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# Effect of grazing and water column nutrient supply on biomass and nutrient content of sediment microalgae

Helmut Hillebrand\*, Maria Kahlert

*Erken Laboratory, Department of Limnology, Evolutionary Biology Centre, University of Uppsala,  
Norr Malma 4200, S 76173 Norrtälje, Sweden*

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## Abstract

Field experiments were conducted to investigate the effects of grazing and nutrient supply on sediment microflora in a freshwater habitat (Lake Erken, Sweden) and at the brackish Baltic Sea coast (Väddö, Sweden). The two sites were of similar productivity, but had contrasting herbivore composition. In a full-factorial experiment design, closed cages excluded macrozoobenthos (>1 mm) from sediment patches, whereas open cages allowed grazer access. The cage design applied here proved to successfully prevent in- and epifauna to access the sediment in closed cages. In half of the treatments, nutrients were added to the water-column by a slow-release fertilizer. The experiments were seasonally replicated four times at Väddö and two times in Lake Erken. After 4–5 weeks, sediment cores were sampled and analyzed for chlorophyll, carbon, nitrogen and phosphorus. The benthic microalgae showed strong seasonal variation in biomass and internal nutrient content. At Väddö, neither grazing nor nutrients affected the algal biomass significantly, but significant grazer effects were detected on C:N:P ratios. In Lake Erken, grazer presence reduced algal biomass by ca. 50%, whereas nutrients were without effect on biomass or on nutrient content. Compared to results from hard substrata at the same sites, sediment microflora was less affected by nutrients and grazing. This may be due to the harsh physico-chemical environment on sediments, to low grazer density at the coastal site and to low availability of water column nutrients to sediment microalgae. In our experiments, sand-dwelling microphytobenthic communities represented a highly dynamic assemblage, which, however, is less structured by biotic interactions than epilithic periphyton. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Nutrients; Grazing; Sediment; Microphytobenthos; Field experiments; Nutrient stoichiometry

\* Corresponding author. Tel.: +46-176-229-070; fax: +46-176-229-315.

E-mail address: helmut.hillebrand@ebc.uu.se (H. Hillebrand).

## 1. Introduction

Sediment microflora is an important contributor to primary productivity in aquatic habitats. In unvegetated areas like intertidal mudflats, the community consisting mainly of diatoms and cyanobacteria is the main source of organic carbon fixation (Cadee, 1980), but even in seagrass meadows, the benthic algae contribute substantially to the autotrophic production (Daehnick et al., 1992; Pinckney and Zingmark, 1993).

However, sediments represent harsh conditions for algal photosynthesis and growth. The substratum is unstable and characterized by strong chemical and physical gradients on small spatial scales. Algal biomass and production on sediments was shown to be highly affected by sand movements (Delgado et al., 1991), grain size (Watermann et al., 1999), current velocity (Berninger and Huettel, 1997) and various physico-chemical gradients such as sulfide, oxygen, temperature (Admiraal, 1984). These factors vary on different temporal and spatial scales and thus, result in distinct temporal and spatial patterns of algal biomass and production (Admiraal, 1984; Underwood and Paterson, 1993; Pinckney et al., 1995; Saburova et al., 1995; Light and Beardall, 1998).

There is less agreement on the importance of competition and grazing on sediments compared to other littoral habitats. Besides the abiotic environment, biotic interactions (competition, grazing, nutrient regeneration) have been found to be important for microalgae colonizing hard substrata in freshwater (Feminella and Hawkins, 1995; McCormick, 1996) and marine habitats (Hillebrand and Sommer, 1997; Hillebrand et al., 2000). For sediments, however, the role of competition was proposed to be minor due to the harsh physical environment and high nutrient concentrations in sediment pore-water (Admiraal, 1984; McIntire and Amspoker, 1986). Still, there has also been experimental evidence of enhanced algal growth in sediments following nutrient addition (Sundbäck and Snoeijs, 1991; Flothmann and Werner, 1992; Pinckney et al., 1995). Grazing has been investigated for both meiofauna and macrozoobenthos. Although, grazing rates by meiofauna and protozoa on sediment microalgae can be high (Blanchard, 1991; Bott and Borchardt, 1999), it is less clear how effective grazing by these small consumers is on a longer time scale allowing numerical responses of prey and grazers (Reise, 1992; Epstein, 1997a,b). Grazing by macroconsumers such as snails (McClatchie et al., 1982), crustaceans (Hargrave, 1970; Gerdol and Hughes, 1994) and annelids (Smith et al., 1996) was shown to have important impact on biomass and community structure of sediment microflora. However, other studies indicated only a minor importance of grazing in the carbon flux of sediment communities (Barranguet et al., 1997). Here, we analyze the impact of nutrient enrichment and macrograzer presence on sediment flora integrated over a period of 4–5 weeks and compare the results to experiments involving epilithic periphyton. We conducted full-factorial field experiments with exclusion of macroconsumers and addition of nutrients in two habitats, a freshwater lake (Lake Erken) and a brackish coastal site (Väddö) in Sweden. The sites were chosen to represent similar habitat productivity but contrasting herbivore fauna. Moreover, at both sites the experiments were placed in shallow bays reducing the impact of physical forces on the algae, since the bays were protected from strong waves or currents and tides are virtually absent from the Baltic coast. We tested the following specific hypotheses:

- ambient grazer densities are able to significantly reduce algal biomass;
- nutrient enrichment increases algal biomass, especially in the absence of grazing;
- algal nutrient stoichiometry reflects the increase of nutrient availability by nutrient enrichment and grazer excretion.

## 2. Methods

### 2.1. Experimental sites

Lake Erken (59°25'N, 18°15'E) covers 23.7 km<sup>2</sup> within a catchment area of 141 km<sup>2</sup>. Mean depth is 9.0 m, pH around 8.0 and mean conductivity 280  $\mu\text{S cm}^{-1}$ . Mean total phosphorus (TP) in the pelagic was 0.94  $\mu\text{mol l}^{-1}$ , mean total nitrogen (TN) 46.1  $\mu\text{mol l}^{-1}$ . The TN:TP ratio, calculated on a molar basis, was 49. Mean phytoplankton biomass was 5.6  $\mu\text{g Chl. } a \text{ l}^{-1}$ . All background data are means of biweekly to monthly measurements from the Lake Erken monitoring program during the experiment period (autumn 1999 to summer 2000). The experiments were placed at the south-eastern shore in a shallow sandy embayment at 50–60 cm water depth.

Väddö (59°56'N, 18°55'E) is an island at the Swedish east coast separated from the mainland by a narrow sound. The southern tip is a restricted military area where the experiments were situated at 50–70 cm water depth in a shallow sandy embayment with salinity around 5.5‰. The habitat productivity is similar compared to Lake Erken, as can be seen from monthly monitoring measurements in nearby Singöfjärden: mean phytoplankton biomass is 7.0  $\mu\text{g Chl. } a \text{ l}^{-1}$ , the mean TP is 0.77  $\mu\text{mol l}^{-1}$  and mean TN is 23.3  $\mu\text{mol l}^{-1}$  resulting in a molar TN:TP ratio around 31.

### 2.2. Experimental setup

The experimental units were cages to exclude macroconsumers from an area of 156 cm<sup>2</sup>. The cages were separated in three parts each (Fig. 1). A basis was constructed from 1 mm thick steel. This basis was pressed into the sediment by the help of four corner pillars. Each side was tightly occluded by metal plates, which did not allow lateral access of burrowing fauna into the inner area of the basis. The basis closed directly at the sediment surface with a flat, horizontal metal frame consisting of 12.5 mm wide steel strips. On top of this, the metal frame of the cage was tightly connected with two screws (Fig. 1). This upper half of the cage consisted of a horizontal frame (matching the size of the basis) and a cubic frame made from 3 mm wide steel strips. From a mesh net with 1 mm mesh size, a hat-like structure was sewn which fitted directly on the metal frame (Fig. 1). Velcro<sup>®</sup> strips were used to tighten the connection. Complete coverage of the cages by the net represented the grazer exclusion treatments (ABSent). In half of the cages, two adjacent sides of the net were cut out to allow meso- and macro-grazers to access the periphyton (PReSent). To estimate the effects of the cages, a control experiment without cages was conducted in the same manner as the caged treatments (CONtrol). These controls allowed grazer access and were, thus, compared to the grazed cages to reveal if a net negative effect of cage presence on algal growth could be detected.

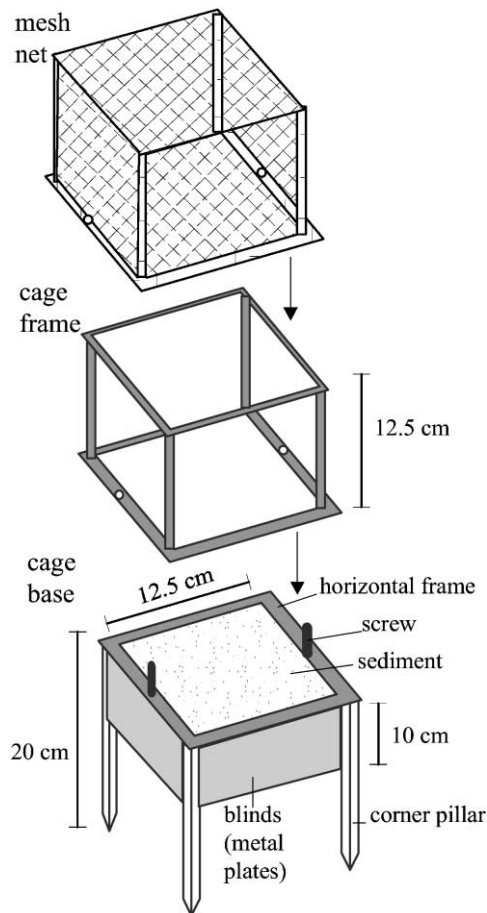


Fig. 1. Design of experimental cage unit. The cage consisted of three parts, the mesh net, a cage frame and a base part. More detailed description in the text.

Nutrients were supplied with a granulose slow-release fertilizer (Agrochem<sup>®</sup>) which adds nitrogen and phosphorus to the water column. The fertilizer was placed in porous plastic boxes 5 cm above the sediment, either adhered to the metal frame of the cages (ABS, PRS) or to a metal pin (CON). The fertilizer enriches the water column continuously for 6 weeks in close dependence on the amount of fertilizer added (Worm et al., 2000). For our experiment, we added 30 g of the fertilizer to half of the cages and the control plots (ENHanced), whereas half of the treatments did not receive additional nutrients (AMBient). Previous investigations showed that the nutrient content in the water column was enhanced for N and P to 2–5-fold of the background level (Hillebrand et al., 2000; Worm et al., 2000; Hillebrand and Kahlert, 2001).

The experiments were conducted with a factorial combination of nutrient enrichment and grazer exclusion. All possible treatment combinations were replicated four-fold, which gave

24 plots for each experiment (three cage types  $\times$  two nutrient treatment  $\times$  four replicates). At Vaddö, we conducted four experiments: autumn (12 Oct–11 Nov 1999, 31 days), early spring (17 Apr–24 May 2000, 38 days), late spring (24 May–19 June 2000, 28 days) and summer (18 July–22 Aug 2000, 36 days). In Lake Erken, we were only able to conduct experiments in autumn 1999 and summer 2000, which were run at the same time as at Vaddö.

At the start of the experiment, the base parts of the cages were pressed into the sediment as described above. The macro-grazers were removed from the experimental plots by taking out the sediment within the base of the cages, sieving it through a 1 mm mesh and pouring it back into the base. This represented a strong disturbance for the algae growing on the sediment, but was necessary to ensure ungrazed conditions. It, furthermore, resembled a naturally occurring turbation disturbance and was equal for all treatments. The porous plastic boxes containing the nutrients were added to all ENH-treatments as described above. Finally, the mesh was put on top of the cage and connected to the Velcro<sup>®</sup> strips. During the course of the experiments, the meshes were carefully brushed to remove algae settling on the net.

Once during each experiment, the abundance of macro-fauna was estimated by taking sediment samples in the vicinity of the experiments with an Ekman Grab (256 cm<sup>2</sup>). Five separate samples were taken at each sampling day and sieved in situ by a sieve with 1 mm mesh size. The organisms were picked up in the laboratory and preserved in 70% ethanol. The organisms were identified to the lowest possible taxonomic level and counted, their abundance calculated to 1 m<sup>2</sup>. For the experiments in autumn 1999, only qualitative data on macrozoobenthos were obtained.

### 2.3. Sampling and analysis

At the end of each experiment, the meshes were removed and we took two cores per treatment with a cut-off syringe (diameter 2.7 cm, depth 4 cm). The two samples were pooled to avoid biased results caused by horizontal heterogeneous distribution of algae within the cages. The samples were immediately transferred to the laboratory. There, we processed the samples to remove sand grains. Whereas other studies used ultrasonication to remove algae from sand grains (Sundbäck and Snoeijs, 1991), we found that this method had strong effects on algal nutrient content (Kahlert, unpublished data). Instead, we suspended the sample in filtered water from the respective sampling site (0.2  $\mu$ m filter-pore size) and stirred the sample thoroughly, waited until most sand grains settled down and decanted the overlying suspension. This was done several times until sand grains were removed. In previous tests, this method was shown to give chlorophyll yields as good as the ultrasonication method, but gave higher particulate P and N yields (Kahlert, unpublished data).

Different sub-samples were taken: (1) an aliquot was filtered on pre-combusted GF/C filters for the analysis of organic carbon (C) and nitrogen (N), (2) an aliquot was filtered on pre-combusted GF/C filters for the analysis of organic phosphorus (P), (3) an aliquot was filtered on GF/C filters for the analysis of Chlorophyll *a* (Chl. *a*), (4) an aliquot was preserved with Lugol's iodine for checking dominant algal groups microscopically. Filters were stored frozen until analysis. Organic P was measured as phosphate after hydrolysis with heating and potassium persulfate (Grasshoff et al., 1983), C and N were measured, simultaneously with a CHN analyzer (LECO CHN-932). Chl. *a* was measured after 24 h acetone extraction and trichromatic measurement controlling for Chlorophyll *b* and *c*, respectively (Strickland

and Parsons, 1972). We are aware that this method is inferior compared to the HPLC-based measurement of pigments in sediments (Wiltshire et al., 1998). To estimate the error introduced by the presence of chlorophyll degradation products in the sample, we checked two out of six experiments for living algal biovolume. At least 400 cells were counted under an inverted microscope ( $400\times$  magnification) and the abundances were transferred to biovolume following the recommendations by Hillebrand et al. (1999). We found a significant correlation between Chl. *a* and algal biovolume ( $r = 0.69$ ,  $N = 46$ ,  $P < 0.001$ ) and are, thus, confident that algal biomass was well reflected by the measurement of Chl. *a*. Moreover, we used organic C as additional estimate of total biomass.

#### 2.4. Statistics

Three-factor analysis of variance (ANOVA) was used to test for significant effects of the manipulations on algal biomass (Chl. *a*) and total biomass (organic C), respectively. The impact of season ( $N = 4$  and  $2$ , for Vaddö and Erken, respectively), grazer presence ( $N = 2$ , ABS and PRS) and nutrient enrichment ( $N = 2$ , AMB and ENH) was tested with a separate analysis for each site. The impact of cages was estimated with an analogous design, where the factor grazer absence was replaced by cage presence ( $N = 2$ , PRS & CON). This design avoided a test between ABS and CON, since these are differentiated by more than one manipulation (Hillebrand et al., 2000). Log-transformed variables were used throughout to assure homogeneity of variances and normal distribution of the dependent variable. Besides calculating the significance levels for our treatments, we calculated the magnitude of the effects using  $\omega^2$  (Winer, 1971).

Three-factor multivariate analysis of variance (MANOVA) was used to test for significant effects on cellular nutrient stoichiometry. Here, the molar ratios representing stoichiometry (C:N, C:P and N:P) were interdependent and had to be tested as multivariate dependent variable. The combination of factors used was as described for the univariate analyses. We used the Pillai's trace statistic, which is recommended for the test of significant effects on multivariate response variables (Scheiner, 1993). Homogeneity of variances was tested with the non-parametric Sen-Puri test.

The stoichiometry of algal nutrient content was also used to indicate nutrient limitation within the sediment flora. Following the recommendations given by Hillebrand and Sommer (1999), N-limitation is characterized by high C:N ratios ( $C:N > 10$ ) in combination with low N:P ratios ( $N:P < 13$ ), whereas P-limitation is characterized by high N:P and high C:P ratios ( $N:P > 22$ ,  $C:P > 180$ ). A one-tailed *t*-test was used to test if algal nutrient values significantly exceeded the indication threshold values. It should be noted that threshold values were established for epilithic microalgae and several factors may interfere with the measurement of C:N:P in sediments. The results should thus be interpreted with care.

### 3. Results

Benthic invertebrate composition differed strongly between the two sites. The fauna in Lake Erken was dominated by insect larvae (Diptera, Trichoptera, Ephemeroptera), whereas at Vaddö, annelid and crustacean grazers were important together with benthic filtration

Table 1

Zoobenthos abundances in grazing experiment in Lake Erken and at Vaddö<sup>a</sup>

	Lake Erken	Vaddö		
	Summer	Early spring	Late spring	Summer
<i>Macoma baltica</i> (Bivalvia)	–	898 (189)	1398 (118)	1078 (132)
<i>Hydrobia</i> sp. (Gastropoda)	273 (139)	547 (28)	1000 (64)	625 (72)
<i>Potamopyrgus jenkinsi</i> (Gastropoda)	31 (15)	86 (31)	–	–
<i>Bithynia tentaculata</i> (Gastropoda)	47 (29)	–	–	–
<i>Gyraulus</i> sp. (Gastropoda)	47 (11)	–	–	–
<i>Valvata pulchella</i> (Gastropoda)	8 (8)	–	–	–
<i>Idotea baltica</i> (Isopoda)	–	31 (31)	–	–
<i>Corophium volutator</i> (Amphipoda)	–	8 (8)	–	–
<i>Mysis mixta</i> (Mysidacea)	–	86 (48)	–	–
Chironomidae (Diptera)	188 (57)	–	–	–
Molanidae and Psychomyidae (Trichoptera)	156 (28)	–	–	–
Caenidae (Ephemeroptera)	211 (78)	–	–	–
<i>Nereis diversicolor</i> (Annelida)	–	844 (130)	422 (78)	102 (34)

<sup>a</sup> Mean abundances (ind. m<sup>-2</sup>) are given with standard errors (in brackets) for experiments conducted in 2000.

feeders (Bivalvia) (Table 1). Only the dominance of hydrobiid snails among the gastropods was similar between both sites, but with higher abundances at Vaddö. The seasonal difference between the experiments at Vaddö was marginal, with the exception of the declining abundance of the annelid *Nereis* during the year (Table 1). The qualitative data obtained in autumn 1999 suggest a composition of the invertebrate fauna similar to the summer data at both sites.

Cyanobacteria and diatoms clearly dominated the algal community at both sites, but green and red filamentous algae were also present. At Vaddö, especially filamentous cyanobacteria (*Oscillatoria* sp.) were important. The main diatoms were the motile *Petroneis humerosa*, *Bacillaria paradoxa* and several *Navicula* species together with attached *Fragilaria* species. In Lake Erken, mobile (*Epithemia*, *Encyonema*, *Navicula*) and araphid diatoms (*Fragilaria*, *Cyclotella*) dominated the community.

Algal chlorophyll and organic C were highly correlated in both habitats (Fig. 2). The correlation on log-transformed variables was significant ( $r = 0.862$ ,  $N = 127$ ,  $P < 0.001$ ). The log–log equation showed that the C:Chl. ratio on weight basis was  $\sim 100$  and the slope only slightly below 1 (Fig. 2).

At Vaddö, slight grazing effects on biomass (Chl. *a*) could be seen in autumn 1999 and summer 2000, mainly at ambient nutrient concentrations (Fig. 3). However, the grazer effect and the interaction between grazing, nutrients and seasons were non-significant throughout (Table 2). There was a significant difference in Chl. *a* concentration and organic C between seasons, with low biomass in autumn and increasingly higher biomass in spring and summer (Fig. 3). Neither nutrient enrichment (Table 2) nor the presence of cages (ANOVA,  $P > 0.15$ ) had significant effects on Chl. *a* or organic C.

The C:N:P ratios measured in the experiments at Vaddö in autumn and early spring (Fig. 4) were low and similar to optimal ratios indicating replete N and P supply. But C:P ratios increased in late spring and were above the indication level for P-limitation in summer,

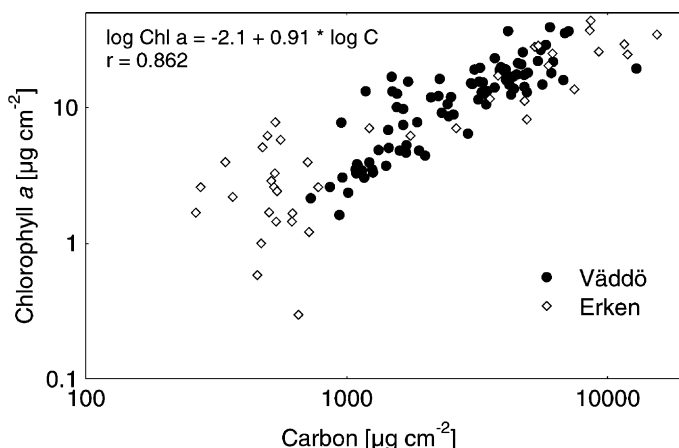


Fig. 2. Correlation between organic C and Chl. *a* in sediment samples from experiments at Vaddö and in Lake Erken. Correlation coefficient and linear equation are given for log-transformed data.

although, the difference was non-significant (one-sided *t*-test,  $N = 19$ , C:P:  $t = -1.64$ ,  $P = 0.055$ ; N:P:  $t = -1.414$ ,  $P = 0.087$ ). This increase in P-deficiency compared to C was clearly reflected by the significant impact of season in the MANOVA on C:N:P ratios (Table 2). Whereas an increased supply of nutrients did not affect the nutrient stoichiometry of the algae, there was a significant impact of grazing (Table 2). The main effects of grazing on the stoichiometry were decreased N content in late spring (mean C:N: ungrazed: 9.2, grazed: 10.4) as well as increased phosphorous content in grazed (mean C:P = 146.4) versus ungrazed (mean C:P = 156.3) treatments in autumn. In both spring experiments, phosphorus content decreased with grazer presence, i.e. mean C:P ratios increased from 180 to 206.1 (early spring) and 181 to 265 (late spring). The presence of cages did not affect the nutrient ratios (MANOVA,  $P > 0.25$ ).

Table 2

Effect of grazer, nutrients and season on sediment microflora at Vaddö<sup>a</sup>

Effect of	Chl. <i>a</i> <i>F</i> -ratio ( <i>P</i> -level) (a)	Organic C <i>F</i> -ratio ( <i>P</i> -level) (b)	C:N:P ratios (c)	
			Pillai's Trace	<i>F</i> -ratio ( <i>P</i> -level)
Season	70.78 (<0.001)	29.51 (<0.001)	1.21	9.18 (<0.001)
Grazer	1.01 (0.321)	1.26 (0.267)	0.25	4.38 (0.009)
Nutrients	0.90 (0.348)	0.06 (0.816)	0.04	0.49 (0.690)
Season × grazer	0.85 (0.472)	1.40 (0.255)	0.33	1.68 (0.100)
Season × nutrients	1.35 (0.271)	0.01 (0.999)	0.09	0.45 (0.908)
Grazer × nutrients	0.26 (0.610)	0.23 (0.639)	0.03	0.42 (0.739)
Season × grazer × nutrients	2.16 (0.105)	2.37 (0.084)	0.13	0.59 (0.799)

<sup>a</sup> The table gives *F*-ratios and significance levels of three-factor ANOVA on algal chlorophyll (*a*), and on organic C (*b*). For C:N:P ratio, a three-factor MANOVA was conducted and Pillai's Trace Statistic, *F*-ratio and *P*-level are given (*c*). Chl. *a* and organic C were log-transformed to achieve homogeneity of variances.



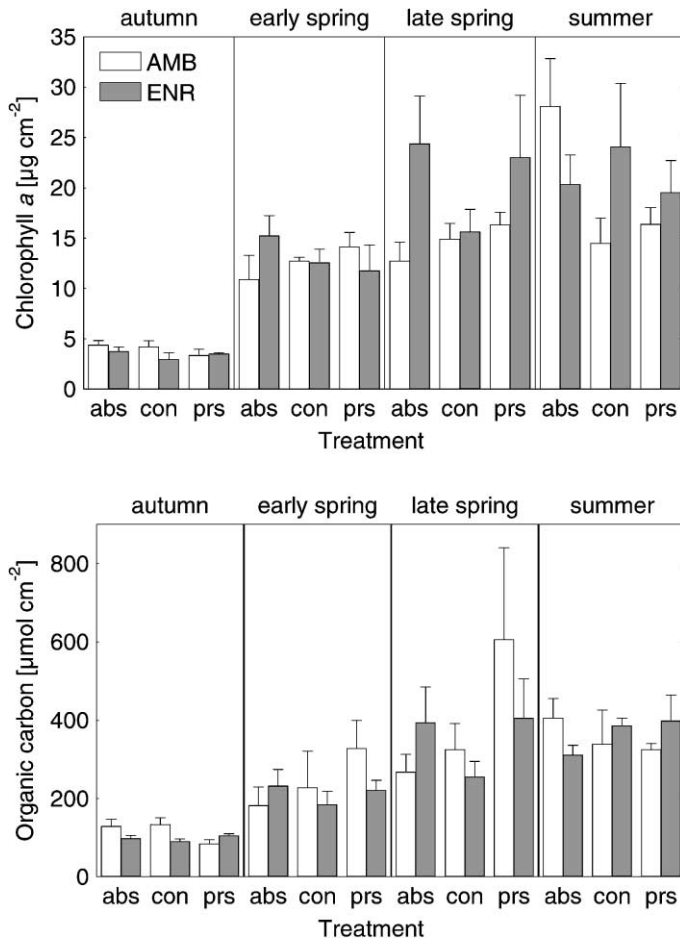


Fig. 3. Chlorophyll *a* (upper panel: mean  $\pm$  S.E.) and organic C (lower panel: mean  $\pm$  S.E.) in sediment samples from experiments at Vaddö. Results are given for experiments conducted in different seasons (autumn 1999, early spring, late spring and summer 2000) for each of three cage treatments (ABS: grazers absent, closed cages; CON: grazers present, cage absent; PRS: grazers present, open cages) and for two nutrient treatments (AMB and ENR).

In Lake Erken, the Chl. *a* and organic C concentrations in the sediment were significantly higher in the summer experiment than in autumn (Fig. 5, Table 3). There was also a significant reduction of biomass due to grazer presence in both experiments (Table 3). Grazer presence led to a reduction of the algal biomass to as low as 50% compared to ungrazed treatments. Neither nutrients (Table 3) nor the presence of cages (ANOVA,  $P = 0.222$ ) did affect the Chl. *a* concentration in the experiments in Lake Erken. For organic C, however, there was a significant grazer  $\times$  nutrient  $\times$  season interaction (Table 3), indicating a slight increase in C with nutrient enrichment in absence of grazer in summer and a slight decrease in presence of grazer (Fig. 5).

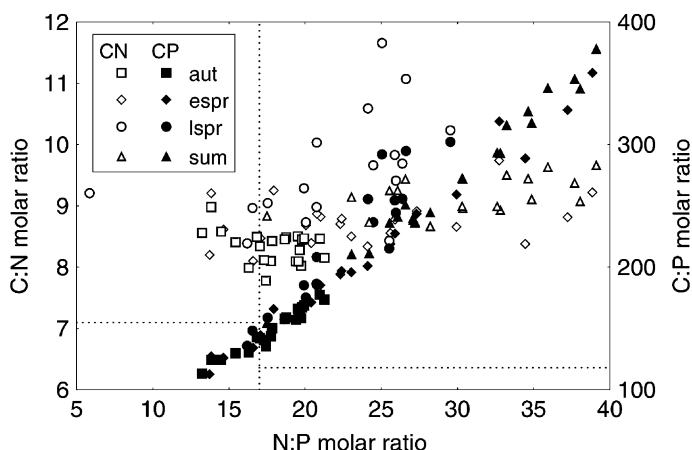


Fig. 4. Stoichiometry of particulate nutrients in sediment samples from experiments at Vaddö. The diagram presents C:N (white) and C:P (black) molar ratios depending on N:P ratios for experiments conducted at different seasons (autumn 1999, early spring, late spring and summer 2000). Dotted lines represent optimal ratios of N:P = 17 (vertical), C:N = 7.1 (horizontal to the left) and C:P = 119 (horizontal to the right) found in nutrient replete benthic microalgae. The graph allows to determine nutrient limitation of benthic microalgae *sensu* Hillebrand and Sommer, 1999. High C:N in combination with low N:P ratios indicate N-limitation (upper left quadrant), whereas high C:P in combination with high N:P ratios indicate P-limitation.

In autumn, the C:N:P ratios indicated high P-supply, since ratios of C:P and N:P were low (Fig. 6). In summer, both C:P and C:N ratios increased, but only for C:N they were significantly higher than the threshold values for indication of N-limitation (one-sided *t*-test,  $N = 19$ ,  $t = -1.88$ ,  $P = 0.038$ ). Accordingly, the C:N:P ratios were significantly different between seasons, but there was no significant effect of grazing or nutrients on the nutrient stoichiometry (Table 3). There was neither a significant effects of cages (MANOVA,  $P = 0.314$ ).

Table 3  
Effect of grazer, nutrients and season on sediment microflora in Lake Erken<sup>a</sup>

Effect of	Chl. <i>a</i> <i>F</i> -ratio ( <i>P</i> -level) (a)	C <i>F</i> -ratio ( <i>P</i> -level) (b)	C:N:P ratios (c)	
			Pillai's Trace	<i>F</i> -ratio ( <i>P</i> -level)
Season	126.87 (<0.001)	447.32 (<0.001)	0.72	17.42 (<0.001)
Grazer	21.61 (<0.001)	4.52 (0.046)	0.14	1.04 (0.395)
Nutrients	0.46 (0.503)	0.89 (0.356)	0.06	0.41 (0.749)
Season × grazer	2.96 (0.100)	7.10 (0.014)	0.20	1.65 (0.209)
Season × nutrients	0.85 (0.368)	2.32 (0.143)	0.10	0.73 (0.545)
Grazer × nutrients	1.48 (0.237)	2.01 (0.171)	0.04	0.26 (0.854)
Season × grazer × nutrients	0.55 (0.466)	6.88 (0.016)	0.10	0.75 (0.536)

<sup>a</sup> The table gives *F*-ratios and significance levels of three-factor ANOVA on algal chlorophyll (*a*) and on organic C (*b*). For C:N:P ratio, a three-factor MANOVA was conducted and Pillai's Trace Statistic, *F*-ratio and *P*-level are given (*c*). Chl. *a* and organic C were log-transformed to achieve homogeneity of variances.

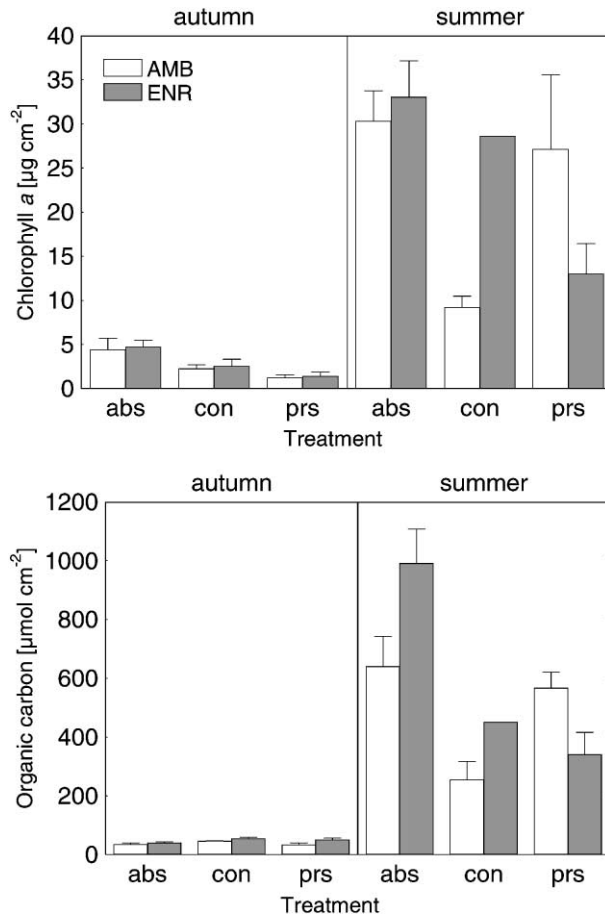


Fig. 5. Algal chlorophyll *a* (upper panel: mean  $\pm$  S.E.) and organic C (lower panel: mean  $\pm$  S.E.) in sediment samples from experiments in Lake Erken. For explanations, see Figure 2.

At both sites, the magnitude of effects on biomass were highest for season. More than 70% of the variance was based on differences between seasons, both for Vaddö ( $\omega^2 = 76.3\%$ ) and Lake Erken ( $\omega^2 = 71.3\%$ ). The grazer influence was comparably minor, but reached some significance in Lake Erken ( $\omega^2 = 11.7\%$ ).

#### 4. Discussion

Our results showed that macrograzers on sediments were able to reduce algal biomass, but to a lesser extent compared to removal rates known from experiments on hard substrata. At Vaddö, the grazing impact was marginal, whereas it was significant at Lake Erken. Grazer presence changed also the C:N:P ratios of benthic samples at Vaddö. Nutrient enrichment

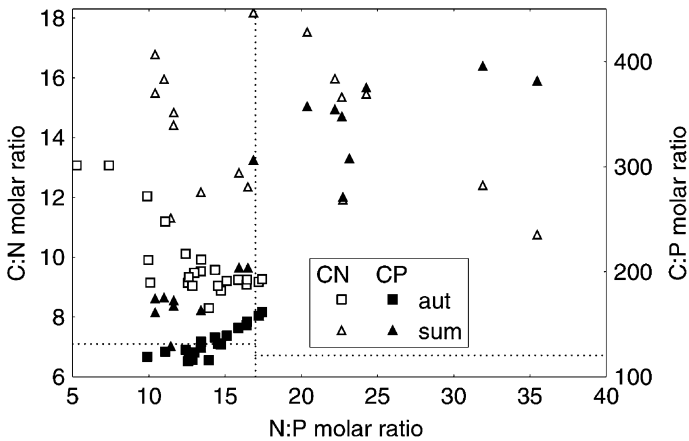


Fig. 6. Stoichiometry of particulate nutrients in sediment samples from experiments in Lake Erken. For details, see Fig. 3.

of the water column had no significant effects on Chl. *a* or nutrient content and only a weak effect on organic C in Lake Erken. This was contrasted by the nutrient stoichiometry in unmanipulated samples, which indicated possible nutrient limitation in summer.

The exclusion of grazers with closed cages was successful and we did not find macroconsumers in the sampled cores. There was also no indication of increased sedimentation in the cages. We found either no significant cage effect for any of the measured variables, except for a weak effect on organic C in Lake Erken. The reduction in flow and light introduced by the mesh nets were obviously of minor importance. However, it should be noted that control treatments do not allow finding significant effects if positive and negative impacts of cage presence canceling each other out.

Nevertheless, we regard the type of cages used as highly suitable for sediment studies, since they allow the exclusion of lateral burrowing fauna and epibenthic macrofauna without using toxic substances. Previous investigations in inter-tidal areas used insecticides to remove grazers (Gerdol and Hughes, 1994; Smith et al., 1996), which may have additional effects than those on the target organisms. An additional advantage of our cage system is the potential use on constantly water-covered sediments. However, more problems with sedimentation may be encountered if wave action and resuspension are more important than in our protected study sites.

The range of Chl. *a* values in our experiments (Fig. 2) was high compared to other studies involving sediment flora, where rarely up to  $25 \mu\text{g cm}^{-2}$  were reported (Daehnick et al., 1992; Hansson, 1992; de Jonge and Colijn, 1994; Barranguet et al., 1997; Hagerthey and Kerfoot, 1998; Lucas and Holligan, 1999). The sediment microflora recovered probably well from the initial disturbance at the start of the experiment and our stirring treatment yielded high biomass values. We are not able to comment on the impact of chlorophyll degradation products on our estimates of algal biomass, but microscopical investigations showed a high percentage of living cells and a good correlation between biovolume and chlorophyll. We are, thus, confident that our results at least allow discussing the differences in algal biomass between seasons, grazer and nutrient treatments.

Although our experiments revealed some impact of grazing on sediment microalgae (both biomass and nutrient ratios), these effects were low compared to experiments conducted on hard substrata, where up to 90% of the available biomass was consumed by herbivores (Feminella and Hawkins, 1995; Hillebrand et al., 2000). We found strong grazing pressure on epilithic benthic algae in experiments conducted at the same sites (Väddö and Lake Erken) and at the same time (Hillebrand and Kahlert, 2001). The difference is especially obvious for the experiments conducted at Väddö, where the absence of significant grazer effects on sediments was contrasted by strong grazer impacts on epilithic periphyton (Hillebrand and Kahlert, 2001). Studies in inter-tidal areas reported strong effects of macroconsumers on microphytobenthos (McClatchie et al., 1982; Gerdol and Hughes, 1994; Smith et al., 1996).

The low grazing pressure revealed at Väddö could have several reasons. The density of hydrobiid snails was low in our experiments (Table 1) compared to reports from intertidal areas, where these species can reach densities up to  $50,000\text{ m}^{-2}$  (Morrisey, 1988; Blanchard et al., 2000). Also other grazers such as *Corophium*, which has been shown to be especially efficient (Morrisey, 1988), were nearly absent in our experiments. The low salinity at Väddö may reduce fecundity and activity of invertebrates from marine origin, such as snails and annelids. There was a quite abundant population of bivalves, but the role of these suspension and deposit feeders is not clear. Page et al. (1992) described a significant reduction of sediment micro-flora due to the presence of the bivalve *Macoma nasuta*, a species congeneric to *M. baltica* found in our experiments. Besides feeding directly on the algae, this effect may also be due to bioturbation and burying of algae during the feeding process of *Macoma*. On the other hand, positive effects of *Macoma baltica* have been found and related to ventilation and fertilization of the sediment (Reise, 1983). The benthic fauna in Lake Erken was present in lower overall animal abundances, but we found a high proportion of insect larvae. Especially trichopteran larvae are regarded as efficient consumers of benthic algae (Feminella and Hawkins, 1995). This may explain the more prominent grazing pressure in Lake Erken compared to Väddö.

Another possible reason reducing the grazing impact of macroconsumers could be a strong grazing impact of small fauna (nematodes, ostracods, copepods, protozoans). These micro and meiofauna had access to both, closed and open cages, and could possibly affect the algal biomass more than macrograzers. We do not have sufficient data to estimate the impact of small fauna, but it has been proposed that micro and meiofauna are able to consume most of the primary production in sediments (Sundbäck et al., 1996; Epstein, 1997a,b). Cattaneo and Kalff (1986) excluded larger grazers on artificial plants and found that smaller fauna (oligochaetes, cladocerans) replaced these macrograzers, resulting in similar total grazer biomass in controls and exclosures. The algal biomass was, consequently, only to a minor degree affected by grazer exclosure in their experiment. However, other studies indicated a low quantitative influence of meiofauna on longer time scales (Admiraal et al., 1983; Montagna, 1984; Reise, 1992). Most studies in sediments measured instantaneous grazing rate (Blanchard, 1991; Bott and Borchardt, 1999), but from these studies it was not clear if the small fauna can efficiently control algal biomass on time scales of several weeks.

Nutrient enrichment of the water column was almost without effect on benthic algal biomass or nutrient content. This may reflect either sufficient nutrient conditions for the algae or the unavailability of water column nutrients to the benthic algae in the time span of our experiments. It has been suggested that the nutrient pool in sediment poor water

should prevent nutrient limitation for the sediment microflora (Admiraal, 1984), but other studies have shown that nutrient addition had a positive effect on algal growth (Nilsson et al., 1991; Sundbäck and Snoeijs, 1991). Furthermore, the nutrient stoichiometry in the summer experiments indicated deficient supply of N and P (Figs. 4 and 6). High C:P and N:P ratios suggested P-limitation at Vaddö, whereas high C:N with high C:P ratios in Lake Erken indicated co-limitation by N and P. However, the transfer of indicator values from epilithon (Hillebrand and Sommer, 1999) to sediment micro-flora has to be done cautiously due to possibly high amounts of non-algal C in sediments. In Lake Erken, both C:P and C:N increased in summer, which may reflect input of additional C instead of deficient N and P. At Vaddö, however, C:N was not raised, whereas C:P and N:P were higher in summer compared to other seasons. Thus, there was no general increase in C but rather a relative reduction in P. With all caution with respect to interfering factors, these results might indicate that nutrient limitation is possible on these sediments. On the other hand, the supply of nutrients to the overlying water column may be responsible for the weak nutrient effects. In previous experiments, benthic microflora profited from nutrients directly supplied from the sediment, either by pore-water nutrient enrichment (Flothmann and Werner, 1992) or by ground-water inflow (Hagerthey and Kerfoot, 1998). Also for epilithic periphyton, substrate-based nutrient supply was highly effective (Hillebrand and Sommer, 1997). Accordingly, water column enrichment affected phytoplankton and periphyton on hard substrates, but not sediment-dwelling algae (Blumenshine et al., 1997; Vadeboncoeur and Lodge, 2000). Nutrients in the water column may, thus, be of limited availability to microphytobenthos.

Variation in Chl. *a* and organic C was much larger between seasons than between grazed and ungrazed or between ENH- and AMB-treatments. This became especially evident by calculating magnitudes of effects, showing that more than 70% of the variance in Chl. *a* was related to seasonal differences. There was a tendency of high summer and low autumn biomass, and intermediate biomass in spring (Vaddö). Besides biomass, also nutrient stoichiometry was different between seasons, whereas treatment effects were weak or absent. Seasonal patterns of microphytobenthic biomass were shown to be pronounced, often with spring peaks and summer biomass minima, but with high variation between patterns at different sites (Underwood and Paterson, 1993; de Jonge and Colijn, 1994; Pinckney et al., 1995). The differences in seasonal biomass variation may, additionally, be interfered by the physico-chemical variability of the environment on short temporal and spatial scales.

In conclusion, a suitable cage design to assess macrozoobenthic grazing on sediment microflora was presented. At both study sites, the sediment communities were only weakly structured by nutrient supply and macrozoobenthic consumption, but were highly variable between seasons. Grazing had an impact on sediment microflora only in Lake Erken, whereas water column nutrient enrichment had no significant effects. For the two sites investigated here, other factors seem to be more important regarding the biomass variation in microphytobenthos.

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