Journal Title: Botanica marina.

Vol: 34 **Iss:** 4

Month/Year: 1991

Pages: 341-358

Article Author: SNOEIJS, P

Article Title: Effects of Nutrient Enrichment on Microalgal Community Composition in a Coastal Shallow-water Sediment System: An Experimental Study

Imprint: Berlin, New York de Gruyter.

If there are any quality control issues with this document please contact us as soon as possible.

ILLiad@wwu.edu

Date Received:

Pages are: Missing Cut Off Other
(Please circle the appropriate description) top bott left right

Page Numbers:

Additional Information:

Botanica Marina
Vol. 34, pp. 341–358, 1991

Effects of Nutrient Enrichment on Microalgal Community Composition in a Coastal Shallow-water Sediment System: An Experimental Study

K. Sundbäck and P. Snoeijs*

Department of Marine Botany, University of Göteborg, Carl Skottsbergs Gata 22, S-41319 Göteborg, Sweden
** Department of Ecological Botany, Uppsala University, Box 559, S-75122 Uppsala, Sweden*

(Accepted 7 April 1991

Abstract

An outdoor flow-through experimental set-up, was used to study the effect of increased concentrations of inorganic nitrogen (IN) and phosphorus (IP) on the microalgae of a sandy sediment. The compositional changes following nutrient addition were investigated in terms of the dominance of major taxonomic groups as well as the species composition of diatoms. Fresh samples were used to assess the viable biomass of major taxonomic and size groups, and cleaned diatom frustules were used for identification to species level. Nutrient addition to the water column stimulated the growth of filamentous cyanobacteria, diatoms and flagellates. Although statistically significant compositional changes with respect to both time and treatment were observed, the microflora was not subjected to any substantial shift in the dominance of major taxonomic groups. Instead, the major change occurred at the macroscopic level as a rapid growth of a filamentous green algal species. Diatoms which accounted for *ca* 50% of the microalgal biomass, were not outcompeted by any other algal group, probably because the Si-pool of the sediment counteracted secondary Si-limitation and because temperature was too low to allow cyanobacteria to fully dominate the biomass. Counts of both living cells and cleaned frustules showed that the diatom flora was dominated by cells in the size group 7–12 µm, however, depending on the counting method and the level of resolution, different compositional changes were noted. Biomass values of size groups of living diatom cells showed that N + P addition favoured larger cells. Frustule counts, which enable identification at the species level, revealed also that some small-sized species were enhanced by the nutrient addition. Canonical correspondence analysis (CCA) based on frustule counts, showed that the succession in both non-enriched and enriched containers was in the same direction, though faster and proceeding further with N + P addition. The CCA revealed that the P + N addition also favoured some small-sized taxa, such as *Nitzschia* and *Amphora* species within the size range 7–12 µm. Oxygen bubbles produced by the stimulated photosynthetic activity at the sediment surface appeared to selectively resuspend large motile diatom species, *e.g. Cylindrotheca.*

Introduction

During the last decade, eutrophication in coastal areas has worried scientists, the general public and local authorities in many countries, including Sweden. One of the symptoms of excessive eutrophication is an increased frequency of algal blooms, that may cause bottom hypoxia (Rosenberg *et al.* 1990). Oxygen deficiency does not only threaten sublittoral areas, but also shallow areas in sheltered bays. Such areas may be increasingly subjected to hypoxia because of the increasing accumulation of allochthonous organic matter such as drifting and decomposing macroalgae and detritus (Sundbäck *et al.* 1990). These symptoms, together with the fact that shallow-water sediment areas are important breeding and foraging areas for macrofauna and many commercial fish species, have drawn our attention to the impact of an increased nutrient load on the food webs of shallow-water sediment communities.

Cultural eutrophication of lakes has caused shifts in the dominance of the major taxonomic groups of phytoplankton, which in turn has induced associated changes in the biomass and composition of other trophic levels (Tilman *et al.* 1982). Parallel to these findings, the question has been raised whether an increasing eutrophication in the marine environment, implying changed ratios of nutrient supply, is currently changing the basis for the pelagic food web from a diatom based system towards a less edible flagellate system (Doering *et al.* 1989). In addition, investigations of nutrient effects on fresh-water periphyton have revealed shifts in the dominance of major taxonomic algal groups in benthic communities (Carrick *et al.* 1988). Less is known about the response of the sediment-associated microflora of shallow marine and brackish-water environments to the increasing load of nitrogen and phosphorus, especially in areas lacking regular tidal flushing of the habitat.

In temperate areas diatoms are important constituents of the microscopic primary producers in shallow-water sediments. Investigations from fresh-water benthic habitats have shown that periphyton diatoms respond to altered concentrations and ratios of inorganic nutrients by changed community composition (Pringle and Bowers 1984, Carrick and Lowe 1988). In the marine environment corresponding changes have been observed for salt-marsh diatom communities (Sullivan 1976, 1981).

This investigation is part of an experimental study, designed to investigate the effect of increased load of inorganic nitrogen and phosphorus on the structure and function of a marine shallow-water sandy sediment system on the west coast of Sweden (Nilsson *et al.* 1991). Since one of the main objectives of the experiment was to investigate the impact of elevated nutrient concentrations on the pathways of carbon between microscopic primary producers and consumers by measuring grazing rates using radioactive labels, we wanted to study in more detail the compositional changes of the microalgae of the sediment system. The changes were studied in terms of:

(1) possible shifts in the dominance of major taxonomic groups and

(2) possible shifts in the species composition of one of the dominant groups of microalgae, the diatoms.

Material and Methods

The experimental set-up

The effect of increased input of inorganic nitrogen (IN) and phosphorus (IP) in the water column above a sediment community, was studied using an outdoor flow-through system (Sundbäck *et al.* 1990) consisting of eight 30-litre containers placed in a floating frame at the Tjärnö Marine Biological Laboratory on the west coast of Sweden (N 58°52′, E 11°09′). This coastal area, in NE Skagerrak, is brackish and virtually non-tidal. Sandy sediment from a nearby shallow bay (0.2 m water depth) was collected in two layers that were treated separately: surface sediment layer (upper 1 cm) and lower sediment layer (1 to 10 cm depth). The sand was sieved (mesh size 0.5 mm) to remove macrofauna, homogenized and spread out in two layers (2 cm of surface sediment on top of a 8 cm thick bottom layer) in each experimental container (area 0.1 m^2). Surface seawater was filtered through 50 µm and 1 µm cotton-filter cartridges, and pumped from two 100-litre cisterns through the experimental containers using a multichannel peristaltic pump. The inflowing water entered the experimental containers 1 cm above the sediment surface and the flow-rate was 1.6 L/h corresponding to a turn-over time of 12 h. The height of the water column above the sediment in the containers was 18 cm. To allow the vertical chemocline in the sediment to stabilize, the containers were allowed to equilibrate for 10 days before the experiment was started.

From the third day of the experiment, the water in one of the two cisterns was enriched with approximately 85 µmol L^{-1} IN and 2.5 µmol L^{-1} IP, using $NaNO_3$ and K_2HPO_4, respectively, thus simulating nutrient levels and N/P ratios found in shallow coastal areas influenced by river or sewage-plant effluents (Granéli and Sundbäck 1985, Granéli *et al.* 1990). Four containers received this enriched water, corresponding to a mean nutrient input of 27.5 mmol IN and 0.74 mmol IP m^{-2} day^{-1}. Four other containers receiving non-enriched water from the other cistern served as controls. The approximate nutrient concentrations of the non-enriched water were: < 0.2 µM PO_4^{3-}, < 1 µM $NO_3^- + NO_2^-$, between 0.35 and 8.5 µM NH_4^+ and between 2.6 and 7.5 µM $Si(OH)_4$. The experiment was run for 4 weeks (12 August to 9 September 1988). The inner walls of the containers were kept clean from algal growth by carefully wiping the surfaces every 3–4 days with clean cloths rinsed in distilled water. Water temperature varied between 16.1 and 18.9 °C and salinity between 14 and 24‰ during the experiment.

Sampling

Sediment cores were taken with transparent PVC tubes designed for radiotracer measurements of primary and bacterial production and meiofaunal grazing rates in undisturbed sediment (*i.d.* 67.8 mm,

length 150 mm; Jönsson in press). Samples were taken on days 1, 7, 14, 21 and 28. At each sampling, one core was taken from each container. The holes in the sediment surface in the containers caused by the core-sampling were filled with autoclaved sediment to prevent leakage of sulfide and other solutes from the anoxic layers. Subsamples for counts of living microalgae as well as diatom frustules were taken with a cut-off 2 mL plastic syringe (*i.d.* 9 mm) from the cores after the radiotracer measurements. Concentrations of NH_4^+, $NO_3^- + NO_2^-$, PO_4^{3-} and $Si(OH)_4$ in the inflowing and the overlying water of each experimental container was analysed as described in Nilsson *et al.* (1991).

Counts and biomass of living microalgae

For each sample used for counts of living microflora, 2 subsamples (*i.d.* 9 mm) from the top 5 mm were pooled. The samples were diluted to 6 mL with filtered seawater and treated in a sonication bath (Sonorex RK 100, 35 kHz) for 9 minutes. This time was selected by plotting number of living (= fluorescing) cells against sonication time. This treatment detached most of the epipsammic cells, but apparently did not break them, since motile cells could be seen in sonicated samples. The autotrophic cells were counted in a Bürker counting chamber using epifluorescence microscopy. Cells were grouped into size and shape classes, and then identified when possible to generic level. Generally cell dimensions of a minimum of 30 cells per size group were measured (for less frequent size groups, a minimum of 15 cells) and average cell volume and carbon content for each group were calculated using geometric formulae according to Edler (1977, 1979); the factor to convert cell volume to carbon varied between 0.085 and 0.11 (diatoms 0.085–0.089). Living cells were counted on four occasions (Days 1, 7, 14 and 28) in samples taken from all 8 containers (4 control and 4 enriched containers). To enable comparison between viable cells and cleaned frustule counts (see below), the absolute numbers of viable cells were converted to relative abundance values (RA, %) by size and shape group.

Analysis of cleaned diatom material

Permanent slides of diatoms were made from the same samples as those used for enumeration of the living microflora. Material from 6 containers (3 control and 3 enriched) were used. The sediment samples were centrifuged for 30 minutes at 1500–2000 rpm. Thereafter 0.5 mL H_2O_2 was added together with a pinch of potassium bichromate to destroy organic material and to detach the diatoms from the sand grains. The samples were washed and centrifuged three times with 10 mL double distilled water. Subsequently, the samples were shaken with 3 mL double distilled water, the sand grains were allowed to sink for 5–10 s and the diatom suspension was collected using a pipette; this procedure was repeated 6 times. Microscopic investigation confirmed that this treatment removed all diatom frustules from the sand grains. One drop of the diatom suspension was put on a cover slip that had been cleaned with 96% ethanol and left to dry in the air. Hyrax (refractive index 1.71) was used as the mounting medium. Diatom frustules were identified, counted and categorized into size groups by phase contrast microscopy using a 100 times oil immersion objective. Five hundred valves were counted along a transect across the middle of the drop. In total, 30 samples were analysed.

Scanning electron microscopy (SEM) was performed on a JEOL JSM-35 Scanning microscope at the Unit for Biological Structure Analysis (Department of Zoology, Uppsala University).

Species were identified using literature cited in Snoeijs (1989) and in addition, Brockmann (1950), Giffen (1966), Williams and Round (1987) and Lange-Bertalot and Krammer (1989). Nomenclature was updated according to the check-list of Hartley (1986) except for those taxa that were identified with the help of newer literature.

Data analysis of frustule counts

Community diversity and dominance were measured by species richness, the Shannon-Weaver index (log base = e) and Pielou's evenness index (Whittaker 1977, Patil and Tailllie 1979, Maarel 1989). The two quantitative indices were calculated from relative abundances.

Constrained ordination (Braak and Prentice 1988) by canonical correspondence analysis (CAA) implemented with the program CANOCO (Braak 1986), was applied to summarize variation in species composition in relation to time and environment. CANOCQ gives a simultaneous ordination with sample and species scores, vectors indicating the direction and rates of change for quantitative predictor variables, and centroids for qualitative predictor variables (class variables). Eigenvalues measure the importance of successive ordination axes. Predictor variables can be used 'actively' (as constraints) in the ordination, or can be added 'passively' later. In both cases, CANOCO performs a multiple regression analysis to indicate which of the variables have significant effects on each axis, though the significance test is rigorous

only for passive variables that were not used in the construction of the ordination. In the analyses class variables for date-treatment combinations were used as constraints. Nutrient concentrations (NO_3 + NO_2, NH_4, PO_4 and $Si(OH)_4$), number of days the experiment had been running (1, 7, 14, 21 and 28 days) and Shannon-Weaver diversity were used as passive quantitative variables. Two CCA analyses were made, one for all 101 identified taxa, and another for clusters of genera.

Statistical analyses

Data on biomass, number of living algae and relative abundances of frustule counts were tested with Taylor's power law (Green 1979) and were transformed by square root or the natural logarithm when necessary. Differences between dates and treatments were tested using Newman-Keuls multiple comparison test (NK) (Zar 1974) with differences accepted as significant when $p < 0.05$.

Results

Visual observations

Four days after the start of the treatment, the sediment surface in the enriched containers was more brownish in colour than in the control containers. In all 8 containers, oxygen bubbles were formed on the sediment surface during daytime. In the enriched containers, flakes of the sediment surface were lifted off by the rising oxygen bubbles towards the end of the experiment. On approximately Day 12, tufts of the filamentous green alga *Enteromorpha clathrata* (Roth) Greville were observed on the sediment in the enriched containers and by Day 28, the filaments started to float up to the water surface.

The relative abundances of different diatom taxa under each treatment are given in Table I.

Living autotrophic microflora

Biomass of major algal groups. The biomass obtained from cell counts of living autotrophic microalgae in the top 5 mm sediment was significantly stimulated by the nutrient addition (Fig. 1). After 2 weeks the microalgal biomass had increased from 113 to 455 mg C m^{-2} in the enriched containers and from 123 to 138 mg C m^{-2} in the control containers. The increase was significantly (NK) higher in the enriched containers. Although there was a trend that the microalgal biomass decreased in the enriched containers towards the end of the experiment, it was still twice as high as in the controls on Day 28 (349 as against 181 mg C m^{-2}).

Initially, diatoms accounted for 47% (= 56 mg C m^{-2}), cyanophytes for 42–47%, and various flagellates for 6–8% of the total living microalgal biomass. When considering the total biomass accounted for by organisms < 0.5 mm (= microflora + bacteria + meiofauna; ciliates and heterotrophic flagellates were not enumerated, but probably constituted a minor proportion; *cf.* Sundbäck *et al.* 1990), diatoms constituted 20% at the start and 13–15% at the end of the experiment. During the first week, the biomass of filamentous cyanobacteria (mainly *Oscillatoria* spp.) increased in both the control and enriched containers. Later their biomass decreased in the control containers and consequently the mean proportion of diatoms increased towards the end of the experiment, comprising on an average 55% on Day 28. In the nutrient-enriched containers, the increase of the biomass was due to both small-sized diatoms and filamentous cyanobacteria, and the relative proportions between these two main algal groups did not change during the course of the experiment. The final increase of the diatom biomass was 2.5-fold in the enriched containers. The proportion of flagellate biomass, in particular those within the size range 4–8 µm, increased significantly (NK) in the enriched containers, though they only comprised 15% of the total living microalgal biomass (Fig. 1).

Cell number of diatom size groups. The initial number of viable diatom cells was 7.5 × 10^5 cm^{-2} and the highest value was reached in enriched containers after

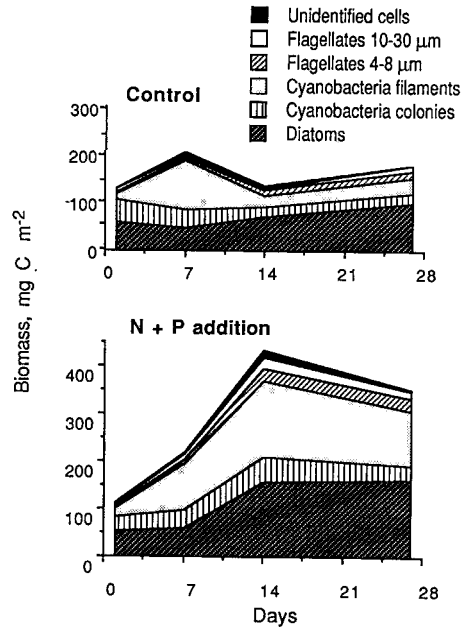

Fig. 1. Cumulative biomass of the major groups of living benthic microalgae in the top 5 mm of sediment in flow-through containers without (control) and with the addition of inorganic phosphorus and nitrogen to the overlying water (N + P addition). The curves show mean values of 4 replicate containers.

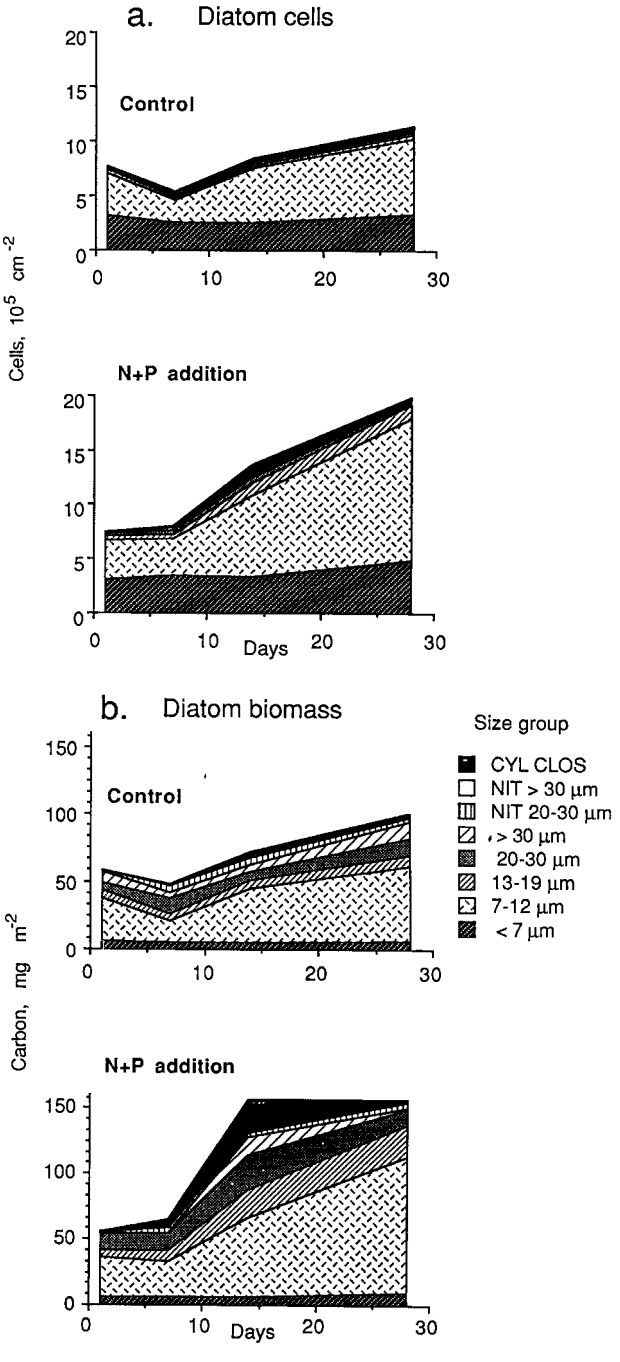

Fig. 2. Cumulative cell number (a) and biomass (b) of size groups of living diatoms in the top 5 mm of sediment. All diatoms are pennate forms; for size groups > 20 µm the genera *Nitzschia* (NIT) and *Cylindrotheca* (CYL) are shown separately. The curves show mean values of 4 replicate containers.

4 weeks (20×10^5 cells cm^{-2}) (Fig. 2a). Pennate taxa within the size group 7–12 µm accounted for most of the increase in enriched containers as well as in control containers, whereas the number of the smallest size group (< 7 µm) was unchanged (Fig. 2a). Cell numbers of the size group 13–19 µm increased significantly (NK) in enriched containers but not in control containers, and were significantly more abundant in enriched containers after 2 weeks (NK). The number of *Cylindrotheca closterium*, one of the few viable taxa identified at the species level, was significantly higher (NK) in the enriched containers after one week (Fig. 2a).

Biomass of diatom size groups. In terms of biomass (Fig. 2b), the smallest size group (< 7 µm) decreased and the larger size groups (> 12 µm) increased, but still the size group 7–12 µm dominated and accounted for the main biomass increase in enriched containers. The size group 13–19 µm increased significantly in enriched containers. This effect was more pronounced in terms of biomass (Fig. 2b) than in terms of cell numbers (Fig. 2a). In the enriched containers, the proportion of *Cylindrotheca closterium* increased from less than 1% to 18% of the total diatom biomass from Day 1 to Day 14 (significant increase with regard to both time and treatment). Furthermore, this diatom was significantly more abundant in enriched than in control containers (NK). At the end of the experiment, however, the biomass of diatom cells > 20 µm decreased in the nutrient enriched containers.

Relative abundance of viable cells and cleaned diatom frustules

Although the comparison of living cell counts and frustule counts was done on size-group level, the *Cylindrotheca closterium* and the genus *Nitzschia* in the size group > 20 µm were distinguished from the rest. On the whole, the proportions of the individual size groups agreed for the two counting techniques (Fig. 3), however, only the frustule counts revealed the increased RA of *Nitzschia* cells within the size range 7–12 µm. This increase was significantly (NK) greater in the enriched containers. The increase of *C. closterium* in the middle of the experiment was slightly less in the frustule counts as compared with the viable counts (Fig. 3).

Assuming that the RA values of frustules reflect RA values in the living diatom community (*cf.* Fig. 3), the results from the two counting methods were combined to illustrate quantitative changes of the 6 principal diatom genera (but including Fragilariaceae in one group) during the experiment (Fig. 4). At the end of the experiment all genera, except for *Cocconeis*, had increased their cell numbers in the control containers (= 'natural succession'); adding the nutrients resulted in more cells of all genera, largely following the pattern of 'natural succession' (Fig. 4a). Relative increases (= % increase from Day 1 to Day 28) (Fig. 4b) show that *Nitzschia* (+ 1300%) and *Amphora* (+ 450%) were the genera that profited most from the nutrient addition.

Table I. List of diatom taxa with mean relative abundance scores per treatment for each date, abbreviation codes and size groups. Separate size groups were used for *Nitzschia* (N) and *Cylindrotheca closterium* (CYL CLOS).

Diatom taxon	Code	Control					Nutrient load					Size group (µm)
		Date 0812	0818	0825	0901	0908	Date 0812	0818	0825	0901	0908	
Achnanthes bahusiensis (Grunow) Lange-Bertalot	ACH BAHU	2.8	1.5	1.5	4.6	1.4	1.5	2.0	1.7	3.2	1.6	13–19
Achnanthes delicatula (Kützing) Grunow	ACH DELI	0.3	0.5	0.3	0.1	0.1	0.9	0.1	0.1	0.2	0.5	13–19
Achnanthes cf. *delicatula* (Kützing) Grunow	ACH CFDE	10.4	9.2	8.0	4.7	5.5	9.5	8.6	6.9	5.0	6.2	7–12
Achnanthes cf. *delicatissima* Simonsen	ACH DECA	14.7	15.6	15.5	11.5	14.5	20.0	17.2	16.1	10.4	10.0	< 7
Achnanthes lemmermannii Hustedt	ACH LEMM	5.4	3.9	4.8	2.1	3.0	3.5	4.4	2.7	2.5	2.1	7–12
Achnanthes minutissima Kützing	ACH MINU	–	0.1	–	0.1	–	0.1	–	–	–	–	13–19
Achnanthes sp.	ACH SP	2.1	4.1	4.4	4.6	6.1	5.0	4.9	4.3	4.5	3.8	7–12
Amphora coffeaeformis (C. A. Agardh) Kützing	AMP COFF	0.5	1.1	0.5	1.3	0.7	0.6	0.5	0.7	0.8	0.9	20–30
Amphora cf. *coffeaeformis* (C. A. Agardh) Kützing	AMP CFCO	0.3	0.2	0.1	0.3	0.2	0.3	–	0.3	0.5	0.5	13–19
Amphora commutata Grunow	AMP COMM	–	0.1	–	–	–	0.1	–	–	–	–	>30
Amphora flebilis Simonsen	AMP FLEB	0.7	0.7	0.2	1.4	1.3	0.5	0.7	0.9	0.8	1.0	13–19
Amphora holsatica Hustedt	AMP HOLS	0.1	0.3	0.2	–	0.1	0.3	0.1	0.2	0.5	0.2	>30
Amphora laevis v. *laevissima* (Gregory) Cleve	AMP LAEV	–	–	–	0.1	–	–	–	0.1	–	–	>30
Amphora lineolata Ehrenberg	AMP LINE	–	–	–	–	–	–	–	0.1	–	–	>30
Amphora ostrearia de Brébisson	AMP OSTR	0.1	0.1	0.5	–	0.2	0.2	0.1	–	–	–	>30
Amphora ovalis (Kützing) Kützing	AMP OVAL	0.1	–	–	–	–	–	–	–	–	–	>30
Amphora staurophora Juhlin-Dannfeldt	AMP STAU	0.4	0.5	0.5	0.8	–	0.1	0.4	0.5	0.3	0.4	7–12
Amphora tenerrima Aleem *et* Hustedt	AMP TENE	0.7	0.9	0.5	1.2	0.7	0.5	0.8	0.5	1.1	1.2	7–12
Amphora veneta Kützing	AMP VENE	–	–	–	–	–	0.3	–	–	–	–	13–19
Amphora sp. 1	AMP SP 1	1.9	4.3	5.1	10.3	6.9	3.1	5.7	7.5	11.2	8.1	7–12
Amphora sp. 2	AMP SP 2	0.1	–	0.1	–	–	–	0.1	0.1	–	–	>30
Amphora sp. 3	AMP SP 3	–	–	–	–	–	0.1	–	–	–	–	>30
Amphora sp. 4	AMP SP 4	0.6	0.1	0.1	0.1	–	–	0.1	–	0.3	0.3	7–12
Anaulus balticus Simonsen	ANA BALT	–	0.1	–	–	0.1	–	0.1	–	–	–	7–12
Auliscus sculptus (W. Smith) Ralfs	AUL SCUL	0.1	–	–	–	–	–	–	0.1	–	–	>30
Berkeleya rutilans (Trentepohl) Grunow	BER RUTI	–	–	–	0.3	0.2	–	–	–	–	0.1	20–30
Catenula adhaerens (Mereschkowsky) Mereschkowsky	CAT ADHA	–	–	–	–	–	0.1	–	–	–	–	7–12
Chaetoceros cyst	CHA CYST	–	0.1	–	–	–	–	–	–	–	–	13–19
Cocconeis cf. *diminuta* Pantocsek	DOC DIMI	0.4	0.5	0.3	0.6	0.1	0.1	0.8	0.3	–	0.1	7–12
Cocconeis peltoides Hustedt	COC PELT	3.5	2.5	2.0	0.8	1.0	2.5	2.9	1.9	0.9	1.5	13–19
Cocconeis cf. *peltoides* Hustedt	COC CFPE	–	0.1	0.1	–	–	0.3	–	0.1	–	–	7–12
Cocconeis placentula Ehrenberg	COC PLAC	0.1	–	–	–	–	–	–	–	–	–	20–30
Cocconeis scutellum Ehrenberg	COC SCUT	–	–	–	–	0.1	–	–	–	0.1	–	20–30
Cocconeis stauroneiformis (W. Smith) Okuno	COC STAU	0.1	–	–	–	–	–	–	–	–	–	20–30
Cocconeis sp.	COC SP	0.1	–	0.1	–	0.1	–	0.2	–	–	–	7–12
Cyclotella atomus Hustedt	CYC ATOM	–	0.3	–	0.1	–	–	–	0.1	–	0.1	< 7
Cyclotella caspia Grunow	CYC CASP	0.1	0.3	0.1	–	–	0.2	–	–	–	0.1	< 7
Cylindrotheca closterium (Ehrenberg) Reimann *et* Lewin	CYL CLOS	0.1	1.3	1.8	2.5	0.3	0.3	1.2	3.7	0.9	0.3	CYL CLOS
Diatoma tenue C. A. Agardh	DIA TENU	0.1	–	–	–	–	–	–	–	–	–	13–19
Dimerogramma minor (Gregory) Ralfs	DIM MINO	0.3	0.7	0.5	0.1	0.3	0.3	0.6	0.1	0.1	0.1	20–30
Diploneis finnica (Ehrenberg) Cleve	DIP FINN	–	–	–	0.1	–	–	–	–	–	–	>30
Entomoneis paludosa v. *duplex* (Donkin) Czarnecki *et* Reinke	ENT PALU	–	0.1	–	–	0.5	0.1	–	0.2	0.3	0.1	20–30
Fragilaria vaucheriae (Kützing) Petersen	FRA VAUC	–	–	–	–	–	0.1	–	–	–	–	7–12
Fragilariaceae sp. 1	FRE SP 1	5.3	3.3	2.6	2.1	2.2	3.8	3.8	2.2	2.9	2.9	< 7
Fragilariaceae sp. 2	FRE SP 2	3.1	2.5	3.9	3.3	4.5	5.1	1.9	1.4	2.5	2.3	< 7
Fragilariaceae sp. 3	FRE SP 3	1.8	1.0	0.7	1.7	0.9	1.7	0.9	0.1	1.1	0.7	7–12
Fragilariaceae sp. 4	FRE SP 4	–	0.1	–	–	–	0.7	0.1	0.1	–	0.3	20–30

Table 1. Continued.

Fragilariaceae sp. 5	FRE SP 5	–	0.1	0.1	–	–	–	–	–	–	–	7–12
Fragilariaceae (rest group)	FRE SPP	0.1	0.1	0.5	0.5	0.3	0.1	0.1	–	0.2	0.1	7–12
Gomphonemopsis exigua (Kützing) Medlin	GOM EXIG	–	0.1	–	–	–	–	–	–	–	–	7–12
Hantzschia marina (Donkin) Grunow	HAN MARI	–	–	–	–	0.1	–	–	–	–	–	>30
Navicula abscondita Hustedt	NAV ABSC	0.3	0.4	0.7	0.5	0.7	0.3	1.1	0.5	0.7	0.1	13–19
Navicula amphipleuroides Hustedt	NAV AMPH	0.1	0.1	–	0.1	0.1	–	0.1	–	0.1	0.3	7–12
Navicula arenaria Donkin	NAV AREN	0.1	–	–	0.3	–	–	0.2	0.1	0.1	0.2	>30
Navicula atlantica (A. Schmidt) H. *et* M. Peragallo	NAV ATLA	–	–	–	0.1	–	0.1	–	0.1	–	–	>30
Navicula cf. *biskanteri* Hustedt	NAV BISK	2.5	2.3	3.1	0.8	1.8	2.3	2.5	1.9	0.4	2.4	7–12
Navicula cincta (Ehrenberg) Ralfs	NAV CINC	–	–	0.1	–	0.1	–	–	–	–	–	13–19
Navicula clamans Hustedt	NAV CLAM	0.2	0.1	–	–	0.3	0.3	–	0.1	–	0.1	13–19
Navicula cryptolyra Brockmann	NAV CRYP	14.7	12.1	9.5	6.9	11.6	13.4	8.1	7.5	5.8	7.4	7–12
Navicula digitoradiata (Gregory) Ralfs	NAV DIGI	–	–	–	0.1	–	–	–	–	–	–	>30
Navicula cf. *flanatica* Grunow	NAV FLAN	–	–	–	–	–	–	–	0.1	–	–	>30
Navicula forcipata Greville	NAV FORC	0.5	0.1	0.3	0.1	0.7	0.4	0.3	0.2	0.4	0.3	20–30
Navicula gregaria Donkin	NAV GREG	0.6	0.7	0.5	0.5	1.5	1.0	1.1	0.3	0.3	0.9	20–30
Navicula halophila (Grunow) Cleve	NAV HALO	–	–	–	0.5	–	–	–	–	–	–	13–19
Navicula heterovalvata Simonsen	NAV HETE	–	–	–	0.2	–	–	–	–	–	–	13–19
Navicula humerosa de Brébisson	NAV HUME	0.1	–	–	–	–	–	–	–	0.1	–	>30
Navicula cf. *jaernefeltii* Hustedt	NAV JAER	0.1	0.1	–	–	–	0.1	0.1	0.3	–	–	13–19
Navicula lanceolata (C. A. Agardh) Ehrenberg	NAV LANC	–	0.1	–	–	0.1	–	–	–	–	0.1	>30
Navicula cf. *perminuta* Grunow	NAV PERM	1.7	2.2	2.9	3.1	1.7	1.2	0.4	1.3	1.7	1.7	7–12
Navicula phyllepta Kützing	NAV PHYL	0.1	1.5	0.6	0.3	0.4	0.4	0.5	0.1	0.3	0.4	7–12
Navicula pygmaea Kützing	NAV PYGM	–	–	–	–	0.4	–	–	–	0.3	0.1	>30
Navicula rhynchocephala Kützing	NAV RHYN	–	–	–	–	–	–	–	–	0.1	–	>30
Navicula salinarum Grunow	NAV SANA	–	–	0.1	–	0.1	–	0.3	–	–	0.3	13–19
Navicula salinicola Hustedt	NAV SANI	2.3	1.1	1.2	0.7	0.9	1.2	0.9	0.5	1.1	1.4	7–12
Navicula subapiculata (Grunow) Hustedt	NAV SUBA	0.1	0.1	0.1	0.3	–	0.3	0.1	0.1	0.1	0.3	20–30
Navicula subinflata Grunow	NAV SUBI	0.3	0.5	0.4	0.2	0.1	0.6	0.6	0.3	0.2	0.3	>30
Navicula wuestii Simonsen	NAV WUES	–	–	0.2	–	–	–	0.3	0.1	–	0.1	7–12
Navicula sp. 1	NAV SP 1	12.1	9.1	8.3	7.9	5.9	8.6	8.9	8.3	7.0	6.9	<7
Navicula sp. 2	NAV SP 2	–	–	–	–	0.1	–	–	–	–	–	13–19
Navicula sp. 3	NAV SP 3	–	–	–	0.1	–	–	–	–	–	–	7–12
Nitzschia aurariae Cholnoky	NIT AURA	1.9	3.9	6.6	11.1	10.6	1.4	6.7	13.9	20.3	21.2	N 7–12
Nitzschia cf. *aurariae* Cholnoky	NIT CFAU	3.0	4.1	6.9	4.6	7.6	3.5	5.2	9.9	8.3	7.4	N 7–12
Nitzschia dissipata (Kützing) Grunow	NIT DISS	0.4	0.5	0.3	0.3	0.5	0.2	0.3	0.1	–	0.4	N 20–30
Nitzschia frustulum (Kützing) Grunow	NIT FRUS	–	–	0.7	–	–	–	–	–	–	–	N 7–12
Nitzschia cf. *linearis* (C. A. Agardh) W. Smith	NIT LINE	–	0.1	–	–	–	–	–	–	–	–	N >30
Nitzschia littorea Grunow	NIT LITT	0.1	–	–	–	0.1	–	0.1	–	–	–	N >30
Nitzschia paleacea (Grunow) Grunow	NIT PALE	0.1	1.0	0.7	3.4	1.1	0.5	1.1	0.6	0.3	–	N 20–30
Nitzschia cf. *spathula* de Brébisson	NIT SPAT	–	0.1	–	0.1	0.1	0.1	–	–	–	–	N >30
Nitzschia thermaloides Hustedt	NIT THER	–	–	0.1	–	–	–	–	0.1	0.1	–	N >30
Opephora olsenii M. Møller	OPE OLSE	0.1	0.1	–	–	–	0.1	0.4	–	–	–	13–19
Opephora sp. 1	OPE SP 1	–	–	–	–	–	0.1	0.1	–	–	–	13–19
Opephora sp. 2	OPE SP 2	0.1	0.1	–	–	–	–	0.1	–	0.1	–	7–12
Pinnularia ambigua Cleve	PIN AMBI	0.1	0.1	0.5	0.1	0.4	0.1	0.1	–	–	–	>30
Pinnularia rectangulata (Gregory) Rabenhorst	PIN RECT	0.1	0.3	–	–	0.3	0.1	–	–	0.1	0.1	>30
Plagiogramma staurophorum (Gregory) Heiberg	PLA STAU	–	–	–	–	0.1	0.1	0.5	–	0.1	0.4	20–30
Pseudostaurosira sp.	PSE SP	2.1	2.4	0.7	0.9	1.1	1.5	1.5	0.3	1.1	1.2	7–12
Rhoicosphenia abbreviata (C. A. Agardh) Lange-Bertalot	RHO ABBR	–	–	–	–	0.1	–	–	–	–	–	>30
Stauroneis salina W. Smith	STA SALI	–	0.3	0.1	0.3	–	0.1	–	0.1	0.5	0.2	>30
Stauroneis tackei (Hustedt) Krammer *et* Lange-Bertalot	STA TACK	–	–	0.3	0.5	–	–	–	–	–	0.1	13–19
Striatella delicatula (Kützing) Grunow	STR DELI	0.1	–	–	0.5	–	–	–	0.1	–	–	13–19
Tropidoneis lepidoptera (Gregory) Cleve	TRO LEPI	–	0.1	0.1	–	0.1	–	–	–	–	–	>30

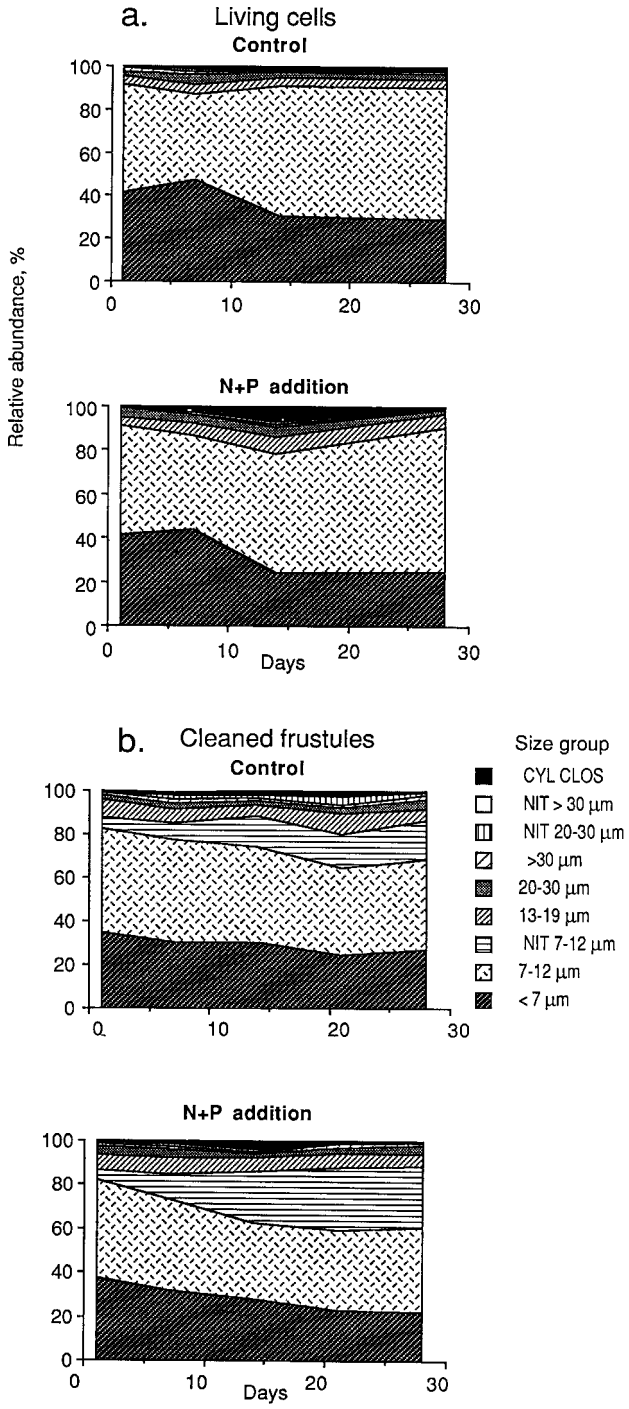

Fig. 3. Relative abundance of size groups of living cells (a) and frustules (b) of diatoms in the top 5 mm of sediment. All diatoms are pennate forms; the genera *Nitzschia* (NIT) and *Cylindrotheca* (CYL) are shown separately. Curves show mean values of 4 replicate containers for living diatoms and 3 replicate containers for frustules.

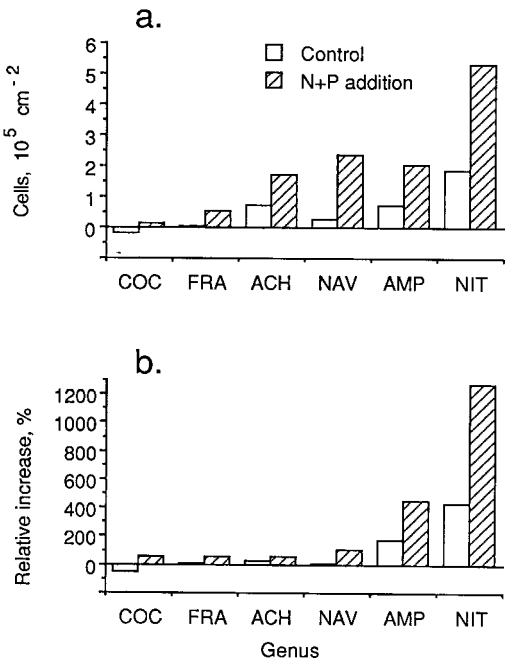

Fig. 4. Absolute (a) and relative increase (b) of the 6 principal diatom genera in the top 5 mm of sediment during the 4-week experiment (differences between first and last sampling date). Absolute cell number = relative abundance of frustules * total number of living cells of a genus. All values are means of 3 replicate containers. COC = *Cocconeis*, FRA = Fragilariaceae; ACH = *Achnanthes*, NAV = *Navicula*, AMP = *Amphora*; NIT = *Nitzschia*.

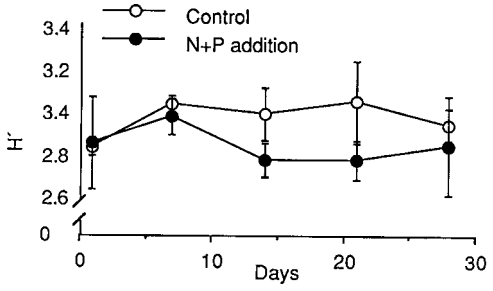

Fig. 5. Shannon-Weaver diversity (H') based on relative abundance of diatom frustules in the top 5 mm of sediment. Curves show means of 3 replicate treatments ± SD.

Diversity and dominance

The diatom flora consisted mainly of small-sized epipsammic and epipelic species; 86% of the cells counted were less than 12 µm long. The dominating taxa (mean RA > 5%) were *Achnanthes* cf. *delicatissima* (mean RA 15%), *Navicula cryptolyra* (mean RA 10%), *Nitzschia aurariae* (mean RA 10%), *Navicula* sp. 1 (mean RA 8%), *Achnanthes* cf. *delicatula* (mean RA 8%), *Amphora* sp. 1 (mean RA 6%) and *Nitzschia* cf. *aurariae* (mean RA 6%) (Table I).

Shannon-Weaver diversity (H') varied between 2.6 and 3.3 and depended about equally on species richness, ($r = 0.83$, $p < 0.001$) and evenness ($r = 0.77$, $p < 0.001$). After one week the H'-values were generally lower for the nutrient enriched containers, but this trend was not statistically significant (NK, $p > 0.05$) (Fig. 5).

CCA of cleaned diatom material

The data set yielded one dominant CCA axis (Fig. 6) with eigenvalue 0.10 (compared with 0.03, 0.02, and 0.02 for axes 2, 3 and 4, respectively). Nitrate + nitrite and phosphate concentrations, number of days and the Shannon-Weaver index gave significant ($p < 0.05$) coefficients in multiple regression analysis of ordination axis 1 on the environmental variables, whereas Si and NH_4 concentrations were not significant on any of the axes.

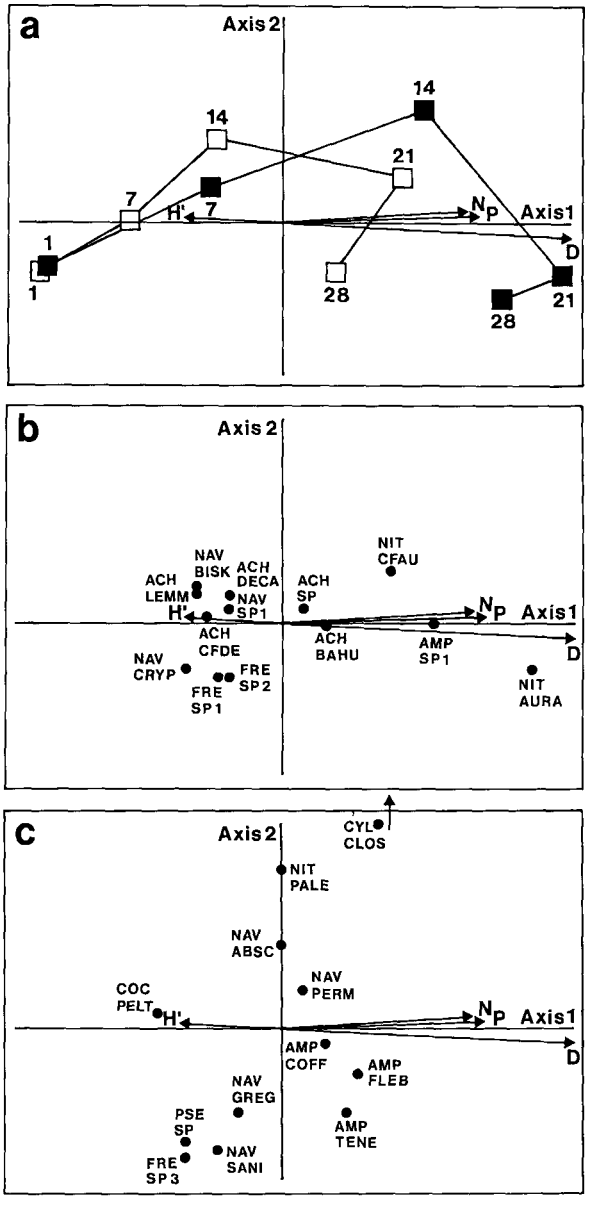

Fig. 6. CCA based on relative abundance (RA) of 101 diatom taxa with respect to number of days and treatment. (a) Ordination showing controids for day-treatment combinations (white squares = control, black squares = N + P addition). The arrows show the direction of variation in time (D for days), NO_3 + NO_2 concentration (N), PO_4 concentration (P) and Shannon-Weaver diversity (H'). (b) Taxon ordination for the 13 taxa with mean RA > 2.0%. (c) taxon ordination for the 12 taxa with mean RA 0.5–2.0%. For abbreviation of taxon names see Table I.

The squares in Figure 6a are centroids of samples taken from 3 replicate containers. They depict the changes in species composition with time in the control and the enriched containers. For Day 1, the centroids for both controls and enriched containers are very close to each other in the left part of the ordination. After nutrient addition, succession was faster in the enriched containers than in the controls, with the direction of the succession being from left to right along the main axis for both (Axis 1). In the last week of the experiment (between Days 21 and 28) succession was reversed for both control and enriched containers.

The vectors (shown by arrows) for environmental variables and community diversity (Fig. 6) are related to the correlations between each variable and Axis 1; they point in the direction of maximum increase of the variables. Those for the added nutrients (N and P) and number of days (D) all point to the right of the ordination in the direction of the succession, whereas the vector for diversity (H') points in the opposite direction.

The taxon scores (Fig. 6b–c) summarize the distribution of the principal diatom taxa with respect to time and nutrient addition. Species succession during the experiment is shown from left to right. The taxa that were most favoured by nutrient addition, notably *Nitzschia aurariae* (Fig. 6b), are located in the far-right end of the ordination. No taxa with mean RA > 0.5% are found in the far-left part, thus indicating that no abundant diatom species were negatively affected by nutrient addition.

A separate CCA on the genus level (but including Fragilariaceae in one group) also produced one main axis of variation (Axis 1, eigenvalue = 0.07) (Fig. 7).

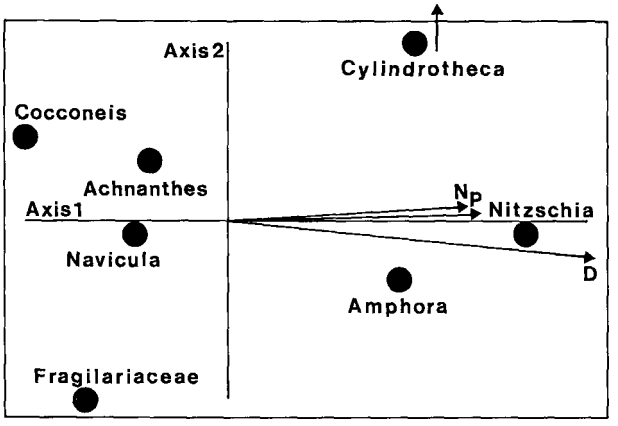

Fig. 7. Taxon ordination from CCA with respect to number of days and treatment. The scores of the seven dominant genera are indicated. The arrows show the direction of variation in time (D), NO_3 + NO_2 concentration (N) and PO_4 concentration (P).

Subsequent axes were of very little importance (eigenvalues < 0.01). The concentration of added nutrients (N and P) and number of days were significant in the ordination whereas Si and NH_4 concentrations were not. The pattern of the centroids (not shown) was similar to Fig. 6a. The taxon scores for the 7 dominating generic groups are shown: *Achnanthes* (mean RA 33%), *Navicula* (26%), *Nitzschia* (17%), *Amphora* (10%), Fragilariaceae including *Fragilaria*, *Pseudostaurosira* and *Staurosira* species (9%), *Cocconeis* (2%) and *Cylindrotheca* (1%). The successional pattern shows that the genera *Amphora*, *Nitzschia* and *Cylindrotheca* were favoured both with regard to time and P + N enrichment.

Notes on taxonomy

Most of the dominant diatom taxa encountered in this study were difficult to identify to species level even at a magnification of x1000 under oil immersion because of their small size and lack of visible structure on the valves. These are probably also the reasons why there are no or few references in the taxonomical literature about these species. Therefore we found it necessary to include some notes on taxonomy and scanning electron micrographs of the dominant taxa.

Achnanthes cf. *delicatula* (Kützing) Grunow (Fig. 8a–c)
Cells are broadly elliptical, sometimes with subrostrate ends, 6–10 × 4–5 μm, and with 10–14 striae in 10 μm on both valves. Sometimes on the rapheless valve there is a larger distance between the middle striae on one side of the valve (Fig. 8b–c). The hyaline area on the rapheless valve can be wider or narrower. The specimen in Figure 8c is similar to *Achnanthes* sp. of Kuylenstierna (1989; plate 35, fig. 349). This taxon is easy to distinguish from *A. delicatula* (Kützing) Grunow *sensu* Lange-Bertalot and Ruppel (1980). It may also be possible that several species are involved.

Achnanthes cf. *delicatissima* Simonsen (Fig. 8d)
Cells are elliptical with subrostrate ends, 6–8 × 3–4 μm, and no striae are visible in the light microscope (LM). Using SEM, the species was found to have 33–36 striae in 10 μm consisting of double rows of pores on the rapheless valve. This taxon appears to be very hyaline and is difficult to study not only by LM but also by SEM. The transmission electron micrograph in plate 36, figs 352–353 of Kuylenstierna (1989), shows an unidentified *Achnanthes* species which most likely is the same taxon and may be conspecific with *A. delicatissima* Simonsen.

Achnanthes sp.
Cells are narrowly elliptical, 6–9 × 3 μm, and with 16–18 striae in 10 μm on both valves. On the rapheless valve there is a larger distance between the middle striae on one side of the valve.

Amphora sp. 1 (Fig. 8e–g)
Valves are 8–15 × 2–3 μm, with no striae visible when observed by LM. Using SEM, the species was found to have 28–30 dorsal striae in 10 μm (double rows of pores), and *ca* 50 ventral striae in 10 μm. It resembles *Amphora coffeaeformis* v. *borealis* (Kützing) Cleve *sensu* Bérard-Therriault *et al.* (1986) in SEM but not when studied by LM. The number of striae is also quite different.

Cocconeis peltoides Hustedt (Fig. 9a–c)
Cells are broadly elliptical, 11–14 × 7–9 μm, with 15–18 striae in 10 μm on the rapheless valve, but in the LM no striae were visible on the raphe valve. Using SEM, the species was found to have ca. 38 striae in 10 μm on the raphe valve. This species is easy to recognize in the LM by the typical features of the rapheless valve. The raphe valve as described by Hustedt (1939) is incorrect as recognized by Simonsen (1987) from LM observations.

Fragilariaceae sp. 1 (Fig. 8h)
Cells are roundish, 2–3 μm in diameter and have *ca.* 22–24 striae in 10 μm. This species is identical with *Fragilaria* sp. A of Kuylenstierna (1989; plate 12, figs 125–126).

Navicula cryptolyra Brockmann (Fig. 9d–e)
Cells are broadly elliptical, 8–13 × 5–6 μm, and have 24–26 striae in 10 μm. The lateral areas are not visible in all frustules in the LM and this sometimes makes identification difficult as previously discussed by Håkansson and Stabell (1977).

Navicula cf. *perminuta* Grunow (Fig. 9f)
Cells are lanceolate with rounded ends, 8–12 × 3–3.5 μm, and have 18–20 striae in 10 μm. This taxon differs from the typical *N. perminuta* by the external raphe endings which in our specimens were sharply bent, while in *N. perminuta* their shape is more rounded (*cf.* plate 61, figs 767–771 in Kuylenstierna 1989).

Navicula sp. 1 (Fig. 9g–h)
Cells are broadly elliptical, 4–7 × 3–3.5 μm, with no striae which are visible in the LM, but the central nodule, the terminal nodules and the raphe canal are clearly distinct. Scanning electron micrographs show a large hyaline area and short marginal striae (*ca* 36 in 10 μm). In the LM the taxon resembles *Navicula nolens* Simonsen.

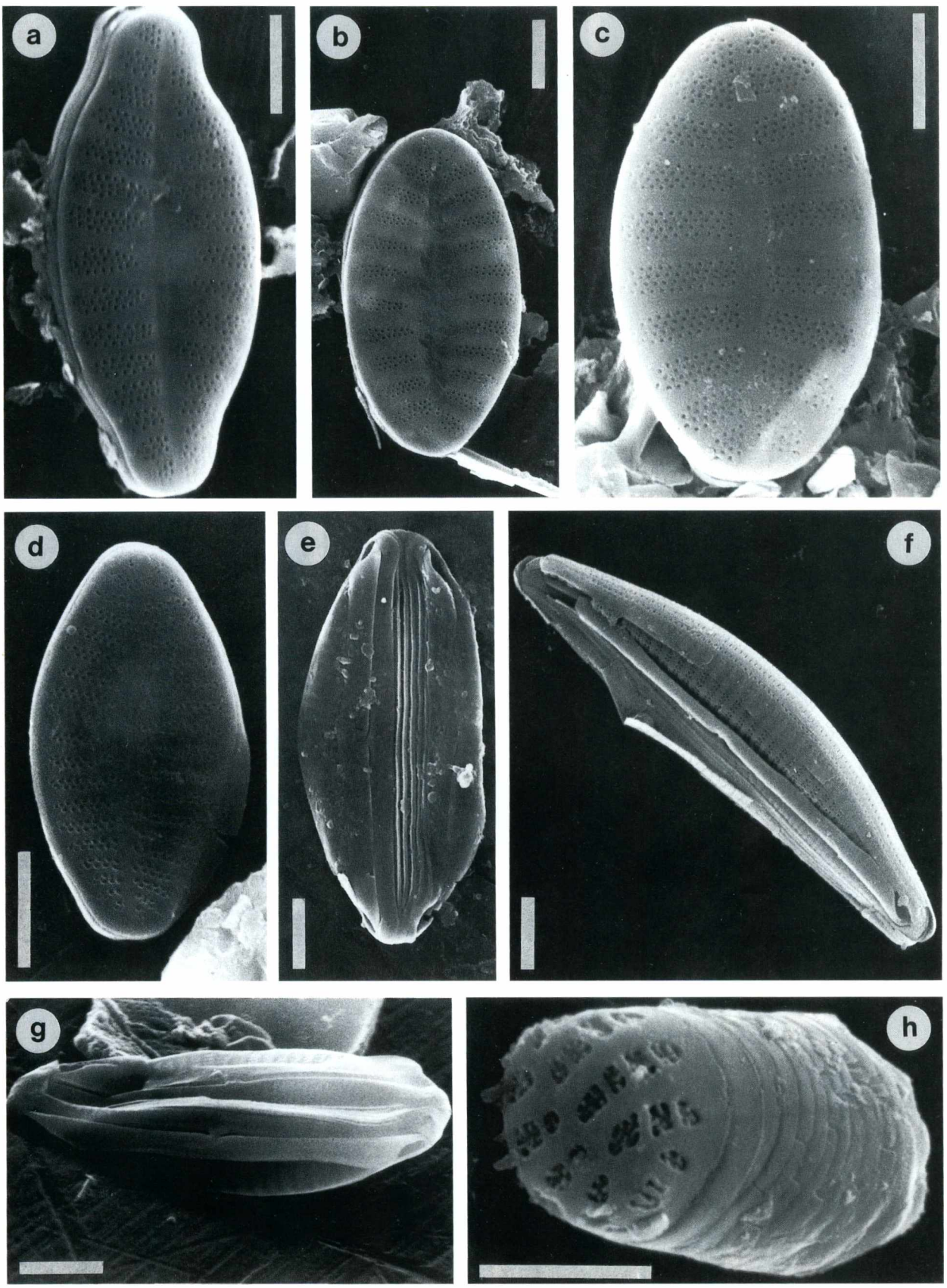

Fig. 8. Scanning electron micrographs of some dominant diatom taxa. Scale bar = 2 µm. a–c: *Achnanthes* cf. *delicatula*. d: *Achnanthes* cf. *delicatissima*. e–g: *Amphora* sp. 1. h: Fragilariaceae sp. 1.

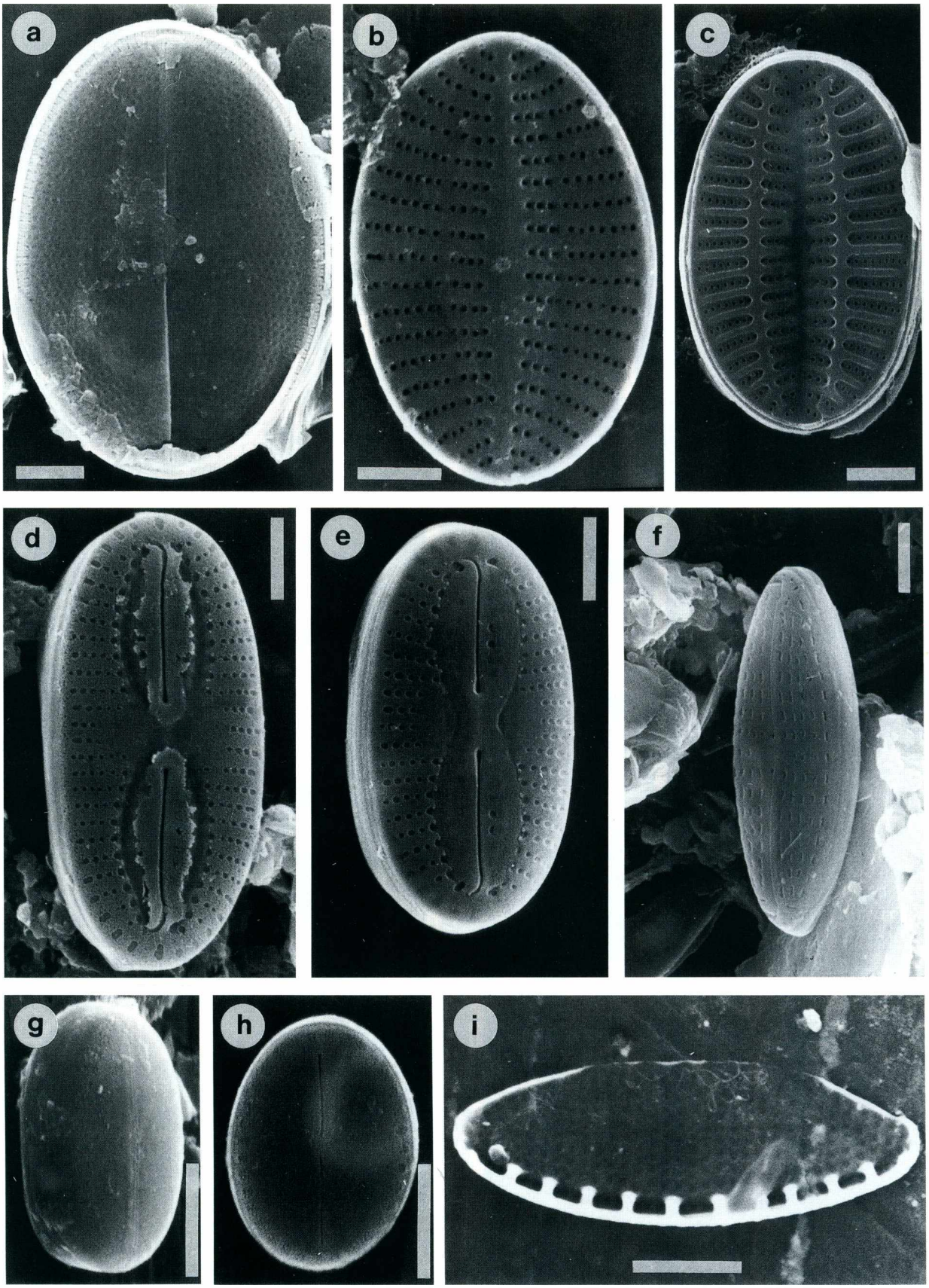

Fig. 9. Scanning electron micrographs of some dominant diatom taxa. Scale bar = 2 µm. a–c: *Cocconeis* peltoides. d–e: *Navicula cryptolyra* (somewhat eroded in d). f: *Navicula* cf. *perminuta*. g–h: *Navicula* sp. 1. i: *Nitzschia aurariae*.

Nitzschia aurariae Cholnoky (Fig. 9i)
Cells are linear-elliptical, 6–11 × 3–4 µm, with 14–18 fibulae and *ca* 45 striae in 10 µm. According to Krammer and Lange-Bertalot (1988), the apices of *N. aurariae* are never rostrate. In part of our frustules there was a tendency for subrostrate ends, and these are called *Nitzschia* cf. *aurariae*.

Discussion

Major algal groups

Nutrient addition to the water column resulted in enhanced microphytobenthic growth. This showed that the microalgae in the sediment were nutrient limited in the non-enriched containers and that nutrients were assimilated from the overlying water. This was also reflected as raised values of primary productivity and chlorophyll *a* content of the sediment (Nilsson *et al.* 1991). The microalgae were also capable, at least initially, to compete with fast-growing filamentous macroalgae for the nutrient pool of the overlying water (the phytoplankton was excluded from the containers by filtration).

One of the most obvious effects of the N + P-enrichment was the stimulated growth of some filamentous algae (the macroscopic green alga *Enteromorpha clathrata* and cyanobacteria). This agrees with previous observations that a proceeding eutrophication will favour opportunistic algal species such as fast growing filamentous macroalgae (Wallentinus 1984, Rosenberg *et al.* 1990). Carrick *et al.* (1988), who carried out experiments on periphyton in Lake Michigan using nutrient releasing substrata, found that N + P enrichment favoured algae other than diatoms, *e.g.* cyanobacteria and green algae, and they categorized these into a 'N + P guild'. It was typical for these filamentous taxa to modify physical aspects of the micro-environment (shading), a situation which was also likely to occur in our enriched containers. In addition, the chemical environment was probably influenced; Lee *et al.* (1975) observed higher concentrations of dissolved organic nitrogen in *Enteromorpha intestinalis* (L.) Link. communities and hypothetized that microscopic epiphytes with heterotrophic properties could be stimulated. Estuarine benthic diatoms have been shown to rapidly assimilate compounds such as amino acids (Admiraal *et al.* 1987).

The nutrient treatment in our experiment involved both a considerable increase of nutrient supply and a changed supply ratio. The concentration of IN increased on an average by a factor of 130 and IP by a factor of 40. No Si was supplied. Between 20 and 50% of this nutrient input dissappeared from the overlying water during the 4-week experiment (Nilsson *et al.* 1991). The nutrient uptake was a sum of algal assimilation, bacterial activity and adsorption processes in the sediment. According to the resource competition theory (Tilman *et al.* 1982), different relative supply rates of two or more limiting resources can be expected to result in (phytoplankton) communities dominated by different species or taxonomic groups. In our study, the N + P addition changed the mean Si : N : P mole ratio of the originally nutrient-poor surface water from 112 : 14 : 1 to 2.7 : 39 : 1. When the sediment microflora is considered, the changed Si : N : P ratio did not, despite the stimulated growth of cyanobacteria, lead to a dramatic shift in the dominance of the major taxonomic groups.

Carrick *et al.* (1988) interpreted that the shift in the algal community in Lake Michigan occurred because the N + P enrichment induced a Si-limitation of diatoms. Although Si was not added in our experiment, diatoms significantly increased their biomass. The optimal Si : N ratio of the medium for marine diatoms is considered to be approximately 1 : 1. The facts that the Si : N ratio in the overlying water of the enriched containers was usually < 0.1, and that the measured uptake from the water could cover between 20 and 90% of the theoretical Si-requirements for diatoms (Nilsson *et al.* 1991), suggest an additional Si-source, which is most probably the sediment itself. On the other hand, despite the generally higher concentrations of Si in sediments than in the overlying water, Si-limitation of sediment-inhabiting diatoms has been documented (Kelderman *et al.* 1988). Ultimately, Si may become a limiting factor in the presence of prolonged raised levels of N and P along the Swedish west coast.

From resource-limited competition experiments using natural phytoplankton communities comprising green algae, diatoms and cyanobacteria, it has been suggested that the nutrient ratios at which dominance shifts from one taxonomic group to another, are temperature dependent (Tilman *et al.* 1986). Our experiment was conducted in late summer, a season favourable for the growth of cyanobacteria in stagnant and shallow brackish-water benthic habitats (Snoeijs and Prentice 1989). Although the growth of cyanobacteria was significantly promoted by the N + P treatment, they did not outcompete the diatoms during the course of our 4-week experiment. One reason may be that the water temperature (16–19 °C) was not high enough to induce the shift. In a previous laboratory experiment with sublittoral marine sediment conducted at 14.5 °C, N + P fertilization also induced the growth of filamentous cyanobacteria, however, diatoms took over again at the end of the

experiment (Sundbäck and Granéli 1988). Filamentous cyanobacteria are usually favoured by N-limitation because of their ability to fix N_2, which has also been found to occur in non-heterocystous species (Jones 1990). The high N/P ratio in the enriched containers would yield secondary P-limitation, and this may also have prevented the shift to a system dominated by cyanobacteria.

Although autotrophic flagellates were initially a minor constituent of the microflora, there was a trend towards increased biomass and this is in accordance with the hypothesis that flagellates become more important as eutrophication continues (Doering *et al.* 1989). Our analysis of the two groups labelled 'flagellates' is incomplete, since they include several taxonomic groups, such as green algae, dinoflagellates, chrysophytes, and probably also prymnesiophytes. Flagellates, both autotrophic and heterotrophic, constitute a rather overlooked functional group in marine sediments (Larsen 1985, Bak and Niewland 1989).

Measurable effects of the N + P treatment on the benthic microflora were observed after 2 weeks. A lag-time of a few weeks has also been observed in other experiments on sediment communities (Levinton 1985, Sundbäck *et al.* 1990). Thus our 4-week experiment probably only reflected the initial phase of the changes in the sediment system, which reacted rather slowly to environmental changes compared with both pelagial systems and non-sediment benthic systems.

The diatom community

Diversity. Shannon-Weaver diversity was rather low (2.6–3.3) compared with previously reported values for diatom communities of sandy substrates (3.2–4.6; Amspoker 1977, Sundbäck 1984) as well as for edaphic diatom communities (*ca* 4–5; Sullivan 1976, Cook and Whipple 1982). The sieving of the sediment may have decreased the initial diversity of the species pool. Algal diversity is expected to be highest at an intermediate level of disturbance, for example mechanical disturbance by grazing (Swamikannu and Hoagland 1989) or exposure to water movement (Sundbäck 1984). Thus, an additional reason for the rather low diversity may be the enclosure of the sediment in the experimental vessels. This enclosure would not only decrease the exposure to water movement but also exclude the immigration of macrofaunal grazers, as well as immigration of diatom species. Macrofaunal grazers were initially excluded from the containers and meiofaunal grazing pressure on autotrophic microalgae was rather low (Nilsson *et al.* 1991).

Diversity and evenness of the diatom community was only slightly negatively affected by the nutrient addition. Nutrient increase has been found to decrease diversity of benthic microalgal communities in fresh water (Carrick *et al.* 1988 and references therein), on salt marshes (Sullivan 1976), and estuarine diatom environments (Admiraal *et al.* 1989). On the other hand, Sullivan (1981) found that nitrogen enrichment could either decrease or increase H′ of edaphic diatom communities and Dam (1982) concluded that diversity indices appear to have no consistent relationship with the degree of water pollution.

Canonical correspondence analysis (CCA). The CCA showed that the succession in both non-enriched and enriched containers was in the same direction, though faster and proceeding further with N + P-addition. Reverse succession at the end of the experiment may be attributed to the loss of larger cells by oxygen bubble transportation. Certain diatom taxa were stimulated more by the nutrient addition than others. However, the CCA did not reveal any fundamental change in community composition that could be attributed to nutrient addition, and the question arises whether there are marine benthic diatom communities typical of eutrophic conditions. More information exists on the response to pollution of benthic microalgae from freshwater than from marine environments (*cf.* Wolf 1982). Using diatoms as freshwater indicator organisms in the 'classic' sense (*e.g.* Sládecek 1973) have been proven to be highly unreliable, whereas measures based on quantitative diatom species composition have been shown to be a useful tool in freshwater quality estimation (*e.g.* Lange Bertalot 1979). Kuhn *et al.* (1981) applied life-form strategies to interpret pollution effects on freshwater periphyton. Sullivan (1976, 1981) conducted *in situ* experiments on the response of edaphic diatom communities of salt marshes to fertilization by N and P. He observed compositional changes in the community, but did not succeed in grouping taxa with similar response patterns with the aid of multivariate methods, partly because the effect was shaded by light and temperature effects. Admiraal *et al.* (1984) and Jong and Admiraal (1984) made competition experiments with both isolated species and natural communities of estuarine diatoms. These workers found that selection mechanisms, such as inorganic carbon-limitation and allelopathy, are more important than inorganic nutrients on the nutrient-rich tidal flat.

In our experiment, the diatom taxa most favoured by the N + P addition were *Nitzschia* and *Amphora* species within the size range 7–12 µm. Despite their

small size these species were important in terms of biomass. Of the 5 *Nitzschia* species listed by Wolf (1982) with a given code for nutrient content spectrum, 4 are categorized as eutrophic and 1 mesoeutrophic. Also others have reported that representatives of the genus *Nitzschia* increase because of fertilization (Sullivan 1981). Sundbäck (1984) found that the *N.* cf. *pusilla* Lange-Bertalot together with a small unidentified *Amphora* species increased as detritus accumulated in a sandy bay. The stimulation of *Amphora* sp. 1, *A. flebilis* and *A. tenerrima* by N + P-enrichment, agrees with the experience that small-sized *Amphora* species tend to rapidly colonize sand grains when trying to isolate and cultivate epipsammic species (Tufail 1987, Sundbäck unpublished). In the middle of the experiment the comparably large-sized *Cylindrotheca closterium* also increased significantly in the enriched containers. According to Admiraal (1977), *Nitzschia* (= *Cylindrotheca*) *closterium* tolerates high concentrations of inorganic N and P and laboratory experiments have shown that its capability to outcompete other tidal-flat diatom species increases with higher salinity and temperature (Jong and Admiraal 1984).

Size, morphology and motility of diatoms. The capability of macroscopic algae to compete for nutrients increases as the surface to volume ratio (S/V) of the species increases (Wallentinus 1984). This has also been shown for phytoplankton (for review see *e.g.* Fogg and Thake 1987). In our experiment the majority of the most abundant taxa were small-sized naviculoid forms, characterized as flat epipsammic diatoms occurring in cavities of sand grains (Sundbäck 1984). These species generally have a high S/V ratio and are capable of fast absorption of nutrients compared with large naviculoids and sigmoid cells (Baille 1987, Hudon and Legendre 1987). Carrick *et al.* (1988) coupled morphology and above all mobility of a diatom species to a certain nutritional guild. In our sandy sediment adnate and less motile epipsammic forms, such as *Achnanthes* species, were common initially. For the two diatom taxa most stimulated by the N + P enrichment, that is the two *Nitzschia* taxa, the morphology implies a lower S/V ratio than *e.g.* *Achnanthes* species, as well as the capability to move faster. Motility was likely to be an advantage when competing for light and nutrients, in particular as the microenvironment in the enriched containers was probably altered by the growth of *Enteromorpha* (*cf.* Hudon and Legendre 1987). The larger-sized, highly motile *Cylindrotheca closterium* cells benefitted from the N + P enrichment. On the other hand, these features facilitate rapid colonization and Hudon and Legendre (1987) suggest that this opportunistic strategy could present a positive trade off against their physical vulnerability.

When considering living cells and biomass, the fact that larger motile diatoms became initially more important in the enriched containers may also be related to the changed environment (changed water movement, shading) induced by the growth of filamentous green algae in combination with the decreased wave impact as a result of the experimental set-up. In addition, colonization space might have limited the growth of adnate epipsammic forms. This conclusion is supported by the significantly decreased chlorophyll *a* values and primary productivity on Day 28 (Nilsson *et al.* 1991). In particular cells of tychoplanktic species such as *Cylindrotheca closterium*, may be transported up from the sediment surface. In addition, the conditions of high oxygen content, high pH and inorganic C-limitation that are created when photosynthetic activity is high, may also function as a selective pressure on the microalgal community (Jong and Admiraal 1984).

Primary producers and higher trophic levels

The recorded changes in the biomass and composition of sediment-associated primary producers following N + P addition were coupled to changes both in functional variables (primary productivity, grazing pressure and nutrient flux) and structural variables, such as the composition of meiofauna. The meiofaunal biomass of, in particular, oligochaetes and copepods, increased significantly in enriched containers (Nilsson *et al.* 1991). As the autochthonous benthic primary production was the only possible source of increase of the organic carbon pool, the biomass increase of consumers must have been directly or indirectly (via bacteria and produced detritus) related to the stimulated algal growth. Since meiofauna only removed a small proportion of the microalgal biomass (*ca* 4–10% of the biomass per day; Nilsson *et al.* 1991), the total amount of microautotrophic biomass did not appear to limit meiofaunal growth. This suggests that the response of meiofauna may rather be connected to a qualitative change of the food sources rather than to the quantity of food. We do not presently know if and how this change is related to the compositional change of the primary producers. More information is needed on both the qualitative value of the food sources of benthic herbivores in sandy sediment (Carman and Thistle 1985), as well as on interactions between microscopic primary producers and bacteria (Hall and Fisher 1985).

Conclusions

Outdoor flow-through systems are useful tools for studying benthic microalgal communities both as a structural and functional component of shallow-water sediment systems. They mimic relatively well natural communities and enable replication and adequate control treatments to test and reveal patterns of change on the lower trophic levels in connection with environmental changes.

Increased concentration of IP and IN in the water column stimulated the growth of sediment-living microalgae. In particular, filamentous cyanobacteria and diatoms, as well as flagellates, were favoured. Although the N + P addition considerably lowered the Si/N ratio, diatoms were not outcompeted by any other microalgal group, probably because the Si-pool of the sediment prevented, at least initially, Si-limitation.

Despite the statistically significant compositional changes, the microflora was not subjected to any dramatic shift in the dominance of major taxonomic groups nor diatom species, but rather the major change occurred on the macroscopic level in the form of a rapid growth of the green alga *Enteromorpha clathrata*. Thus, in the long run enhanced growth of filamentous species can be expected to fundamentally change the character of bare sandy habitats and further contribute to the accumulation of drifting macroalgae and detritus in shallow areas.

Counts of both living cells and frustules showed that the diatom flora was dominated by cells within the size range 7–12 µm, however, depending on the method and level of resolution, different aspects of compositional changes were detected. Thus, both methods were necessary for the interpretation of the changes in composition.

The increasing abundance of small *Nitzschia* and *Amphora*-species with N + P enrichment supports previous findings that nutrient enrichment affects the composition of benthic diatom communities.

In stagnant conditions, a highly stimulated photosynthetic activity at the sediment surface may in itself function as a selective pressure on the microalgal community by selectively transporting away easily suspendible species.

Acknowledgements

Tjärnö Marine Biological Laboratory (Strömstad) provided excellent working facilities during the experiment. The Department of Marine Botany (Göteborg), and Department of Ecological Botany (Uppsala) provided facilities for subsequent analyses and data treatment. We thank Mats Kuylenstierna for valuable advice on identification of some diatom taxa and Gary Wife for help with SEM and preparing the photographs for publication. KS thanks her colleagues Benno Jönsson, Inger Lindström Swanberg and Per Nilsson for inspiring co-operation during the experiment. Financial support was provided by The Swedish Natural Science Research Council, The National Swedish Environmental Protection Agency and the Royal Society of Arts and Sciences in Gothenburg.

References

Admiraal, W. 1977. Tolerance of estuarine benthic diatoms to high concentrations of ammonia, nitrite ion, nitrate ion and ortophosphate. *Mar. Biol. 43*: 307–315.

Admiraal, W., H. Peletier and T. Brouwer. 1984. The seasonal succession patterns of diatom species on an intertidal mudflat: an experimental analysis. *Oikos 42*: 30–40.

Admiraal, W., C. Riaux-Gobin and R. W. P-M. Laane. 1987. Interactions of ammonium, nitrate, and D- and L-amino acids in the nitrogen assimilation of two species of estuarine benthic diatoms. *Mar. Ecol. Prog. Ser. 40*: 267–273.

Admiraal, W., E. D. de Ruyter van Steveninck and H. A. M. de Kruijf. 1989. Environmental stress in five ecosystems in the floodplain of the River Rhine. *The Science of the Total Environment 78*: 59–75.

Amspoker, M. C. 1977. The distribution of intertidal epipsammic diatoms on Scripps Beach, La Jolla, California, USA. *Bot. Mar. 20*: 227–232.

Bak, R. P. M. and G. Nieuwland. 1989. Seasonal fluctuations in benthic protozoan populations at different depths in marine sediments. *Neth. J. Sea Res. 24*: 37–44.

Baille, W. P. 1987. Diatom size distribution and community stratification in estuarine intertidal sediments. *Estuar. coast. Shelf Sci. 25*: 193–209.

Berárd-Therriault, L., A. Cardinal and M. Poulin. 1986. Les Diatomées (Bacillariophyceae) benthiques de substrats durs des eaux marines et saumâtres du Québec. 6. Naviculales: Cymbellaceae et Gomphonemaceae. *Naturaliste can. (Rev. Écol. Syst.) 113*: 405–429.

Braak, C. J. F. ter. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology 67*: 1167–1179.

Braak, C. J. F. ter and I. C. Prentice. 1988. A theory of gradient analysis. *Adv. Ecol. Res. 18*: 271–317.

Brockmann, C. 1950. Die Watt-Diatomeen der Schleswig-Holsteinischen Westküste. *Abh. Senckenb. naturf. Ges. 478*: 1–26.

Carman, K. R. and D. Thistle. 1985. Microbial food partioning by three species of benthic copepods. *Mar. Biol. 88*: 143–148.

Carrick, H. J. and R. L. Lowe. 1988. Response of Lake Michigan benthic algae to in situ enrichment with Si, N and P. *Can. J. Fish. Aquat. Sci. 45*: 271–279.

Carrick, H. J., R. L. Lowe and J. T. Rotenberry. 1988. Guilds of benthic algae along nutrient gradients: relationships to algal community diversity. *J. N. Am. Benthol. Soc. 7*: 117–128.

Cook, L. L. and S. A. Whipple. 1982. The distribution of edaphic diatoms along environmental gradients of a Louisiana salt marsh. *J. Phycol. 18*: 64–71.

Dam, H. van. 1982. On the use of measures of structure and diversity in applied diatom ecology. *Nova Hedwigia, Beih. 73*: 87–115.

Doering, P. H., C. A. Oviatt, L. L. Beatty, V. F. Banzon, R. Rice, S. P. Kelly, B. K. Sullivan and J. B. Frithsen. 1989. Structure and function in a model coastal ecosystem: silicon, the benthos and eutrophication. *Mar. Ecol. Prog. Ser. 52*: 287–299.

Edler, L. 1977. Phytoplankton and Primary Production in the Sound. Ph. D. thesis, Department of Marine Botany, University of Göteborg, pp. 82.

Edler, L. (ed.). 1979. Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. *Baltic Mar. Biol. (BMB) Publ. No. 5*, p. 1–38.

Fogg, G. E. and B. Thake. 1987. *Algal Cultures and Phytoplankton Ecology*. The University of Wisconsin Press, Madison, pp. 269.

Giffen, M. H. 1966. Contributions to the diatom flora of South Africa. III. Diatoms of the marine littoral regions at Kidd's Beach near East London, Cape Province, South Africa. *Nova Hedwigia 13*: 245–293.

Granéli, E. and K. Sundbäck. 1985. The response of planktonic and microbenthic algal assemblages to nutrient enrichment in shallow coastal waters, southwest Sweden. *J. Exp. Mar. Biol. Ecol. 85*: 253–268.

Granéli, E., K. Wallström, U. Larsson, W. Granéli and R. Elmgren. 1990. Nutrient limitation of primary production in the Baltic Sea area. *Ambio 19*: 142–151.

Green, R. H. 1979. *Sampling Design and Statistical Methods for Environmental Biologists*. John Wiley and Sons, New York.

Håkansson, H. and B. Stabell. 1977. Identification of some small species of *Navicula*. *Bot. Not. 130*: 477–481.

Hall, S. L. and F. M. Fisher, Jr. 1985. Annual productivity and extracellular release of dissolved organic compounds by the epibenthic algal community of a brackish marsh. *J. Phycol. 21*: 277–281.

Hartley, B. 1986. A check-list of the freshwater, brackish and marine diatoms of the British Isles and ajoining coastal waters. *J. mar. biol. Ass. U. K. 66*: 531–610.

Hudon, C. and P. Legendre. 1987. The ecological implications of growth forms in epibenthic diatoms. *J. Phycol. 23*: 434–441.

Hustedt, F. 1939. Die Diatomeenflora des Küstengebietes der Nordsee vom Dollart bis zur Elbemündung. I. Die Diatomeenflora in den Sedimenten bei der unteren Ems sowie auf den Watten der Leybucht, des Memmert und bei der Insel Juist. *Abh. Naturwiss. Vereins Bremen 31*: 572–677.

Jones, K. 1990. Aerobic nitrogen fixation by *Lyngbya* sp., a marine tropical cyanobacterium. *Br. phycol. J. 25*: 287–289.

Jong, L. de and W. Admiraal. 1984. Competition between three estuarine benthic diatom species in mixed cultures. *Mar. Ecol. Prog. Ser. 18*: 269–275.

Jönsson, B. H. 1991. A ^{14}C-incubation technique for measuring microphytobenthic primary productivity in intact sediment cores. *Limnol. Oceanogr.*: In press.

Kelderman, P., H. J. Lindeboom and J. Klein. 1988. Light dependent sediment-water exchange of dissolved reactive phosphorus and silicon in a producing microflora mat. *Hydrobiologia 159*: 137–147.

Krammer, K. and H. Lange-Bertalot. 1988. Bacillariophyceae, 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. *Süßwasserflora von Mitteleuropa, Bd 2/2*. pp. 596.

Kuhn, D. L., J. L. Plafkin, J. Cairns Jr. and R. L. Lowe. 1981. Qualitative characterization of aquatic environments using diatom life-form strategies. *Trans. Am. Microsc. Soc. 100*: 165–182.

Kuylenstierna, M. 1989. Benthic Algal Vegetation in the Nordre Älv Estuary (Swedish west coast). Vol. 2. Plates. Dissertation, Dept. of Marine Botany, University of Göteborg, 72 plates.

Lange-Bertalot, H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. *Nova Hedwigia, Beih. 64*: 285–304.

Lange-Bertalot, H. and M. Ruppel. 1980. Zur Revision taxonomisch problematischer, ökologisch jedoch wichtiger Sippen der Gattung *Achnanthes* Bory. *Arch. Hydrobiol., Suppl. 60*: 1–31.

Lange-Bertalot, H. and K. Krammer. 1989. *Achnanthes*, eine Monographie der Gattung. *Bibliotheca Diatomologica 18*. J. Cramer, Berlin. pp. 393.

Larsen, J. 1985. Algal studies of the Danish Wadden Sea II. A taxonomic study of psammobious dinoflagellates. *Opera Bot. 79*: 14–37.

Lee, J. J., M. E. McEnery, E. M. Kennedy and H. Rubin. 1975. A nutritional analysis of a sublittoral diatom assemblage epiphytic on *Enteromorpha* from a Long Island salt marsh. *J. Phycol. 11*: 14–49.

Levinton, J. S. 1985. Complex interactions of a deposit feeder with its resources: roles of density, a competitor, and detrital addition in the growth and survival of the mudsnail *Hydrobia totteni*. *Mar. Ecol. Prog. Ser. 22*: 31–40.

Maarel, E. van der. 1989. Species diversity in plant communities in relation to structure and dynamics. *In*: (H. G. During, M. G. A. Werger and G. H. Willems, eds) *Diversity and Pattern in Plant Communities*. S. P. B. Academic Publishing, The Hague. pp. 1–14.

Nilsson, P., B. Jönsson, I. Linström Swanberg and K. Sunbäck. 1991. Response of a marine shallow-water sediment system to an increased load of inorganic nutrients. *Mar. Ecol. Prog. Ser.*: In press.

Patil, G. P. and C. Taillie. 1979. An overview of diversity. *In*: (J. F. Grassie, G. P. Patil, W. Smith and C. Taillie, eds) *Ecological Diversity in Theory and Practice*. International Publishing House, Fairland. pp. 3–27.

Pringle, C. M. and A. Bowers. 1984. An in situ substratum fertilization technique: Diatom colonization on nutrient-enriched, sand substrata. *Can. J. Fish. Aquat. Sci. 41*: 1247–1251.

Rosenberg, R., R. Elmgren, S. Fleischer, P. Jonsson, G. Persson and H. Dahlin. 1990. Marine eutrophication. Case studies in Sweden. *Ambio 19*: 102–108.

Sládecék, V. 1973. System of water quality from the biological point of view. Ergebnisse der Limnologie, *Arch. für Hydrobiol. 7*: 1–218.

Simonsen, R. 1987. *Atlas and Catalogue of the Diatom Types of Friedrich Hustedt* (1–3). J. Cramer, Berlin–Stuttgart. 1: pp. 525, 2: Pl. 1–395, 3: Pl. 396–772.

Snoeijs, P. J. M. 1989. A check-list of the benthic diatoms at Forsmark (northern Baltic Sea). 1. Epilithic and epiphytic taxa. *Ann. Bot. Fennici 26*: 427–439.

Snoeijs, P. J. M. and I. C. Prentice. 1989. Effects of cooling water discharge on the structure and dynamics of epilithic algal communities in the northern Baltic. *Hydrobiologia 184*: 99–123.

Sullivan, M. J. 1976. Long-term effects of manipulating light intensity and nutrient enrichment on the structure of a salt marsh diatom community. *J. Phycol. 12*: 205– 210.

Sullivan, M. J. 1981. Effects of canopy removal and nitrogen enrichment on a *Distichlis spicata*-edaphic diatom complex. *Estuar. coast. Shelf Sci. 13*: 119–129.

Sundbäck, K. 1984. Distribution of microbenthic chlorophyll-*a* and diatom species related to sediment characteristics. *Ophelia, Suppl. 3*: 229–246.

Sundbäck, K. and W. Granéli. 1988. Influence of microphytobenthos on the nutrient flux between sediment and water: a laboratory study. *Mar. Ecol. Prog. Ser. 43*: 63–69.

Sundbäck, K., B. Jönsson, P. Nilsson and I. Lindström. 1990. Impact of accumulating drifting macroalgae on a shallow-water sediment system: an experimental study. *Mar. Ecol. Prog. Ser. 58*: 261–274.

Swamikannu, X. and K. D. Hoagland. 1989. Effects of snail grazing on the diversity and structure of a periphyton community in a eutrophic pond. *Can. J. Fish. Aquat. Sci. 46*: 1698–1704.

Tilman, D., S. S. Kilham and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. *Ann. Rev. Ecol. System. 13*: 349–372.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham and F. A. Johnson. 1986. Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch. Hydrobiol. 106*: 473–485.

Tufail, A. 1987. Microbial communities colonising nutrient-enriched marine sediment. *Hydrobiologia 148*: 245–255.

Wallentinus, I. 1984. Comparison of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. *Marine Biol. 80*: 215–225.

Whittaker, R. H. 1977. Evolution and measurement of species diversity. *Taxon 21*: 213–251.

Williams, D. M. and F. E. Round. 1987. Revision of the genus *Fragilaria*. *Diat. Res. 2*: 267–288.

Wolf, H. de. 1982. Methods of coding ecological data from diatoms for computer utilization. *Mededelingen Rijks Geologische Dienst. 36*: 95–98.

Zar, J. H. 1974. *Biostatistical Analysis*, Prentice-Hall, INC, Englewood Cliffs, H. J. pp. 620.