

Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity

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Abstract

The impact of grazing and nutrient supply on epilithic periphyton was investigated in factorial field experiments in four seasons at three Swedish sites of different productivity and herbivore composition (Lake Limmaren, Lake Erken, and Vaddö, a low salinity coastal site). Nutrient supply was enhanced by a granulose fertilizer containing nitrogen (N) and phosphorus (P), and grazer density was manipulated by exclusion cages. Algal biomass was increased by nutrient enrichment and reduced by grazer presence, but effects were highly variable between sites and seasons. Generally, grazers had stronger effects on algal biomass than nutrient enrichment, but there was no overriding effect of either grazing or nutrients. This indicated a simultaneous top-down and bottom-up control of algal biomass. Taxonomic composition of the periphyton was more affected by grazer presence than by nutrients. Internal nutrient ratios of the algae indicated N limitation at two of the sites. At all sites, the content of N and P in the periphytic assemblage was enhanced by the experimental nutrient enrichment, resulting in decreased C:N and C:P ratios. The presence of herbivores also increased periphytic nutrient content (decreased N:P and C:P ratios) in some experiments, suggesting an increase in algal P due to excretion. The effect strength of grazers and nutrients on periphyton was affected by different abiotic characteristics such as light availability, nutrient concentrations, and temperature. However, single environmental characteristics were not sufficient to explain the relative importance of grazing and nutrients.

Almost all kinds of substrata in aquatic habitats are covered by periphyton, which in euphotic littorals is often dominated by algae. The biomass and productivity of periphyton is affected by abiotic factors (e.g., light, waterflow, nutrients, substrata) and by biotic interactions (resource competition, herbivory). There is a considerable body of evidence for the importance of both grazing (Feminella and Hawkins 1995; Steinman 1996) and nutrient supply (Borchardt 1996; McCormick 1996) on the biomass, productivity, species composition, diversity, and physiognomy of periphytic assemblages.

Experiments in freshwater (Fairchild et al. 1985; Pringle 1990) and in coastal habitats (Sundbäck and Snoeijis 1991; Hillebrand and Sommer 1997) have shown that the addition of nutrients often results in an increase in benthic algal biomass and changes in community composition. On the other hand, grazers can be very efficient in periphytic communities, and most studies report significant decreases of algal biomass due to grazer activity (Feminella and Hawkins 1995; Steinman 1996). Several herbivore types (especially

gastropods and trichopteran larvae) can dramatically reduce periphytic biomass, often to only a few percent of the ungrazed biomass (Lamberti et al. 1987; Hill et al. 1992; Hillebrand et al. 2000). In addition to biomass consumption, however, grazing may result in increased nutrient content or increased biomass-specific productivity of grazed compared to ungrazed periphyton (McCormick and Stevenson 1991; Rosemond et al. 1993). This positive effect of consumers on their prey can be the result of excretion of nutrients, removal of senescent cells, or increased uptake of nutrients by the remaining cells (Lamberti et al. 1987; McCormick and Stevenson 1991; Rosemond et al. 1993; Kahlert and Baunsgaard 1999). In pelagic systems, a strong influence of nutrient regeneration by herbivores—and their N:P ratios—on phytoplankton community composition has been found (Sterner et al. 1992; Elser and Hassett 1994; MacKay and Elser 1998). In periphyton, producers and consumers live in tighter spatial connection (Kahlert and Baunsgaard 1999), but similar stoichiometric effects on benthic food webs have not been documented (Steinman 1996).

Although nutrient and grazing effects have been separately studied, few investigations have considered them simultaneously. Almost all of these have been conducted in streams (McCormick and Stevenson 1991; Hill et al. 1992; Rosemond 1993; Rosemond et al. 1993; Pan and Lowe 1994) or in seagrass-epiphyte systems (Neckles et al. 1993). Generally, these studies showed highly interacting top-down and bottom-up effects. However, transferring the results from these studies to other habitats is questionable. Streams are strongly influenced by the unidirectional waterflow and disturbance events like spates (Biggs et al. 1998), and the interaction between host and epiphyte may play a dominant role in macrophyte-dominated systems (Brönmark 1985). Further, the studies in streams are highly biased with regard to regions and to seasons (Feminella and Hawkins 1995).

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Table 1. Background information on three experimental sites, Lake Erken, Lake Limmaren, and Vädö. Monitoring data are given for each season (autumn 1999 [Aut], early spring 2000 [E Spr], late spring 2000 [L Spr], summer 2000 [Sum]) and represent means of weekly samplings throughout the experimental period (provided by the Erken Laboratory's monitoring program). For Vädö, data from the nearby Singofjärden were used. TN and TP denote total nitrogen and phosphorus concentrations, DIN and DIP are dissolved inorganic N (nitrate + nitrite + ammonium) and P (phosphate). All nutrient concentrations are given in $\mu\text{mol L}^{-1}$. Additionally, water temperature ($^{\circ}\text{C}$), phytoplankton biomass ($\mu\text{g Chl } a \text{ L}^{-1}$), and Secchi depth (m) are given. Additionally, nitrate and DIP concentrations (mean \pm SE, $n = 12$) were measured in the experiments for ambient and enriched treatments. n.m.: not measured.

Season	Monitoring							Ambient		Enriched	
	TN	TP	DIN	DIP	$^{\circ}\text{C}$	Chl	Secchi depth	Nitrate	DIP	Nitrate	DIP
Lake Erken (59°50'N, 18°37'E)											
Aut	45.8	1.35	4.07	0.87	9.4	6.3	3.8	2.18 (0.05)	0.75 (0.06)	4.95 (2.80)	3.09 (2.36)
E Spr	42.5	0.68	0.87	0.46	8.7	2.8	5.1	0.10 (0.01)	0.03 (0.002)	0.21 (0.10)	0.14 (0.10)
L Spr	47.0	0.61	2.44	0.12	13.5	3.5	5.4	0.10 (0.02)	0.08 (0.01)	1.50 (0.22)	1.15 (0.24)
Lake Limmaren (59°44'N, 18°43'E)											
Aut	81.7	2.15	4.64	0.29	7.0	28.2	1.6	7.14 (0.16)	0.78 (0.05)	9.46 (2.90)	2.08 (1.61)
E Spr	63.5	1.35	0.02	0.01	9.5	16.0	2.9	0.11 (0.02)	0.08 (0.01)	1.97 (1.37)	0.64 (0.38)
L Spr	69.8	1.87	0.40	0.11	14.6	12.8	1.9	0.27 (0.14)	0.20 (0.10)	0.66 (0.27)	0.41 (0.20)
Sum	82.1	1.86	1.79	0.04	17.4	31.9	1.5	n.m.	n.m.	n.m.	n.m.
Vädö (59°56'N, 18°55'E)											
Aut	19.5	1.11	1.31	0.42	8.4	6.2	2.0	n.m.	n.m.	n.m.	n.m.
E Spr	23.8	0.86	2.58	0.19	7.0	3.2	2.2	n.m.	n.m.	n.m.	n.m.
L Spr	17.3	0.52	0.30	0.01	11.8	8.5	2.8	n.m.	n.m.	n.m.	n.m.
Sum	22.1	0.66	1.31	0.03	14.9	5.5	2.5	n.m.	n.m.	n.m.	n.m.

For lakes, Marks and Lowe (1989) showed an increase of grazing effects on periphyton composition with nutrient enrichment. Hillebrand et al. (2000) reported significant and counteracting effects of both factors in a spring grazing experiment at the Baltic coast. However, these studies did not determine the conditions under which nutrients or grazers are more important. Furthermore, no attempt was made to compare the negative (consumption) and positive (nutrient regeneration) effects of grazing under different levels of nutrient availability.

In this study, we experimentally manipulated grazing intensity and nutrient supply at three sites and in four seasons to encompass different abiotic conditions (background nutrient availability, temperature, light) and changes in algal and grazer community composition. We analyzed algal biomass, community composition, and internal nutrient content to test the following hypotheses: (1) The biomass of algal assemblages is reduced by grazing and increased by nutrient enrichment. These contrasting effects are either interactive (grazing reduces fertilization effects and vice versa) or independent (grazing reduces biomass with or without fertilization). (2) The internal nutrient content of the benthic algae is enhanced by external nutrient enrichment and by grazer presence. Grazer presence is more important with regard to algal nutrient content if ambient nutrient concentrations are low. (3) The relative importance of grazing and nutrients for periphyton biomass and composition depends on the background nutrient concentrations. At high nutrient availability, algal biomass is not affected by nutrient addition, but easily consumed by grazers. At low nutrient availability, the effects of nutrients are more pronounced.

Methods

Experimental sites—The field experiments were conducted at three different sites in Sweden: two lakes and a shallow embayment at the low salinity Baltic coast (Table 1). At all three sites, the littoral consists of bedrock, cobblestones, and sediments. The sites were chosen to encompass different levels of productivity and different grazer compositions. Lake Erken covers 23.7 km² within a catchment area of 141 km². The mean depth is 9.0 m, the pH is around 8.0, and mean conductivity is 280 $\mu\text{S cm}^{-1}$. The experiments were placed at the southeastern shore of the lake in a cobblestone area. Lake Limmaren covers an area of 6.5 km² within a catchment area of 18 km². Mean depth is 4.3 m, pH is around 8.2, and conductivity around 250 $\mu\text{S cm}^{-1}$. The experiments were conducted at the southern shore of the lake in an area composed of large cobblestones. The two lakes, Lake Erken and Lake Limmaren, have similar pH and conductivity values, but Lake Limmaren is much more productive than Lake Erken (Table 1). Lake Erken and the coastal site at Vädö are of similar productivity, but the slightly saline environment changes the components of the grazer fauna (*see results for more details*) and the algal community. Vädö is an island at the Swedish east coast separated from the mainland by a narrow sound. The southern tip is a military area with restricted access, where the experiments could be conducted without disturbance by human activities. The experiments were placed in a shallow embayment (mean depth 1.5 m) with salinity around 5.5‰ and a pH varying between 7.2 and 8.0. None of the sites in our study was oligotrophic, but the range from moderate to high total nutrient content

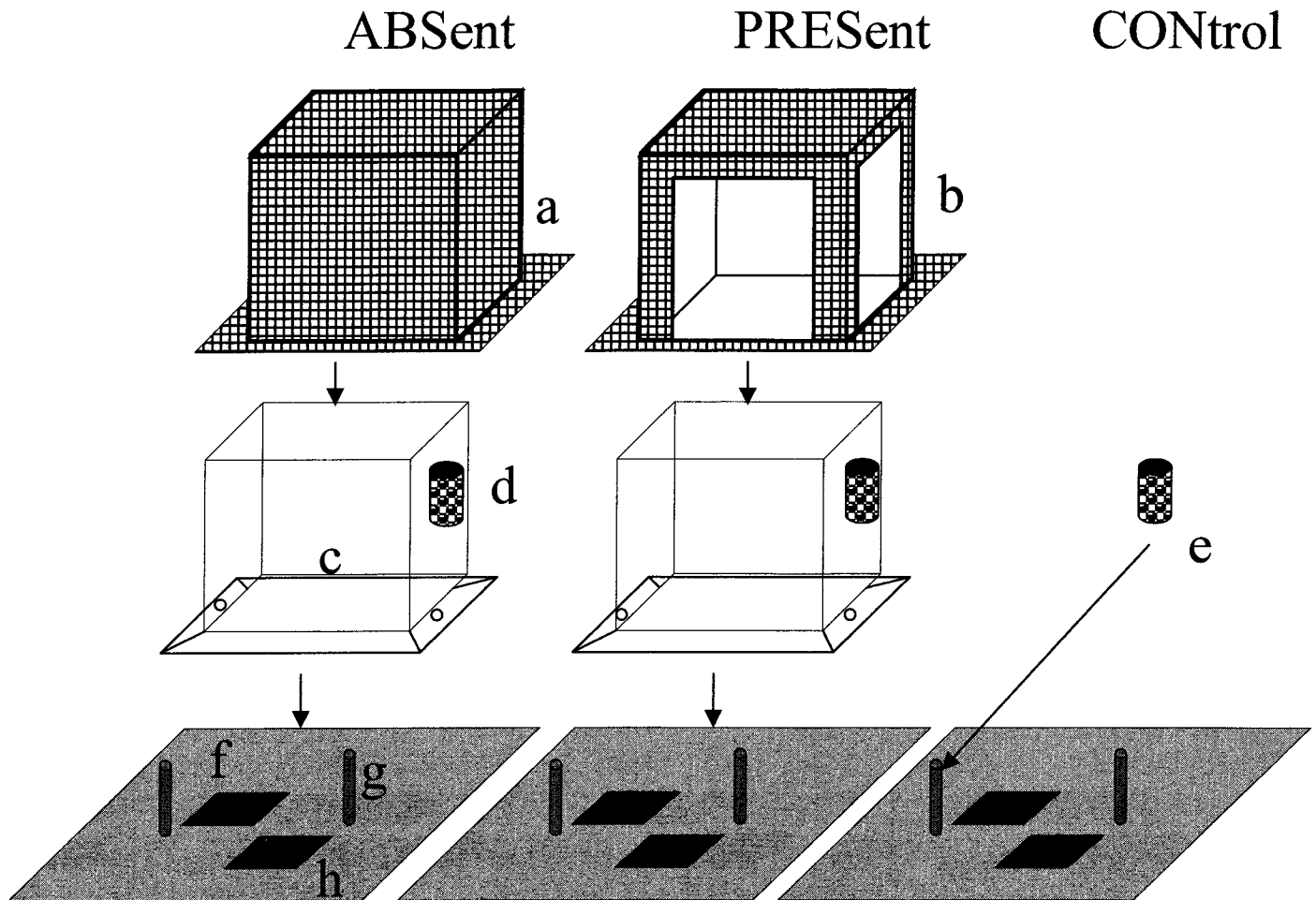


Fig. 1. Experiment design: Grazers were excluded (absent) by a 1-mm mesh in the form of a hat (a). All sides were closed and the net was put on top of a metal frame (c). The dimensions of the cube were $15 \times 15 \times 15$ cm. The metal frame was mounted on top of a 40×40 cm concrete plate (f) by means of two screws (g). Grazer access treatments (present) were constructed in a similar way, except that two adjacent sides of the net were cut out (b). For the analysis of cage artifacts (control), we used concrete plates (f) without cages. To fertilize plots, we fixed porous plastic containers (d) to the metal frame or—for control plots—directly to the screws on top of the concrete plate (e). Please note that only half of the replicates received nutrient enrichment. Standard substrates for each replicate were two unglazed ceramic tiles (h).

resulted in a range of 2 orders of magnitude in dissolved nutrient concentrations (Table 1).

Experimental setup—The experiments were conducted with a factorial combination of nutrient enrichment and grazer exclusion. All possible treatment combinations were replicated fourfold, which gave 24 plots for each experiment (3 cages \times 2 nutrient levels \times 4 replicates). At each site, we conducted experiments in each season: autumn (12 October–11 November 1999, 31 d), early spring directly after ice break (17 April–24 May 2000, 38 d), late spring (24 May–19 Jun 2000, 28 d), and summer (18 July–22 August 2000, 36 d). The experiments all lasted more than 4 weeks, which was stated to be a threshold for the analysis of grazing effects in streams (Feminella and Hawkins 1995), and less than 6 weeks, the time period of continuous nutrient enrichment (see below).

Grazer density was manipulated with metal-frame cages ($15 \times 15 \times 15$ cm), which were mounted on top of concrete

plates (40×40 cm) and covered by a net with 1-mm mesh size (Fig. 1). These nets were in the form of hats, which were tightly attached to the cage frame by Velcro strips. This allowed the exchange of the hats during the experiments in order to prevent shading from algal colonization of the nets. Nets were replaced by clean nets every 5–10 d, after visual inspection of the experiments. We believe that changing the mesh is better than cleaning the cages by brushing, which introduces a strong moment of disturbance and transfers organic matter into the cages. Complete coverage of the cages by the net (Fig. 1) represented the grazer exclusion treatments (absent). In half of the cages, two adjacent sides of the net were cut out (Fig. 1) to allow mesograzers and macrograzers to access the periphyton (present). To estimate the effects of the cages, plots without cages were established in the same manner as the caged treatments (control). Throughout this text, grazer treatments will be referred to as absent, present, and control; on the figures these treatments are abbreviated as ABS, PRES, and CON.

Nutrients were supplied with a granulose slow-release NPK fertilizer (Plantacote™ Depot 6M, Urania Agrochem) which adds nitrogen (N, both as NH_4^+ and NH_3^-), phosphorus (P, as water-soluble P_2O_5), and potassium (K, as K_2O) to the water column. Previous investigations showed that this fertilizer continuously enriched the water column for 6 weeks. For our experiment, we added 30 g of the fertilizer to half of the cages and to half of the control plots (enriched); the other half did not receive additional nutrients (ambient). For enriched treatments, the porous plastic boxes containing the nutrients were connected to the frame of the cages (absent, present) or to a screw on the concrete plates (control) (Fig. 1). Nutrient treatments will be referred to as enriched and ambient throughout the text; on the figures they are abbreviated as ENR and AMB.

Since the efficiency of nutrient enrichment was only known for brackish and marine environments (Hillebrand et al. 2000; Worm et al. 2000a,b), we estimated the amount of nutrients added to the water column by taking a water sample from each treatment once during each of the first three experiments. The water sample was taken in the centre of each cage or at a similar position for control plots, i.e., always 5–10 cm from the nutrient source. This was done in both freshwater environments, Lake Limmaren and Lake Erken. The samples were directly filtered through 0.2- μm syringe filters and stored frozen until the analysis.

To minimize the impact of the microtopography on the results (Nicotri 1977), we used unglazed ceramic tiles (5 × 5 cm) as standard substrata in all experiments. The tiles were precolonized at each site for 6 to 12 months to allow the establishment of natural periphyton communities. At the start of the experiment, the macrograzers were removed from the algal-covered tiles and two tiles were glued to each concrete plate (Fig. 1), either within the cages (absent, present) or outside (control). After adding the nets, the plates with the cages and the control treatments were placed along the shoreline at a depth of 70–90 cm. Although the Baltic coast is virtually nontidal, wind-induced water level changes occur and the experiment at Vaddö thus experienced water levels between ca. 40 and 130 cm.

Sampling and analysis—During the experiments, the nets were exchanged as described above and the closed cages (absent) were checked for invasion of grazers. Additionally, the ambient grazer density was estimated by sampling five cobblestones from the vicinity of the experiment once during each experiment (May, June, and July 2000). The stones were transferred to the laboratory, the macrozoobenthos was picked and identified, and the surface area of each stone was estimated by covering the stone with aluminum foil and measuring the total area by weighing the foil. The results were adjusted to m^{-2} . In autumn 1999, the ambient grazer density was determined directly from the basal concrete plates of the cages and open plots.

At the end of each experiment, the tiles were sampled from each replicate and immediately transferred into plastic bags with water that had previously been taken from the surrounding of the experiment and filtered (0.2- μm filter). For each replicate, two tiles were used and pooled afterward for the analysis in order to minimize the impact of random

effects during the colonization history. The plastic bags were coded and transferred to the laboratory. All processing was completed within 6 h, and samples were stored in the dark and kept cool (4°C) during this time. First, the periphyton was removed from the tiles with razor blades, algal conglomerates were carefully separated with scissors and forceps, and the suspension was adjusted to a defined volume. This suspension was divided into six different subsamples for each replicate. (1) An aliquot was filtered on precombusted GF/C filters for the analysis of particulate carbon (C) and particulate nitrogen (N); (2) an aliquot was filtered on precombusted GF/C filters for the analysis of particulate phosphorus (P); (3) an aliquot was filtered on GF/C filters for the analysis of chlorophyll *a* (Chl *a*) (all filtered samples were stored frozen until analysis); (4) an aliquot was preserved with Lugol's iodine for determination of algal abundance and biovolume; (5) an aliquot was digested with H_2O_2 for the mounting of permanent diatom slides; and (6) an aliquot was used directly for live identification of unsilicified algae.

The identification of algae was done to species whenever possible, otherwise to genus. For identification, permanent slides and high magnification were used for diatoms. Live algal material was checked for filamentous and other non-silicified algae as well as additional information on diatoms. Counting was done with 3-ml Utermöhl chambers under an inverted microscope at 400× magnification, and at least 1,000 cells were counted per sample. Species not identifiable in preserved samples were put into groups, which were used consistently throughout the experiments. Biovolume for each species or group was calculated with best fitting geometric models (Hillebrand et al. 1999). Particulate P was measured as phosphate after hydrolysis with heating and potassium persulfate (Grasshoff et al. 1983), and Chl *a* was measured after acetone extraction according to Strickland and Parsons (1972). C and N were measured simultaneously with a CHN analyzer (LECO CHN-932). Water samples for the assessment of nutrient enrichment were analyzed for phosphate and nitrate plus nitrite for all replicates, whereas the background silicate concentrations were measured only in control treatments. Dissolved nitrate plus nitrite were analyzed with the sulfanilamide method and dissolved phosphate with the ammonium-molybdate method (Grasshoff et al. 1983) in a Flow Injection Analyser (FIA). Ammonium could not be measured in the samples from our experiment because samples were frozen (*see above*). It should be noted that our calculations of enrichment were based on nitrate plus nitrite, whereas the fertilizer also supplied ammonium. However, previous studies showed that enrichment was similar for both nitrogen compounds (Hillebrand et al. 2000).

Statistical analysis—The efficiency of nutrient enrichment was tested with a two-factor analysis of variance (ANOVA) on dissolved phosphate and nitrate concentrations, which comprised season and nutrient enrichment as independent factors. Three different measures of biomass were obtained (particulate C, Chl *a*, biovolume) and the correlation (Pearson's *r*) between these measures was used to indicate the reliability of the results for these different parameters.

Full-factorial ANOVA was used to test for significant im-

pacts of three factors (season, grazer or cage presence, nutrient enrichment) on the biomass and C:Chl ratios of the algae, respectively. The analysis was done for each site separately. Independent factors for the analysis of grazing and nutrient effects comprised season ($n = 4$), grazer presence ($n = 2$, absent and present), and nutrient enrichment ($n = 2$, enriched and ambient). The impact of cages was estimated with an analogous design, where the factor grazer absence was replaced by cage presence ($n = 2$, present and control). This design avoided a test between absent and control (Hillebrand et al. 2000; Worm et al. 2000a). Homogeneity of variances (Bartlett's χ^2) and normal distribution of the dependent variable (Kolmogorov-Smirnov test) were confirmed. Tukey's honest significant difference (HSD) was used to distinguish between significantly different treatment levels.

Full-factorial multivariate analysis of variance (MANOVA) was used to test for significant effects of three factors (season, grazer or cage presence, nutrient enrichment) on multivariate dependent variables. The first MANOVA was conducted on the relative nutrient content of the periphyton, which was represented by the interdependent ratios C:N, N:P, and C:P. The second MANOVA was conducted on taxonomic composition of the periphyton, which was represented by the proportional contribution of Cyanobacteria, Bacillariophyceae, Chlorophyceae, Zygnematophyceae, Rhodophyceae, and Phaeophyceae to total biovolume. We used the Pillai's trace statistic, which is recommended to test for significant effects on interdependent response variables (Scheiner 1993). For the MANOVA, the homogeneity of variances was tested with the nonparametric Sen-Puri test.

C:N:P ratios were also used to indicate nutrient limitation of the periphyton. A one-sided t -test was used to test significant differences between the measured ratios and threshold values for the indication of N limitation (C:N > 10, N:P < 13) and P limitation (C:P > 180, N:P > 22). The threshold values were obtained from laboratory studies with natural periphyton (Hillebrand and Sommer 1999). To account for variation of optimal ratios in natural periphyton, e.g., due to the proportion of detritus, the thresholds were calculated as optimal ratios \pm standard error in the laboratory study (Hillebrand and Sommer 1999). These threshold ratios are higher than optimal ratios calculated from field data (Kahlert 1998), and we are thus confident that a significant deviation can serve as an indication of nutrient limitation. For this test, the ambient-absent treatments of each experiment were used ($n = 4$).

We intended to compare the magnitude of effects between sites and between factors and to relate the effects to site characteristics. Therefore, we calculated standardized mean difference (D) as effect sizes for various treatment combinations. Differences between treatment means have been used before to infer grazing effects across different experiments (Rosemond et al. 1993; Feminella and Hawkins 1995). Since variation often increases with increasing mean, it is advantageous to take the variation between replicate treatments into account. For D , the difference between two treatment means is standardized by the pooled standard deviation of both treatments (Gurevitch and Hedges 1993).

D is calculated as

$$D = \frac{\bar{X}_E - \bar{X}_C}{S} J$$

where X_C is the mean of the control, X_E the mean of the treatment, S is the pooled standard deviation, and J corrects for a bias due to small sample size. The pooled standard deviation is calculated as

$$S = \sqrt{\frac{(n_E - 1)(SD_E)^2 + (n_C - 1)(SD_C)^2}{(n_E + n_C - 2)}}$$

where n is the number of replicates and SD is the standard deviation of effects and controls, respectively. J is calculated as

$$J = 1 - \frac{3}{4(n_E + n_C - 2) - 1}$$

The response of total algal biovolume to nutrient enrichment was calculated as D_{nut} between the treatment combination absent-enriched and absent-ambient. Positive values indicate an increase in algal biomass with nutrient enrichment. The response to grazer presence was calculated as $D_{\text{graz+}}$ between the treatment combination present-enriched and absent-enriched and as $D_{\text{graz-}}$ between the treatment combination present-ambient and absent-ambient. Negative values indicate a reduction of the biomass due to grazer presence. The same calculations were performed for C:N, C:P, and N:P ratios.

Spearman rank correlation (r_s) was used to test for significant relationships between nutrient and grazer effects (D_{nut} vs. D_{graz}) and between grazing effects and total herbivore abundance. For herbivore density, only the abundances measured with the same method (all experiments in 2000) were used ($n = 8$). The impact of site characteristics (TP, TN, dissolved P and N concentrations, Secchi depth, temperature) on the effects of grazer exclusion and nutrient enrichment was tested with multiple regressions on effect sizes (D). The multiple regression was conducted as a forward stepwise regression (F -to-enter = 2.0), adding independent variables to the model as long as they contribute significantly to the explained variance.

Results

Efficiency of experimental manipulations—The experimental manipulations were highly successful, both for grazer exclusion and nutrient enrichment. Only one experiment (Lake Erken, summer) had absent treatment plots invaded by macrozoobenthos (gastropod *Theodoxus fluviatilis*) and was excluded from the analysis. Elsewhere such invasions were rare and led to removal of single replicates from the analysis.

The three sites differed greatly in ambient grazer densities (Table 2). Chironomids were numerically dominant in the two lakes, but gastropods and trichopteran larvae are probably the most effective grazers (Steinman 1996). The gastropod *Theodoxus fluviatilis* was important in both lakes, other main grazers were trichopteran larvae (Lake Erken) and ephemeropteran larvae (Lake Limnaren). At Vaddö, *Hydrobia* species (Gastropoda) and crustaceans (Isopoda and

Table 2. Abundance of main grazer taxa on stones at the three different experimental sites. Abundances are given as individuals m^{-2} (standard error in brackets). Note that sampling techniques differed between the experiments in 1999 and 2000 (*see methods*), and values for November 1999 are presumably underestimated. not det.: Group was present but not determined in the samples. First line for each site represents total abundance (in bold), which was not determined for autumn 1999 due to the differences in sampling.

Site and major taxa	Nov 1999	May 2000	Jun 2000	Jul 2000
Erken	not det.	1257	1193	838
Gastropoda (all species)	22 (3)	187 (44)	140 (42)	153 (54)
<i>Theodoxus fluviatilis</i>	21 (3)	166 (42)	133 (42)	112 (53)
<i>Lymnaea</i> sp.	—	15 (6)	8 (8)	12 (12)
Isopoda; <i>Asellus aquaticus</i>	8 (1)	21 (5)	29 (14)	104 (65)
Diptera; Chironomidae	not det.	603 (106)	427 (41)	224 (107)
Ephemeroptera (all species)	<1 (<1)	6 (4)	126 (52)	127 (38)
Trichoptera (all species)	1 (<1)	424 (116)	450 (56)	221 (50)
Limmaren	not det.	751	1056	1507
Gastropoda (all species)	16 (2)	148 (26)	54 (22)	125 (50)
<i>Theodoxus fluviatilis</i>	15 (2)	132 (15)	41 (12)	107 (50)
<i>Lymnaea</i> sp.	<1 (<1)	—	9 (9)	—
Isopoda; <i>Asellus aquaticus</i>	2 (1)	69 (29)	55 (28)	97 (41)
Diptera; Chironomidae	not det.	243 (61)	545 (239)	1049 (386)
Ephemeroptera (all species)	—	88 (21)	358 (57)	188 (83)
Trichoptera (all species)	2 (1)	172 (11)	31 (20)	48 (27)
Väddö	not det.	182	870	421
Gastropoda (all species)	12 (2)	131 (39)	541 (184)	189 (73)
<i>Hydrobia</i> sp.	—	14 (9)	327 (173)	161 (59)
<i>Theodoxus fluviatilis</i>	1 (<1)	102 (43)	128 (60)	12 (12)
<i>Lymnaea</i> sp.	8 (2)	14 (9)	5 (5)	—
Isopoda; mainly <i>Idotea baltica</i>	—	23 (18)	137 (52)	117 (80)
Amphipoda; <i>Gammarus</i> and <i>Monoporeia</i>	32 (7)	7 (7)	55 (23)	not det.
Diptera; Chironomidae	not det.	17 (10)	132 (76)	115 (71)
Trichoptera (all species)	5 (5)	—	—	—

Amphipoda) dominated the grazer fauna. To minimize disturbance, we avoided direct counts in the cages, but we checked for qualitative and obvious quantitative differences between the grazer density around the plots, in open cages (present), and in uncaged controls at each sampling date. No such differences were observed.

A two-factor ANOVA on log-transformed dissolved nutrient concentrations revealed that the enriched treatments were significantly enriched in nitrate plus nitrite ($p = 0.007$) and phosphate ($p < 0.001$). The enrichment was not affected by cage presence (insignificant main cage effects and interactions, $p > 0.6$). Nutrient concentrations measured in un-enriched (ambient) treatments of the experiment were well correlated with the dissolved nutrient concentrations measured in the monitoring program (P : $r = 0.798$, $p = 0.057$; N : $r = 0.874$, $p = 0.023$) (Table 1). Enriched treatments always had higher concentrations of N and P than ambient treatments (Table 1). The median enrichment factor was 2.4 for N (calculated for nitrate) and 5.0 for P. Ambient silicate concentrations were always high ($>6 \mu M$) and were probably nonlimiting.

Algal biomass—In log–log space, the various measures of biomass (total biovolume, particulate C, and Chl *a*) were always highly correlated (Pearson's $r > 0.6$, $p < 0.001$) with

slopes slightly less than 1 (0.78–0.94). Although these parameters reflect different aspects of biomass (Chl *a* can be influenced by light and nutrient conditions, biovolume by the size distribution of the algae), they were consistent during our study. Therefore, we conducted the statistical analysis of significant treatment effects on algal biomass using only total biovolume.

At all three sites, the algal biovolume varied significantly between seasons (Table 3), whereas there was no constant difference between the sites (Fig. 2). Ungrazed algal biovolume (mean of the treatment combination absent–ambient) was not significantly correlated to either dissolved or total nutrient concentration, except for a positive correlation to DIN ($r_s = 0.733$, $p = 0.010$, $n = 11$). The seasonal setting of the experiment also influenced the effects of nutrient and grazer manipulations (Table 3).

In Lake Erken, grazers reduced and nutrient enrichment increased algal biovolume. Significant interactions were detected between seasons and grazers and between grazers and nutrients, respectively (Table 3). Grazer presence decreased algal biovolume in autumn and early spring (Fig. 2), but there was no significant effect in late spring (HSD, $p = 0.802$). Nutrients increased the algal biovolume significantly, especially in the absence of grazers (HSD, $p < 0.001$), whereas the grazer impact was stronger under enriched con-

Table 3. Results of univariate three-factorial ANOVA on total periphytic biovolume ($\text{mm}^3 \text{cm}^{-2}$). The table gives the F ratios (with significance levels in parentheses) for the main factors and all interactions in separate analyses for each of the three sites. The degrees of freedoms for the effect terms are given in parentheses for each effect (note: for Erken, all terms involving seasons have 2 d.f.), for the error term in the row on top of the analysis. Effects significant at $p < 0.05$ are printed in bold, trends with $p < 0.1$ are printed in italic. Untransformed data were homoscedastic and normal distributed for Erken. Log-transformed data were used for Limmaren and Vaddö.

Factor	Erken	Limmaren	Vaddö
Degrees of freedom	31	43	47
Season (3)	4.277 (0.023)	43.206 (<0.001)	45.260 (<0.001)
Grazing (1)	14.695 (<0.001)	28.254 (<0.001)	19.697 (<0.001)
Nutrient (1)	12.359 (0.001)	0.881 (0.353)	3.866 (0.055)
Season \times grazing (3)	10.249 (<0.001)	1.055 (0.378)	2.331 (0.086)
Season \times nutrient (3)	1.167 (0.325)	0.275 (0.844)	1.536 (0.218)
Grazing \times nutrient (1)	5.177 (0.030)	2.420 (0.127)	0.001 (0.970)
Season \times grazing \times nutrient (3)	0.498 (0.613)	1.994 (0.129)	0.696 (0.559)
Degrees of freedom	29	35	42
Cage (1)	15.475 (<0.001)	10.752 (<0.001)	16.006 (<0.001)
Season \times cage (3)	0.961 (0.394)	0.179 (0.590)	3.530 (0.023)
Nutrient \times cage (1)	2.463 (0.127)	0.022 (0.884)	0.073 (0.788)
Season \times nutrient \times cage (3)	2.534 (0.097)	1.621 (0.202)	2.336 (0.087)

ditions (Fig. 2, autumn and early spring). In Lake Limmaren, grazers reduced algal biovolume throughout the year (Table 3, Fig. 2). There was no significant interaction between grazers and nutrient enrichment or season, respectively. However, grazing impacts were higher in autumn and summer compared to the spring experiments (Fig. 2). Nutrients had no significant effects on periphyton biovolume. At Vaddö, the loss of biovolume due to grazers was also highly significant (Table 3), with strongest impact in summer (Fig. 2). Increasing biovolume with nutrient enrichment was found in late spring and summer, but the nutrient effect was insignificant (Table 3).

At all three sites, the presence of cages reduced the algal biovolume (Table 3, Fig. 2). This effect was not consistent throughout the year, but was especially strong in autumn and summer (Fig. 2). Generally, the algal assemblage reached an intermediate biovolume in control treatments compared to absent and present treatment (Fig. 2).

Algal nutrient content—The comparison of algal nutrient content to optimal ratios and limitation indices derived for benthic microalgae (Hillebrand and Sommer 1999) indicated a slight prevalence of algal N deficiency in Lake Erken and at Vaddö (Fig. 3). High C:N ratios (>10) with low N:P ratios were found in Lake Erken in early and late spring and at Vaddö in early spring. Internal C:N ratios were significantly higher than the threshold values at both sites in early spring (one-sided t -test, $p < 0.01$) but not in late spring in Lake Erken ($p = 0.16$). At Vaddö, a slight but insignificant ($p = 0.22$) tendency toward P deficiency could be seen in summer, i.e., N:P and C:P ratios were both high. In Lake Limmaren, nutrient content was generally high and there was no indication of limitation (Fig. 3). Generally, C:N ratios in ambient-absent treatments were well correlated to TN ($r_s = -0.664$, $p = 0.026$, $n = 11$) and the nitrate measured directly in the experiments ($r_s = -0.812$, $p = 0.049$, $n = 6$).

Mean C:P ratios also decreased when there was more phosphate ($r_s = -0.841$, $p = 0.036$, $n = 6$), whereas the correlation to TP was negative but insignificant ($r_s = -0.305$, $p = 0.361$, $n = 11$).

At all sites, the stoichiometry of the algal biomass was affected by the experimental manipulations and varied significantly between seasons (Table 4). In Lake Erken, C:N and C:P ratios were reduced by the addition of nutrients, i.e., the content of both N and P relative to C was higher in enriched treatments (Table 4). The presence of grazers also increased the content of nutrients in the periphyton (Table 4). The significant season \times grazer interaction resulted from grazer presence decreasing C:P ratios in autumn and late spring and decreasing C:N ratios in late spring. The N:P ratio decreased with grazer presence in autumn 1999 and in enriched treatments of the spring experiments (Fig. 4), but N:P ratios did not decrease in the ambient treatments in the spring experiments. The presence of cages had a significant influence only in Lake Erken in autumn (Table 4), where the increase of nutrient content in enriched treatments was confined to caged treatments.

In Lake Limmaren, nutrient enrichment had no significant effects on periphytic nutrient content (Table 4). However, a decrease of N:P ratios in the enriched treatments (Fig. 4, autumn and early spring) was observed as well as a decrease of C:P ratios. The presence of grazers increased phosphorus content, i.e., decreased N:P ratios (Fig. 4, autumn and summer) and also C:P ratios, but these effects were not statistically significant.

At Vaddö, the C:N ratios were reduced in the enriched treatments in autumn, resulting in an interaction between nutrient enrichment and season (Table 4). The interactions between grazing effects and nutrient enrichment or season, respectively, were insignificant (Table 4), and the effects of grazers were complex: In enriched treatments, grazers reduced N:P ratios (Fig. 4) and accordingly also C:P ratios,

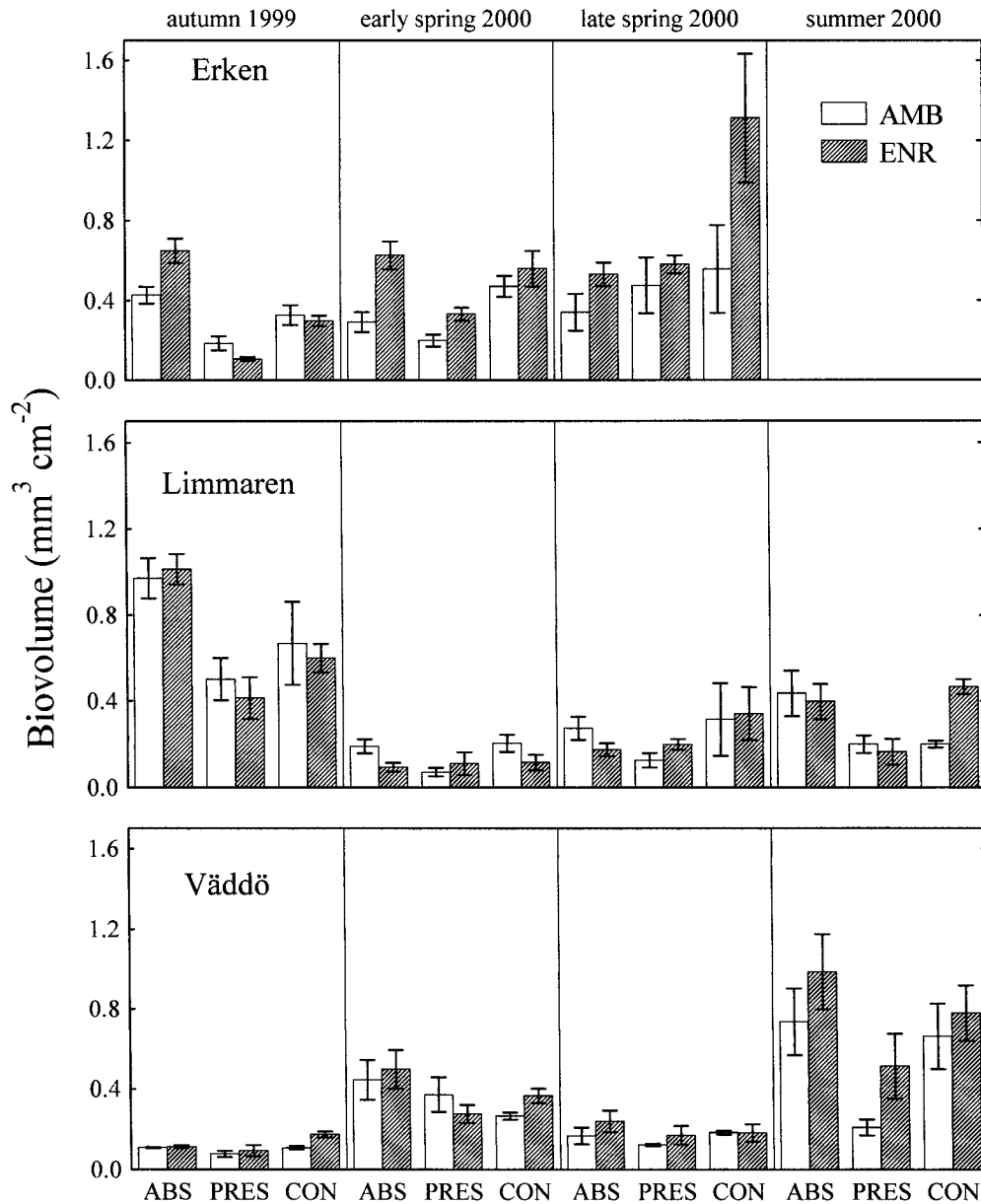


Fig. 2. Total biovolume of periphytic algae (mean \pm standard error) in experiments at three different sites and in different seasons. Each row gives the results for one site for both nutrient levels (ambient and enriched) and for the different seasons. In each diagram, the grazer and cage treatments separate the columns (grazer absent, grazer present, and control plots without cages).

except for the summer experiment. C:N ratios were lowered by grazer presence in ambient treatments in autumn and early spring, otherwise the effects of grazers on N were weak.

Like the nutrient ratios, the chlorophyll content per unit carbon and per unit biovolume, respectively, were significantly affected by nutrient enrichment and grazing pressure. Nutrients decreased the C:Chl ratio significantly in Lake Erken (ANOVA, $p = 0.032$) and in Lake Limmaren (but not in summer and not in all treatments, therefore there was a significant three-way interaction season \times nutrient \times grazing, $p = 0.042$). At none of the sites was the C:Chl ratio affected

by grazing (ANOVA, $p > 0.15$). The ratio of Chl *a* per unit biovolume was significantly increased by grazer presence at Väddö (ANOVA, $p = 0.003$) and in Lake Erken ($p = 0.023$). In Lake Limmaren, the ratio was also increased in most experiments and treatments (three-way interaction, $p = 0.052$).

Algal taxonomic composition—The periphyton found on the ceramic tiles showed high species richness (>200 in total across all sites and seasons) and a high differentiation of growth types and cell sizes. The most important groups were filamentous green algae (Zygnematophyceae and Chlorophy-

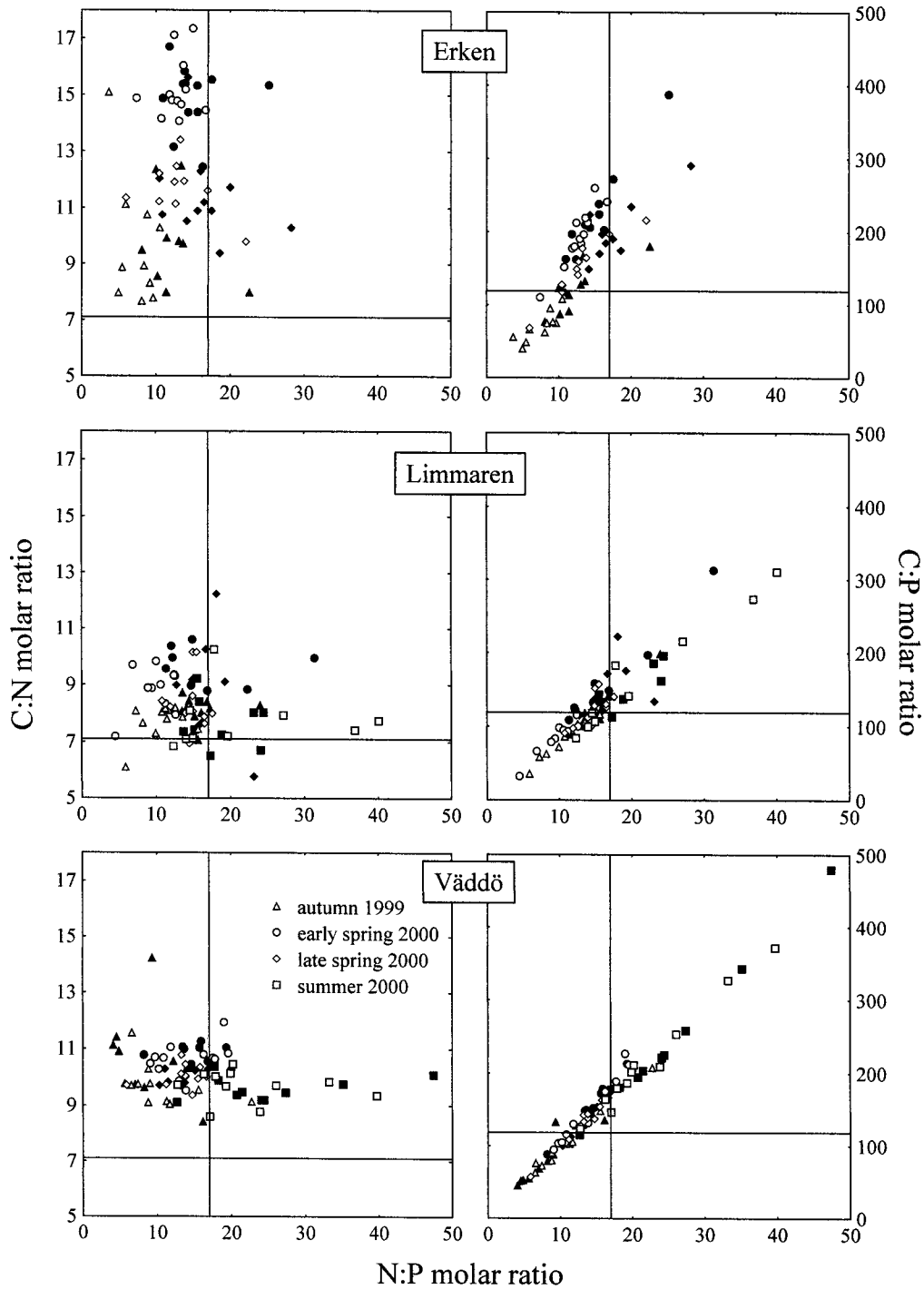


Fig. 3. Cellular stoichiometry of C:N:P in periphytic algae in experiments at three different sites and in different seasons. Each row represents one site, the left column shows the C:N ratios and the right the C:P ratios. Black symbols represent ambient treatments, white symbols enriched treatments. The lines are the optimal ratios for periphyton derived from Hillebrand and Sommer (1999). The presentation allows the indication of nutrient limitation. High C:N values together with low N:P values indicate N limitation (left column: quadrant at the top left), whereas high C:P values in combination with high N:P values indicate P limitation (right column: quadrant at the top right).

Table 4. Results of multivariate three-factorial ANOVA on periphytic nutrient content (C:N, N:P, C:P ratio). The table gives Pillai's trace (PT) values and the F ratio (with significance levels in parentheses) for the main factors and all interactions for each of the three sites. Effects significant at $p < 0.05$ are printed in bold, trends with $p < 0.1$ are printed in italic. Untransformed data were homoscedastic for Limmaren and Vaddö, whereas for Erken homogeneity of variances was achieved by square root transformation. Degrees of freedom for the F statistics are $F_{k \times a, a \times n}$, where a = factor degree of freedom (Table 3), n = error degree of freedom (Table 3), and k = number of dependent variables (3).

Factor	Erken		Limmaren		Vaddö	
	PT	F	PT	F	PT	F
Season	1.14	12.69 (<0.001)	0.67	3.96 (<0.001)	0.84	5.18 (<0.001)
Grazing	0.29	3.76 (0.022)	0.15	2.35 (0.088)	0.06	0.86 (0.471)
Nutrient	0.51	9.87 (<0.001)	0.10	1.51 (0.227)	0.11	1.63 (0.199)
Season \times grazing	0.76	5.90 (<0.001)	0.26	1.29 (0.251)	0.34	1.72 (0.092)
Season \times nutrient	0.23	1.23 (0.302)	0.33	1.72 (0.093)	0.37	1.90 (0.058)
Grazing \times nutrient	0.14	1.50 (0.235)	0.04	0.52 (0.671)	0.16	2.49 (0.075)
Season \times grazing \times nutrient	0.20	1.09 (0.380)	0.09	0.40 (0.931)	0.31	1.55 (0.138)
Cage	0.46	7.78 (<0.001)	0.28	0.59 (0.625)	0.14	1.99 (0.132)
Season \times cage	0.44	2.63 (0.026)	0.22	1.19 (0.314)	0.36	1.79 (0.076)
Nutrient \times cage	0.33	4.41 (0.012)	0.01	0.11 (0.953)	0.06	0.76 (0.522)
Season \times nutrient \times cage	0.27	1.43 (0.220)	0.20	0.83 (0.592)	0.27	1.30 (0.242)

ceae), cyanobacteria, and diatoms. Red and brown algae were also important at Vaddö (Fig. 5). The proportional contribution of these classes to total biovolume was highly affected by the seasonal setting and the presence of grazers, whereas nutrient enrichment effects were insignificant throughout (Table 5). At all sites, the seasonal pattern was high diatoms in autumn, high chlorophytes in spring, and high cyanobacteria in summer (Limmaren and Vaddö), autumn (Vaddö), or late spring (Erken).

There were highly significant season \times grazer interactions at all sites (Table 5), reflecting shifts in seasonal dominance and grazing susceptibility of different algal taxa. Grazing had a strong negative effect on the proportion of filamentous green algae in spring (Erken and Vaddö), but Zygnematomyceae were less affected than Chlorophyceae. At Vaddö, filamentous cyanobacteria (mainly Oscillatoriales and Nostocales) profited slightly from enrichment and in some experiments also from grazer presence (Fig. 5). In Lake Limmaren, a decrease in cyanobacterial proportion (mainly coccal forms) was detected in present treatments compared to absent treatments, connected to a subsequent increase of diatoms. Phaeophytes gained importance in late spring and summer; this was more prominent in nutrient-enriched treatments (Fig. 5). Crustose rhodophyte species gained importance with grazer presence in Lake Erken.

Although cages did not reduce species richness (ANOVA, $p > 0.3$), the proportion of algal groups was clearly affected, mainly by a reduction of filamentous green algae in the cages. In Lake Erken, the reduction of filamentous algae was confined to spring experiments (Fig. 5, Table 5), and at Vaddö this effect was more pronounced for Zygnematomyceae than for Chlorophyceae (Fig. 5).

Comparison of effect sizes—In most experiments, both grazing and nutrients were important (Fig. 6). Three out of 11 experiments (all from Limmaren) showed an impact of grazing without a positive impact of nutrients, and in one

experiment (Lake Erken, late spring) there was a strong nutrient effect but no grazing effect (Fig. 6). The magnitude of the effect sizes was higher for grazing than for nutrient enrichment ($D_{\text{graz}} > D_{\text{nut}}$). The nutrient and grazer effects were not correlated at the ambient ($r_s = 0.19$, $p = 0.574$, $N = 11$) or at the enriched nutrient level ($r_s = -0.34$, $p = 0.312$, $N = 11$). Thus, grazers and nutrients were effective at the same time and there was no overriding effect by either top-down or bottom-up regulating factors.

There were also strong effects of both factors on the stoichiometry of internal nutrients (Fig. 7). Nutrient enrichment consistently decreased C:N and C:P ratios ($D_{\text{nut}} < 0$), and thus increased the internal content of both nutrients (Fig. 7A,B). This resulted in increasing or decreasing N:P ratios with enrichment (Fig. 7C). The effect of grazing on C:N ratios was variable and inconsistent (Fig. 7A), although in ambient treatments C:N ratios tended to be slightly lower in present than in absent treatments ($D_{\text{graz-}} < 0$). However, for C:P and N:P ratios the effect sizes were variable but most often negative, indicating enhanced periphytic P content in grazer presence (Fig. 7B,C). This increase in P content was shown in ambient as well as enriched treatments.

The effects of nutrients and grazers on nutrient stoichiometry were not correlated (Spearman rank correlation r_s , $p > 0.1$). Although grazing increased P content (Fig. 7B,C), this did not result in higher biomass compared to ungrazed treatments (Fig. 6). Grazers thus had two effects, the consumption of algal biomass and the increase in nutrient content (mainly P), but these two effects were not significantly correlated (r_s , $p > 0.1$).

The effect of site and season on grazing and nutrient effects—Several parameters characterizing the sites (background nutrient concentrations, light and temperature) were related to the effect sizes of grazing and nutrient enrichment (Table 6). However, the single parameters and the complete models were weakly significant or nonsignificant and the ex-

plained variance was always below 55%. The negative impact of grazing on biovolume was more pronounced if TP or DIN were high (Table 6). Higher TP reduced the effect of nutrient enrichment, whereas increasing DIN concentrations resulted in higher effectiveness of nutrient enrichment. The most important influence on nutrient effects was by Secchi depth, which indicated that nutrient effects were low when light penetration was low. Increasing temperature resulted in an increase of nutrient effects on C:N and a decrease in grazing effects on C:N. Increasing DIP concentrations made the nutrient enrichment less effective for C:P, whereas increasing TP increased grazing effects on C:P. There was also a weak but interesting relation between DIN concentrations and the effects of grazing and nutrients on the N:P ratio (Table 6). If DIN was high, the effects on N:P became more negative, and thus more P relative to N was included in the periphyton.

Discussion

We found strong impacts of grazing and variable impacts of nutrient supply on periphytic biomass. Often both factors were important at the same time, but the magnitude of their effects was not correlated. Algal taxonomic composition was clearly more affected by grazing than by nutrient supply. Moreover, grazers and nutrient enrichment influenced periphytic nutrient content. Although effects were not significant throughout, grazers tended to decrease C:P and N:P ratios, whereas nutrients increased both N and P content in the periphyton. In the following, we will first discuss the experimental setup before we evaluate the relative impacts of the manipulated factors on algal biomass and composition (hypothesis 1) and on nutrient stoichiometry (hypothesis 2), as well as the dependence of the different effects on seasons and sites (hypothesis 3).

Experimental setup and analysis—Our experiments were designed in accordance to recommendations by Feminella and Hawkins (1995), who suggested conducting experiments for at least 4 weeks and expanding the experiments beyond the most favorable seasons (late spring, summer) and the temperate regions of North America. The manipulations of grazing intensity and nutrient supply were effective, i.e., grazers were successfully excluded, and dissolved N and P concentrations were higher in enriched than in ambient treatments. The semipermeable coating of the fertilizer granules allowed a continuous nutrient enrichment (Worm et al. 2000b). Thus, we are confident that our experiments revealed reliable estimates of grazing pressure and nutrient effects, but possible biases may be introduced by the use of artificial substrates, by cage artifacts, and by the choice of only one location at each site.

Periphyton on ceramic tiles was similar in composition to the natural flora, with the notable exception of the long-lived *Cladophora* sp., which is more abundant on natural stones in Lake Erken (Kahlert, unpubl. data). *Cladophora* was present in our experiments, but the preincubation period of at least 6 months was seemingly not sufficient to establish the dominance of this species.

Cages not only excluded grazers but also changed some abiotic conditions (mainly light availability and waterflow).

However, the mesh we used reduces light availability by no more than 10% (Hillebrand et al. 2000), and shadowing by microalgal colonization of the nets was minimized by changing the nets in regular intervals. The reduced waterflow did not result in visibly higher sedimentation in the cages. We conducted a thorough control experiment to estimate the effect of cages and found significant negative impacts on algal biomass at all three sites but often confined to one or two seasons per site. Different algal colonization probably caused the differences between present and control treatments, since we found a slightly reduced amount of filamentous green and red algae in the cages. Since those filamentous algae are supposed to be highly susceptible to grazing (Steinman 1996), we are very confident that our results are not generally flawed by this artifact. Other cage effects than on biomass were accordingly rare. In the autumn experiment in Lake Erken, some stormy days probably decreased the effectiveness of the enriched treatment in uncaged plots, explaining the lack of nutrient impact on algal stoichiometry in the control treatments.

Because we measured grazing on small substrates at only one littoral location at each site, extrapolation to larger areas must be done with care. Differences between littoral zones may affect the distribution and grazer activity of different invertebrate groups (Harrison and Hildrew 1998). However, we used typical habitats within the three sites, and our enclosure design provided the comparison of ungrazed to naturally grazed periphyton.

Further, the use of nutrient ratios to detect nutrient limitation and the impact of grazing on nutrient conditions has to be interpreted with caution. C:N:P ratios are widely used to indicate nutritive status of phytoplankton (Hecky et al. 1993), and this concept has recently been established also for benthic microalgae (Kahlert 1998; Hillebrand and Sommer 1999). However, detritus or heterotrophic components in the periphyton could influence C:N:P ratios. Additionally, several factors can interfere with the experimental manipulations to produce similar results on nutrient stoichiometry. Nutrients may increase either the N or P content or change the algal composition to species with a different C:N:P ratio, whereas grazers may reduce the detritus content, change the composition of algae, or increase the supply of nutrients to the algae. However, we have good indication that changes in nutrient supply reflect changed nutrient content in algae rather than changed proportions of heterotrophs, detritus, or algal taxonomic groups, respectively.

To analyze the importance of heterotrophic components, we counted bacteria, ciliates, and meiofauna in five out of 11 experiments. The community was highly dominated by autotrophs: bacteria contributed mainly between 1 and 10% of the algal biomass and ciliates ~1% (Hillebrand, Haglund, Nagel unpubl. data). The significant correlation between C and algal biovolume also indicated that C was largely influenced by algal material. Moreover, we found that C:N and C:P ratios well reflected total and dissolved nutrient concentrations (see *Algal nutrient content*). A removal of C-rich, nutrient-poor material such as detritus by grazers cannot explain the pattern we found, since grazer presence reduced N:P and C:P ratios, but not C:N and C:Chl ratios. The taxonomic composition is an unlikely explanation for chang-

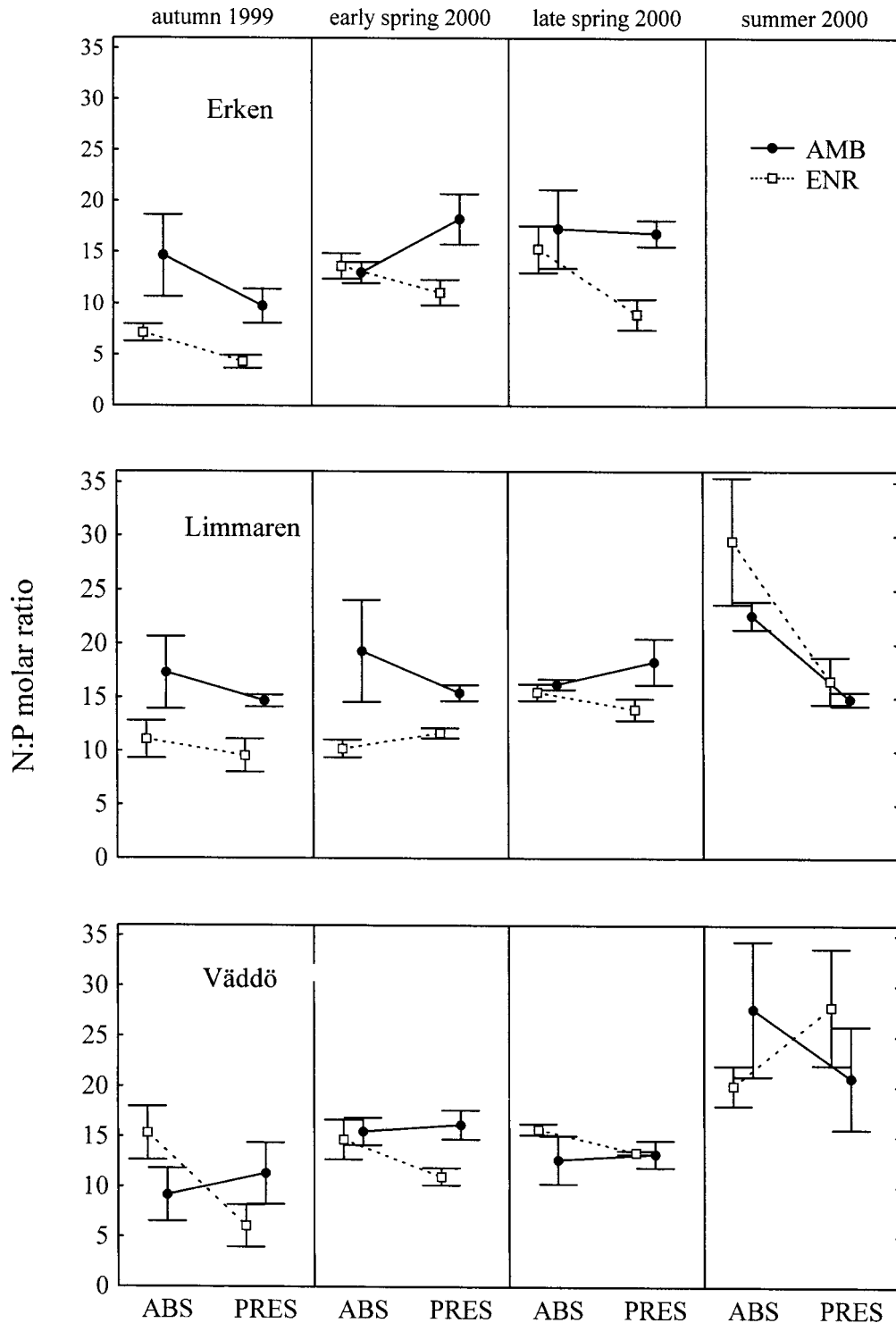


Fig. 4. Effect of grazing and nutrient enrichment on molar N:P ratios in periphyton (mean \pm standard error) at three different sites (rows) and in four different seasons (columns). For abbreviations, see Fig. 2.

es in P and N content, since decreasing N:P ratios could not be related to changed proportions of taxa with supposedly high P demand such as cyanobacteria or low P demand such as chlorophytes (Sommer 1996).

We are thus confident that significant deviation from the indication thresholds can be interpreted as nutrient limitation, since the threshold values (Hillebrand and Sommer 1999) take into account that nonalgal material can affect the

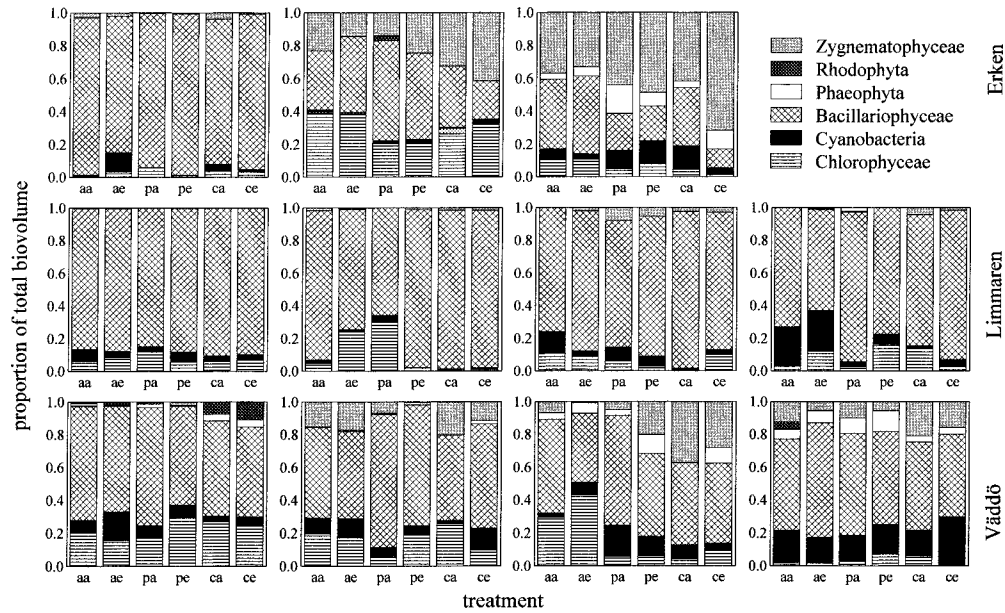


Fig. 5. Proportional contribution of algal classes to total periphytic biovolume, shown for different sites (rows) and seasons (columns). The treatments are abbreviated as “aa” (absent–ambient), “ae” (absent–enriched), “pa” (present–ambient), “pe” (present–enriched), “ca” (control–ambient), and “ce” (control–enriched).

C:N:P ratios. Instead our method may even overestimate cellular P because particulate inorganic P may be found in the particulate P measured (Stelzer and Lamberti 2001). With all caution related to the abovementioned artifacts, we also assume that the effects of grazer presence and nutrients on stoichiometry were based at least in a large part on changes in algal nutrient content.

The impact of grazing and nutrients on periphytic biomass and composition—Our first hypothesis—that grazer presence reduces and nutrient enrichment increases algal biovolume—was confirmed. Our findings are consistent with previous results from streams (McCormick and Stevenson 1991; Hill

et al. 1992; Rosemond 1993) and coastal sites (Hillebrand et al. 2000), but in our experiments both effects were highly variable between seasons and sites (*see Grazer and nutrient effects in connection to habitat and season*). In contrast to our hypothesis, however, we did not find evidence for either interactive or independent effects of nutrients and grazers.

A significant interaction between grazer presence and nutrient enrichment was found in Lake Erken, with nutrients being more effective in absent treatments and grazer impact stronger at enriched treatments. This corroborates previous reports of more pronounced effects of grazing under nutrient-enriched conditions (Marks and Lowe 1989; Rosemond 1993; Hillebrand et al. 2000) and more effective nutrient

Table 5. Results of multivariate three-factorial ANOVA on periphytic taxonomic composition. The table gives Pillai’s trace (PT) values and the *F* ratio (with significance levels in parentheses) for the main factors and all interactions for each of the three sites. Effects significant at *p* < 0.05 are printed in bold, trends with *p* < 0.1 are printed in italic. Untransformed data were homoscedastic for Limmaren and Erken, whereas for Vaddö homogeneity of variances was achieved by square root transformation. Degrees of freedom for the *F* statistic are as in Table 4, but with *k* = 6.

Factor	Erken		Limmaren		Vaddö	
	PT	<i>F</i>	PT	<i>F</i>	PT	<i>F</i>
Season	1.50	17.00 (<0.001)	0.74	3.52 (<0.001)	1.20	5.91 (<0.001)
Grazing	0.21	1.45 (0.238)	0.20	2.61 (0.047)	0.16	1.67 (0.147)
Nutrient	0.08	0.44 (0.815)	0.06	0.63 (0.644)	0.17	1.68 (0.144)
Season × grazing	0.68	2.87 (0.006)	0.59	2.65 (0.003)	0.92	3.91 (<0.001)
Season × nutrient	0.16	0.47 (0.901)	0.18	0.69 (0.760)	0.31	1.01 (0.450)
Grazing × nutrient	0.13	0.79 (0.566)	0.13	1.50 (0.219)	0.09	0.84 (0.543)
Season × grazing × nutrient	0.31	1.03 (0.452)	0.33	1.34 (0.202)	0.43	1.47 (0.108)
Cage	0.38	3.04 (0.028)	0.22	2.33 (0.076)	0.37	4.53 (0.001)
Season × cage	0.55	1.99 (0.054)	0.33	1.10 (0.392)	0.37	2.26 (0.004)
Nutrient × cage	0.10	0.58 (0.714)	0.07	0.62 (0.653)	0.16	1.50 (0.200)
Season × nutrient × cage	0.26	0.77 (0.659)	0.34	1.10 (0.367)	0.38	1.15 (0.314)

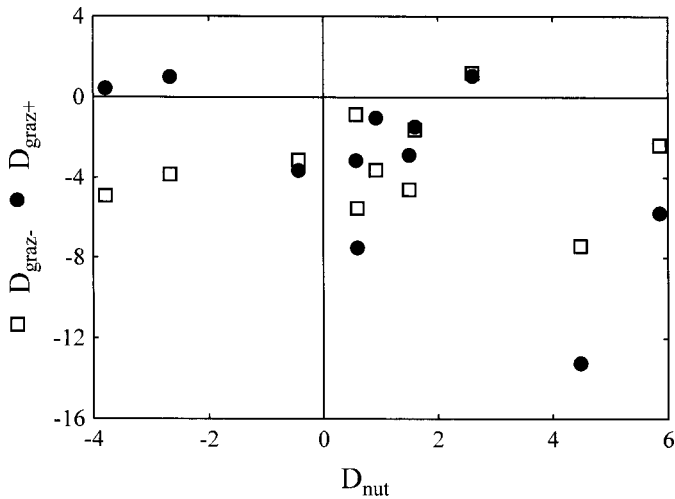


Fig. 6. Effect sizes (standardized mean difference D) for the effects of nutrients (D_{nut}) and grazer (D_{graz}) on periphytic biovolume. Lines represent zero effect sizes, negative values of D_{graz} represent the decrease of periphyton due to grazing, and positive values of D_{nut} represent the increase of periphyton with nutrient addition.

treatments in ungrazed periphyton (Hill et al. 1992; Hillebrand et al. 2000). Under enriched conditions, food-limited herbivores may increase grazing pressure (functional response) at higher food availability (Hart and Robinson 1990; Sommer 1999). Furthermore, mobile grazers may actively select high-quality food patches and increase local density, thus showing a numerical response based on distribution rather than on reproduction (Hill et al. 1992; Nisbet et al. 1997; Cruz-Rivera and Hay 2000).

Nutrient and grazing impacts were interactive to some degree, and seven out of 11 experiments showed a combination of both grazer and nutrient effects (see Fig. 6). Nevertheless, there was no significant correlation between grazer and nutrient effect sizes. Top-down or bottom-up factors thus neither consistently excluded nor reinforced each other. During sampling, we observed that grazing marks were often confined to parts of the substrate (e.g., in the form of grazing tracks). Although no direct evidence is available for our experiments, we assume that our substrates integrated bottom-up and top-down controlled portions of the periphyton, i.e., some parts were highly affected by grazing, whereas other parts were not and were possibly controlled by nutrient supply. In benthic communities, grazing effects often are spatially explicit (Nisbet et al. 1997; Poff and Nelson-Baker 1997), i.e., grazing pressure is patchily distributed, either horizontally (DeNicola et al. 1990; Sommer 2000) or vertically (DeNicola et al. 1990; Steinman 1996; Sommer 1997). The spatial distribution of grazing pressure very much depends on grazer abundance, feeding behavior, mobility, and mouthpart morphology (Sommer 2000).

Grazer effects were generally stronger than nutrient effects in our study. This was evident for algal biomass and even more for taxonomic composition. Similar results were obtained from experiments in streams and seagrass meadows (Neckles et al. 1993; Rosemond et al. 1993; Pan and Lowe 1994). There could be several reasons for the smaller effects

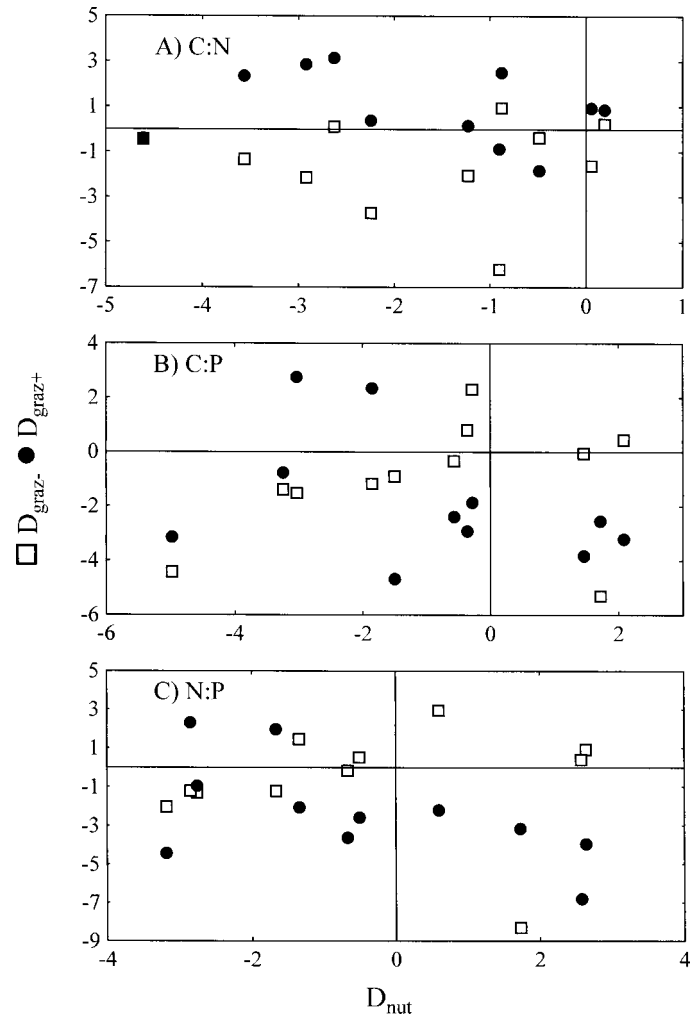


Fig. 7. Effect sizes (standardized mean difference D) for the effects of nutrients (D_{nut}) and grazer (D_{graz}) on periphytic nutrient content: (A) C:N ratio, (B) C:P ratio, (C) N:P ratio. Lines represent zero effect sizes.

of nutrient enrichment. First, the total nutrient concentrations (TN, TP) were moderate to high at all three sites, which could reduce the importance of nutrient addition. However, at all three sites dissolved nutrient concentrations varied between seasons and were low in some times of the year. Generally, the nutrient concentrations in our experiment were much lower than thresholds (ca. $1.6 \mu\text{M P}$ and $7 \mu\text{M N}$) proposed to indicate the absence of nutrient limitation in mature periphyton (Borchardt 1996). Periphyton assemblages are embedded in a mucilage matrix and surrounded by a boundary layer, which slows down the rate of diffusion of nutrients into the community and limits the access to water column nutrients (Bothwell 1989; Burkholder et al. 1990; Hill et al. 1992). Periphyton may thus be less affected by water column nutrient enrichment, and stronger nutrient effects were reported from experiments with addition of nutrients directly into the periphyton (Hillebrand and Sommer 1997). However, the observed change of nutrient ratios with nutrient enrichment indicated that added P and N were incorporated in the periphyton. Therefore, we assume that the

Table 6. Multiple regression on effect sizes representing the impact of nutrient enrichment (D_{nut}) and grazing (D_{graz}) on biovolume, C:N, C:P, and N:P ratios, respectively. The table lists explained variance (r^2), F ratio, and significance levels (p) for the complete regression model. Furthermore, estimates of the regression coefficient B are given for the intercept and each independent variable included in the model, together with significance levels. The independent variables comprise temperature, Secchi depth, and dissolved and total nutrient concentrations. For two effect sizes (D_{graz} on N:P and C:P, respectively), no regression model was found. Degrees of freedom for the F statistics are $F_{a,n-a-1}$, where a = number of factors included in the model, and $n = 11$.

Effect	On	r^2	F	p	Intercept	Variable included	B
D_{nut}	Biovolume	0.546	5.00	0.037	-2.43	Secchi depth	1.22†
						DIN	0.88*
						TN	-0.04
$D_{\text{graz-}}$		0.369	3.92	0.065	-0.29	TP	-1.90
						DIP	-4.06
$D_{\text{graz+}}$		0.450	9.18	0.014	0.31	DIP	-7.33
						DIN	-1.18
D_{nut}	C:N	0.192	3.38	0.099	-4.31†	Temperature	0.23*
$D_{\text{graz-}}$		0.259	4.49	0.063	-5.22†	Temperature	0.33*
$D_{\text{graz+}}$		0.287	5.03	0.052	2.78†	TN	-0.04*
D_{nut}	C:P	0.177	3.15	0.110	-0.10	DIP	-4.40
$D_{\text{graz-}}$		0.104	2.16	0.176	0.93	TP	-1.68
D_{nut}	N:P	0.094	2.04	0.187	0.59	DIN	-0.61
$D_{\text{graz-}}$		0.347	3.66	0.075	7.23†	Temperature	-0.56†
						DIN	-0.94

* $p < 0.1$.

† $p < 0.05$.

time scale of our field experiments (~4 weeks) may favor the detection of direct effects of complete grazer exclusion compared to the indirect effect of relative nutrient enrichment, which is mediated by uptake and growth kinetics.

Regarding taxonomic composition, filamentous algae (mostly chlorophytes, but also rhodophytes) were reduced most by grazing, whereas the relative importance of filamentous cyanobacteria was often enhanced by grazer presence (see comparable results in Lowe and Hunter 1988; Rosemond et al. 1993; Sommer 1997). Moreover, the grazer presence increased the Chl:biovolume ratio, as was previously reported from streams (Hill and Knight 1987). Although other explanations are possible, the decrease in filamentous algae and the increase in Chl:biovolume ratio presumably reflects a preferential removal of upright, large species by grazing herbivores. This is a consistent pattern emerging in periphyton grazing experiments (streams: Hill and Knight 1987; Steinman et al. 1987; DeNicola et al. 1990; lakes: Lowe and Hunter 1988; coasts: Nicotri 1977; Hillebrand et al. 2000). In contrast to the small effects of nutrients on taxonomic composition, other studies reported distinct shifts between taxonomic groups following enriched nutrient supply (Marks and Lowe 1989; Sommer 1996). The insignificant effects may be due to the fact that we changed both N and P supply simultaneously and thus to a lesser degree the supply ratios, which may have an important impact on species composition (Sommer 1996; Stelzer and Lamberti 2001).

The impact of grazing and nutrient enrichment on algal nutrient stoichiometry—Our second hypothesis—that both nutrients and grazers increase the internal nutrient content of

the algae—was accepted, although effects were not constant throughout.

Nutrient enrichment reduced C:P, C:N, and C:Chl ratios. The nutrient effect was significant for Lake Erken, and the nutrient \times season interaction was nearly significant for Väd-dö. Thus, the NPK fertilizers in our experiments evidently increased the incorporation of both P and N. Decreasing C:N and C:P ratios in benthic algae following enhancement of nutrient supply were also found for groundwater-influenced lake sediments (Hagerthey and Kerfoot 1998), in streams (Peterson et al. 1993; Stelzer and Lamberti 2001) and at coasts (Hillebrand and Sommer 1997).

Grazing effects on the relative algal nutrient content were variable but distinct. A general positive effect of grazing on biomass-specific productivity, C:N, and C:Chl ratios has been previously described for freshwater (Cuker 1983; Lamberti et al. 1987; Rosemond 1993; Rosemond et al. 1993) and marine intertidal periphyton (Hunter and Russell-Hunter 1983). However, the positive effect of grazing on nutrient content did not outweigh consumptive losses, i.e., algal biomass was not increased by grazer presence (cf. Steinman 1996; De Mazancourt et al. 1998). We found strongest effects of grazers on C:P and N:P ratios, whereas C:N and C:Chl were less or not affected. For these grazing effects, cautious interpretation of the results is necessary due to several confounding indirect effects (see *Experimental setup and analysis*). However, an important role of changed nutrient supply by grazing can be proposed, which can be mediated by removal of the canopy layer leading to a more efficient nutrient uptake or by nutrient excretion (McCormick and Stevenson 1991). Our data do not allow disentangling these two effects, but excretion of P is probably an

important factor, since our findings are consistent with results from laboratory experiments with periphyton and grazers from Lake Erken. These experiments revealed high P content of fecal pellets excreted by the dominant gastropod *Theodoxus* (C:N:P = 67:5:1) and consecutively an increased P content in the algae exposed to fecal pellets even without the presence of grazers (Stendera, unpubl. data). N may thus be retained in the herbivore biomass if it is in short supply compared to the demand of the herbivores. Alternatively, it may be excreted in liquid form (Grimm 1988) and thus dispersed into the water column. Thus, grazers increased algal P content, whereas algal C:N:P ratios indicated a slight N deficiency.

With all caution in relation to measurement artifacts, this stoichiometric imbalance indicated a dynamic feedback mechanism influencing microbenthic communities (*see also* Stelzer and Lamberti 2001). Reports on the effects of imbalanced nutrient regeneration have been published for zooplankton and phytoplankton (Sterner et al. 1992; Elser and Hassett 1994) showing strong feedback of the regeneration stoichiometry on autotrophic composition (MacKay and Elser 1998; Elser and Urabe 1999). In benthic systems, these effects could be even stronger than in pelagic systems, due to the short recycling pathways and close spatial connection in this habitat (Cuker 1983; Kahlert and Baunsgaard 1999) and the importance of nutrient regeneration for periphyton growth (Bothwell 1989; Burkholder et al. 1990). However, to our knowledge there are no systematic studies on the stoichiometry of food web interactions involving periphyton.

Grazer and nutrient effects in connection to habitat and season—Our third hypothesis—that total nutrient concentrations determines the relative effects of grazers and nutrients—was not confirmed. Although nutrients and light influenced the effects of nutrients and grazing, they did not determine a trend from top-down to bottom-up control of periphyton biomass.

Total nutrient concentrations did not determine the amount of periphytic biomass, which is consistent with previous reports stating that periphyton is not well correlated to total productivity due to the asymmetric competition for light with phytoplankton (Cattaneo 1987; Hansson 1992). In fact, Lake Limmaren had highest TN and TP concentrations, but also highest phytoplankton biomass and lowest Secchi depth (Table 1), and thus not consistently higher periphyton biomass.

The nutrient ratios in the absent-ambient treatments indicated slight nitrogen limitation (Lake Erken, Vaddö), slight P limitation (Vaddö), or the absence of nutrient limitation (Lake Limmaren). For Lake Erken, the N limitation in the periphyton is contrasted by a tendency toward P limitation described for phytoplankton in spring and early summer (Istvanovics et al. 1992); however, later in the year N may become limiting also for pelagic microalgae (Vrede et al. 1999). Guildford and Hecky (2000) supposed that TN:TP ratios indicate which nutrient may become limiting for phytoplankton, with TN:TP < 20 indicating N deficiency and TN:TP > 50 indicating P deficiency. This could not be established for our experiments; the rather high TN:TP ratios (Table 1) were contrasted by low periphyton N:P ratios (Fig.

3). Thus, the C:N and C:P ratios reflected total and dissolved nutrient concentrations, but the algal N:P ratio did not reflect the high TN:TP ratios of the environment. We interpret this to mean that not only nutrient ratios but also nutrient concentrations (Stelzer and Lamberti 2001) and uptake ability (Burkholder et al. 1990) are important for periphyton nutrient content.

Light, temperature, and nutrient concentrations influenced the response of algal biomass and nutrient content to nutrient enrichment and grazer presence. The importance of Secchi depth for nutrient effects on biovolume indicated that nutrient supply is less important if light availability is low, which is also shown by the low C:N:P ratios found in periphyton from Lake Limmaren. The negative impact of TN on D_{nut} also reflects the reduced importance of nutrient supply in this highly productive environment. The rather counterintuitive positive impact of DIN on D_{nut} can be attributed to the fact that the fertilizer enriched the nutrients in relation to the background concentrations, i.e., higher background DIN resulted in higher nutrient addition. Grazer effects generally tended to increase if nutrient concentrations were higher, whereas temperature affected the effect sizes inconsistently.

The direct impacts of single abiotic parameters on the effect sizes were generally weak and insignificant. Even in combination, the abiotic factors explained at maximum 55% of the variance. Possible reasons are that our sites spanned too narrow of a range of abiotic characteristics, that other factors are more important than those measured, or that biotic interactions within the food web are important. Although our study did not include oligotrophic sites, we found a wide range of dissolved nutrients and the ranges in temperature and light penetration reflected the variation found at high northern latitudes. However, we could not include a variety of abiotic factors, which are possibly very important for grazing effects, such as currents, wind stress, allochthonous material input, and disturbances in general (Steinman 1996), or life-history traits like reproduction in insect larvae (Harrison and Hildrew 1998). Previous studies emphasized that grazer effects are highly density dependent (Hill and Knight 1987; Steinman et al. 1987; Sommer 2000). We found no relation between grazer impact and grazer density, i.e., grazer impact was low in some spring experiments, although grazer densities were high. Since temperature did not significantly influence grazer effects on algal biovolume, other factors must be reducing grazer activity without reducing grazer density. Nonlethal effects of predators are a possibility, since predator presence can alter herbivore behavior and activity and may thus reduce grazing pressure on periphyton (McCollum et al. 1998; Diehl et al. 2000; Turner et al. 2000). We lack proper estimates of predator abundance and type to test this hypothesis. However, our inability to explain changes in grazing effects from single habitat characteristics indicates the importance of complex feedback mechanisms in benthic food webs.

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