differences in algal growth rates between the light treatments. Instead of alleviating grazing effects by helping plants to regrow after grazing, higher light intensities increased the strength of grazing effects in our experiment. On the other hand, the combined effects of high light intensities and nutrient enrichment compensated for grazing effects on algal diversity. Thus, the combination effect of increased light intensities and nutrient addition can have stronger effects than either factor alone.

Similar to our study, Rosemond (1993) and Rosemond et al. (2000) found that grazing more than nutrient and light regime determined benthic algal biomass and community composition. When grazer densities were high, changes in nutrient and light levels did not affect algal biomass. At no grazing, the combined effects of nutrients and light were strong. In these studies as well as in our study, benthic algal diversity increased when snails were removed, since grazing lead to an algal community dominated by only a few algal species. On the other hand, even though light and nutrient regime had no effects on benthic algal biomass they might still have affected algal species diversity, as in our study.

Evidence from periphyton nutrient state

Grazing, but not nutrients and light, changed the nutrient ratios of periphyton. Contrary to some grazing studies (Hillebrand and Kahlert 2001; Frost and Elser 2002b; Frost et al. 2002; Liess et al. 2006), but in accordance with another very similar grazing study (Liess and Hillebrand 2006), grazing increased the C:P and N:P ratios of periphyton, especially in the ambient nutrient treatments. Thus, the negative grazing effect on algal diversity in our study was not offset by grazer-mediated nutrient excretion.

Periphyton in our study had mostly lower C:P ratios than the snail grazer *V. viviparus* with an average C:P ratio of 190 (Liess and Hillebrand 2005). According to stoichiometric theory (Sterner and Elser 2002), grazers

should increase the C:nutrient ratio of their food only when food C:nutrient ratio is higher than grazer C:nutrient ratio. However, since we used juvenile snails, their P requirements might have been especially high due to high growth rates (Elser et al. 2003). Thus, the effects of grazing on periphyton C:P and N:P ratios might have been caused by the high P demand of snails and the incorporation P from the periphyton into snail body tissue, while at the same time P excretion was reduced to a very small amount (see also Liess an Hillebrand 2006 for further discussion).

When nutrients were added, the negative effects of grazing on periphyton C:P ratios were alleviated. According to our hypotheses, this results in a similar pattern in algal diversity: Nutrient enrichment decreased the negative effects of grazing on algal diversity, since algae with sufficient stores of nutrients could regrow faster after being grazed. On the other hand, the nutrient content of the periphyton in the ungrazed treatments did not change due to addition of N or P, suggesting that periphyton was already nutrient saturated. According to our hypotheses, the addition of N and P in the ungrazed treatments encouraged the growth of opportunistic, fast growing, algal species and thus reduced diversity through the takeover of by few fast growing opportunist species.

Light did not change periphyton nutrient stoichiometry, in contrast to an earlier study (Frost and Elser 2002a), since the strong negative effects of grazing on periphyton nutrient content masked the light effects. However, in the treatment without nutrient nor grazer addition, we can see almost a doubling of the mean C:P and N:P ratios with higher light intensities (Fig. 5) and, accordingly, the diversity index was highest in the ungrazed, unenriched, high light treatment. Here, stronger nutrient competition and no grazing were advantageous for slow growing species.

Stoichiometry versus diversity

Grazing and its interaction with nutrient enrichment influenced periphyton nutrient ratios as well as algal diversity. Probably, factors strong enough to affect the nutrient state of the autotroph community are also strong enough to affect algal diversity. Thus, changes in grazing pressure strong enough to affect the nutrient content of autotrophs will probably also change their taxonomic composition and diversity. At optimal nutrient condition for periphyton growth, species with fast growth rates are more likely to dominate primary producer assemblages than when either N or P is limiting. Under N limiting conditions, a taxonomic shift towards N-fixing cyanobacteria can be expected. Under P limiting condition, species with slow growth rates and high uptake efficiencies for P will probably become more dominant. Knowing more about this correlation between nutrient ratios and primary producer taxonomy and diversity could be useful in quickly determining the potential effects of changes in grazing pressure, nutrients or irradiance on primary producer diversity. However, the Simpson diversity index was not correlated with any of the C:N, C:P and N:P ratios when all periphyton samples were compared. Other factors, such as algal growth and grazing pressure, as well as overall nutrient condition, may more strongly determine algal diversity under intermediate C:N, and C:P ratios. Diversity and periphyton nutrient content are probably correlated only when extremely high or low C:nutrient ratios are considered. Periphyton nutrient conditions can correlate with dissolved nutrients in the water column. Thus, low C:N and C:P ratios are often a result of high concentrations of dissolved N and P (Liess and Hillebrand 2006). Under these conditions only few extreme r- or k-strategists will be able to utilize all resources or persist, thus leading to low diversity under extreme nutrient conditions. A correlation between the C:nutrient ratios and diversity will therefore probably be a wide bell-shaped curve, where diversity will be high in the middle (but also depend on other factors) and low at the extremes. Similar effects of C:nutrient ratios can be expected for primary producer communities, such as phytoplankton, or even terrestrial primary producer communities, such as grasslands. Especially for extreme environments, it is of importance to determine the effects of changes in nutrient status on diversity and the subsistence of rare species. Thus, for example, in terrestrial arctic mountain regions, many extreme k-strategists will be driven to extinction by opportunistic species due to climate warming and increased deposition of nitrogen (Molau and Alatalo 2003). Where k-strategists are out competed, the diversity of the resulting community will depend on the grazing pressure that the new dominant species of the community are subjected to. Our experiments did not include extreme conditions, but more research is needed here to determine how especially extreme environments are affected by changes in C:nutrient ratios driven by light and nutrient input, and how these factors interact with other factors such as grazing.

Conclusions and recommendations for future research

This study shows that factors affecting the nutrient status of the primary producer can also affect their

diversity. This mechanism might be of more general importance and applicable to other primary producer assemblages as well. Studies in other ecosystems are needed to test whether factors that can influence primary producer nutrient stoichiometry are also likely to change primary producer taxonomic composition and diversity. In previous phytoplankton studies it has been found that increased light intensities could decrease autotroph nutrient content (Hessen et al. 2002; Urabe et al. 2002). It is very likely that a change in irradiance, high enough to affect primary producer nutrient content will also affect primary producer species composition and diversity. Merging biodiversity research, food-web theory and ecological stoichiometry is an important frontier for ecologists, with implications for biodiversity conservation.

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