Influence of sediment on pelagic carbon and nitrogen turnover in a shallow Danish estuary

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ABSTRACT: The impact of sediment on pelagic microbial processes was examined for 3 different sediment types (a bare sandy sediment, an eelgrass sediment and a diatom sediment) in a shallow Danish estuary. Diel and seasonal variations in the *in situ* fluxes and pelagic turnover of various C and N compounds were measured in plexiglas chambers incubated on top of the sediment and related to parallel control incubations without sediment contact. In April, the microbial activity was low, and the sediment had relatively little influence on the pelagic C and N turnover. In August, however, the eelgrass and diatom sediments had a significant stimulatory effect on the bacterial production, which was 2 to 4 times higher than at the bare sediment station. The measured effects of the sediment on pelagic C and N turnover, thus, appeared to be related to the presence and activity of benthic microalgae and macrophytes. Stimulation of bacterial production by the sediment was in all experiments associated with a net flux of dissolved organic carbon (DOC) from the sediment. We suggest that the release of biodegradable DOC from benthic microalgae and macrophytes caused a stimulation of the bacterioplankton production. Since 2 to 4 times more nitrogen was retained in the pelagic bacterial biomass at the eelgrass and diatom sediment than at the bare sediment location, we suggest that the presence of benthic microalgae and macrophytes may cause a temporary immobilization of N in bacterioplankton biomass.

KEY WORDS: Sediment effect · Bacterioplankton · C and N turnover · DOC release

INTRODUCTION

In oceanic environments, phytoplankton primary production is the dominant source of organic matter for bacterioplankton (e.g. Ducklow et al. 1993). In coastal waters, sediment and benthic macrophytes may also affect the pelagic nutrient cycling (Pakulski 1986, Turner 1993). Sedimentation of organic matter often leads to a rapid mineralization that causes a flux of inorganic nutrients back into the water column (Sloth et al. 1995). Despite a rapid turnover of dissolved organic nitrogen and carbon (DON and DOC) in

sediment, the pore water often contains residual pools of labile organic compounds, such as dissolved free amino acids, in concentrations that are 5 to 100 times higher than in the water column above the sediment (Boon 1986, Lomstein et al. 1995). Considering the concentration gradients in the sediment-water interface, a transport of organic nutrients from the sediment to the overlying water would be expected. Efflux of DON has been found to be equal to or higher than the amount of dissolved inorganic N (DIN) released from the sediment (Christensen et al. 1983, Lomstein & Blackburn 1992, Sloth et al. 1995).

The efflux of organic compounds from sediments may be influenced by factors other than diffusion. The composition of the sediment and the temperature have also been found to influence the efflux (Holmer 1996). Bacteria, benthic microalgae and macrophytes

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may retain C and N in the sediment by incorporating nutrients into their biomass and, hence, reduce the efflux of nutrients (Pedersen & Borum 1992, Kairesalo et al. 1996). On the other hand, benthic micro- and macrophytes may contribute to the DON and DOC pools in the water column by releasing dissolved organic compounds (Hough & Wetzel 1975, Jørgensen et al. 1981, Pakulski 1986, Turner 1993, Mann & Wetzel 1996).

The quantitative importance for pelagic processes of nutrient fluxes across the sediment-water interface is poorly studied. It has previously been hypothesized that leachates from benthic macrophytes may influence the pelagic microbial processes and community respiration (Pakulski 1986, Turner 1993). The actual effects of the release of DOM and inorganic nutrients from the sediment on the pelagic bacterial C and N turnover will depend on the amount and composition of the released material, and on the growth limiting factors for the bacterioplankton.

The purpose of this study was to measure fluxes of dissolved organic and inorganic material from 3 different sediments (a bare sediment, an eelgrass-covered sediment and a diatom-covered sediment), and to examine the effects of these fluxes on the bacterioplankton nutrient turnover. This is, to our knowledge, the first attempt to directly measure effects of different sediment types on pelagic processes on a diel and seasonal time scale.

MATERIALS AND METHODS

Study area and experimental design. The studies were carried out in the Limfjord, Denmark, south of the Aggersund Bridge in April and August 1995. *In situ* fluxes and pelagic turnover of various C and N compounds were measured in triplicate 29 l transparent plexiglas chambers ($29 \times 29 \times 34.5$ cm) placed on the sediment at 1 to 1.5 m depth. Chambers were incubated for 2 to 8 h on a bare sandy sediment ('bare sediment'), on eelgrass-covered sediment ('eelgrass sediment'), and on sediment covered with a diatom biofilm ('diatom sediment'; only August). *In situ* water circulation was simulated by an electric stirring rod fitted on the lid of the chamber. Duplicate or triplicate 2 l polycarbonate bags or 1 l glass bottles without sediment contact were used as controls. The controls were suspended in the water column ca 10 cm above the sediment surface. Incubations were performed both day and night.

Subsamples for chemical analyses and microbial activity (see below) were taken from the chambers at time zero and by the end of the incubation. In the diatom sediment experiment, additional samples for

chemical analyses were taken every half hour during the incubation.

Dilution cultures. To estimate the bacterial content of C per unit of biovolume, batch cultures were set up in April. In these cultures, growth medium was prepared by filtering water from the field location through 142 mm Millipore GV filters (0.22 µm pore size) (Millipore, Bedford, USA), using a Millipore stainless steel filter holder and a Millipore peristaltic pump. Water used for bacterial inoculum was filtered through 47 mm Nuclepore polycarbonate membranes (0.8 µm pore size) (Nuclepore, Cambridge, USA) to remove bacterivores. Cultures were set up by inoculating the 0.22 µm filtered water with 10% by volume of the 0.8 µm filtered water. The dilution of the inocula ensured that the cultures were free of particulate detritus. Cultures were incubated in the dark at room temperature. Samples for particulate organic carbon (POC), bacterial counts and biovolumes were taken at 24 h intervals (see below).

Chemical analyses. Samples for dissolved free amino acids (DFAA), dissolved combined amino acids (DCAA), urea and inorganic nitrogen (NH_4^+ and NO_3^-) were filtered through 0.2 µm sterile filters (Minitan, Sartorius, Göttingen, Germany) and stored frozen. Concentrations of DFAA were measured by high performance liquid chromatography (HPLC) after precolumn derivatization with *o*-phthaldialdehyde (Lindroth & Mopper 1979, Jørgensen et al. 1993). For measurements of DCAA, 1 ml subsamples were freeze-dried and hydrolyzed in vapor phase under nitrogen atmosphere for 20 min at 150°C by a microwave technique (Jørgensen & Jensen 1997). The hydrolyzed material was redissolved in borate buffer at pH >10 after which the amino acid content was quantified as individual free amino acids by HPLC.

Samples for total organic carbon (TOC) were stored frozen in precombusted (500°C overnight) glass tubes and measured by high temperature catalytic oxidation (oxidation at 680°C, Pt-covered alumina beads as catalyst) on a Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Japan) after removal of inorganic carbon by acidification with conc. HCl (Suprapur®, Merck, Darmstadt, Germany) and purging with nitrogen gas for 10 min (Kroer 1993). Concentrations of DOC were calculated by subtraction of POC from TOC.

Total dissolved N (TN) was measured by high temperature catalytic oxidation (oxidation at 800°C, Pt-covered alumina beads as catalyst) and detection by chemiluminescence upon reaction with ozone on a Mitsubishi Total Nitrogen Analyzer TN-05 (Mitsubishi Kasei Corp., Japan). DON concentration was calculated by subtracting amounts of dissolved inorganic N (see below).

Ammonium and nitrate were measured using standard procedures (Technicon, Tarrytown, USA). Concentrations of urea were determined by the diacetyl monoxime method according to Price & Harrison (1987).

Chlorophyll a (chl a) of sediment cores (27 mm diameter) was extracted in 6 ml of 96% ethanol at −18°C for 2 to 3 d. Chl a in 100 ml water samples, filtered onto 25 mm Whatman GF/F filters (Whatman, Maidstone, England), was extracted in 3 ml 96% ethanol. Chl a concentrations were determined spectrophotometrically.

Biomass and biovolume determination. Bacterial samples were preserved with 0.2 μm filtered formaldehyde (2% final concentration) and counted after staining with acridine orange (Hobbie et al. 1977). In the April experiment the sizes of the bacteria were determined. Acridine orange stained cells were photographed using a Zeiss Axiovert 100TV epifluorescence microscope fitted with a Photometrics AT200 CCD camera (Photometrics Ltd, Arizona, USA) and analyzed by the NIH Image 1.59 Software. The image analysis method was based on an edge detection algorithm described by Viles & Sieracki (1992) and B. H. Hygum (unpubl.). Photographs were taken from randomly selected fields. The biovolume of the cells was calculated according to the formula:

$$Volume = 8.5 \times A^{2.5} \times P^{-2}$$

where A is the area and P the perimeter of the cells. A total of 200 to 8000 cells were measured per sample.

The photomicrographs of bacteria were calibrated with photomicrographs of YG Fluoresbrite™ microspheres (Polysciences, Inc., Warrington, USA) of 0.21, 0.51 and 1.07 μm diameter. The bacterial size did not change systematically during the experiments, and the mean bacterial cell volume was 0.048 ± 0.011 μm^3.

For bacterial POC analysis in the dilution culture experiment in April, 200 to 400 ml of water was filtered though precombusted (450°C, 4 h) 25 mm Whatman GF/F filters. After filtration, filters were dried and stored in a heated vacuum desiccator at 45°C until assayed on a Perkin Elmer 240C CHN Analyzer (Perkin Elmer, Norwalk, USA). To correct for carbon-containing particles in the culture medium and for adsorption of dissolved organics to the filters, 350 to 400 ml of culture medium was filtered onto Whatman GF/F filters and the carbon content subtracted from the bacterial POC values. Based on the POC measurements and the measured bacterial biovolumes an average carbon conversion factor of 0.412 ± 0.310 pg C μm^{-3} was calculated.

Total POC was determined in triplicate on precombusted 13 mm Whatman GF/F filters by high temperature catalytic oxidation according to Søndergaard & Middelboe (1993).

Correction for carbon on blank filters (no water filtered) was performed for all POC values.

Measurements of bacterial activity. Bacterial uptake of DFAA was determined by incubating samples with an equimolar mixture of 4 [^{14}C]-labelled amino acids (glu, ser, gly, ala) with an individual specific activity between 58 and 282 mCi mmol^{-1} (Amersham, Buckinghamshire, England). Six nCi, corresponding to about 10 nM amino acids, was added to 5 ml duplicate subsamples and 1 killed control (2% formaldehyde, final concentration). Samples were incubated for 0.3 to 2 h to ensure a maximum uptake of 10% of the added tracer. The incubations were stopped with formaldehyde (2% final concentration). Samples were then filtered onto 0.2 μm cellulose nitrate filters, rinsed 3 times with Milli-Q water (Millipore) and radioassayed by liquid scintillation counting. Respiration of [^{14}C]-labelled amino acids during the incubations was assumed to be 50% of the uptake (Middelboe et al. 1995).

Turnover of DCAA was estimated by use of the fluorogenic substrate analog L-leucine-4-methyl-coumarinylamide hydrochloride (Leu-MCA) (Hoppe et al. 1988, Middelboe et al. 1995). Triplicate 3 ml subsamples were incubated with 500 nM Leu-MCA (final concentration) for 2 to 10 h. The increase in fluorescence was linear with time for the incubation times used. Leu-MCA turnover rate was calculated from the increase in fluorescence as the non-fluorescent Leu-MCA was hydrolyzed to the highly fluorescent 7-amino-4-methyl-coumarin (AMC). Background fluorescence at time zero was measured for each triplicate sample. Total DCAA uptake was estimated from concurrent measurements of Leu-MCA turnover and DCAA concentrations assuming 50% respiration of the uptake (Middelboe et al. 1995).

Uptake (hydrolysis) of urea by the bacteria was measured from production of $^{14}CO_2$ in triplicate 20 ml water samples to which 60 nCi of ^{14}[C]urea (56 mCi mmol^{-1}, DuPont NEN Products Research Products, Denmark) was added. Samples were incubated in 100 ml serum bottles with rubber septa to which plastic cups with accordion-folded paper wicks were fitted. Uptake was stopped after incubation periods of 2 to 3 h by injection of formaldehyde (2% final concentration) through the membrane. Samples were then acidified with 10% phosphoric acid, to drive off $^{14}CO_2$, and the wicks soaked with 500 μl of Carbosorb CO_2 absorber (Packard Instruments, Groningen, The Netherlands). After 1 h on a shaking table, the CO_2 traps were transferred to 20 ml scintillation vials and radioassayed.

Bacterial cell production was measured as bacterial incorporation of [^{3}H]-thymidine (Fuhrman & Azam 1980), using a conversion factor of 1.1×10^{18} cells mol^{-1} ^{3}H-thymidine incorporated (Riemann et al. 1987).

Table 1. Basic chemical and physical data (measured concentration ranges are shown). nd: not determined

	NH_4^+ (μM)	NO_3^- (μM)	Urea (μM)	DFAA (μM)	DCAA (μM)	TOC (μM)	POC (μM)	DON (μM)	Pelagic chlorophyll (μg chl a l^{-1})	Sediment chlorophyll[a] (μg chl a cm^{-2})
April	1.2–3.1	25.6–33.3	1.4–1.9	0.30–0.81	1.1–1.4	235–335	46–75	nd	1.1–1.7	nd
August	0.2–4.2	0.3–1.7	1.2–1.5	0.21–1.3	2.7–7.3	495–700	57–109	30–100	4.2–6.5	2.37 ± 0.83

[a]Measured only at the diatom sediment station

RESULTS

Concentrations and turnover of C and N

Pelagic concentrations of ammonium, urea and DFAA were within the same range in April and August (Table 1). DCAA concentrations, however, were significantly higher ($p < 0.05$) and nitrate concentrations significantly lower in August than in April ($p < 0.01$; Table 1). The concentration of TOC increased by a factor of 2 to 6 from April to August, while POC concentrations only showed a slight increase (Table 1), suggesting that the DOC concentration had increased. DFAA and DCAA constituted less than 1% of DOC at both sampling periods. POC constituted from 10 to 20% of the total pool of organic carbon. Pelagic chlorophyll concentrations were 3 to 4 times higher in August than in April. The chlorophyll concentration in the diatom sediment was 2.4 μg chl a cm^{-2} (Table 1). Unfortunately, the chlorophyll concentration in the bare sandy sediment was not measured.

The bacterial C and N demand and the uptake of DFAA were 5 to 10 times higher in August than in April, while DCAA and urea utilization were more than 50-fold higher (Fig. 1, Tables 2 & 3). Thus, compared to DFAA, the relative importance of the DCAA and urea as C and N sources for the bacteria increased from April to August.

Effect of sediment type on C and N fluxes and bacterioplankton activity

In April, the effect of sediment on the pelagic microbial activities was limited (Table 2). The bare sediment significantly ($p < 0.01$) stimulated the pelagic DFAA uptake during the night (Table 2), while the bacterial production was positively affected by the eelgrass sediment at night ($p < 0.05$; Table 2). Other parameters were not significantly affected (Table 2).

Bacterial uptake of C and N (DFAA + DCAA + urea) did not differ significantly ($p > 0.05$) between bare sediment and eelgrass sediment in April (Fig. 1A–D). Measured C uptake rates varied between 160 and 240 nmol C l^{-1} h^{-1}. The bacterial C and N demand, however, was almost 3 times higher in the eelgrass sediment incubation than in the bare sediment incubation (Fig. 1A–D). A net flux of 2.7 ± 0.1 μmol DOC l^{-1} h^{-1} from the eelgrass sediment into the water column (Fig. 1B) may have supported the increase in C demand (34 nmol l^{-1} h^{-1}; Table 2), which corresponded to 1.3% of the DOC input. The discrepancy between the N uptake and the N demand probably indicates a mineralization of DON, as discussed later.

In August, the eelgrass sediment significantly stimulated the DFAA ($p < 0.05$) and DCAA ($p < 0.01$) assimilation in addition to the bacterioplankton production ($p < 0.05$; Table 3). The bare sediment significantly stimulated the DCAA uptake ($p < 0.05$; Table 3). This stimulation, however, was approximately 4 times smaller than for the eelgrass sediment (Table 3).

Similar to in April, bacterial C and N demands in August were significantly higher ($p < 0.05$) in the eelgrass sediment incubation (3250 ± 352 nmol C l^{-1} h^{-1}) than in the bare sediment incubation (1033 ± 135 nmol C l^{-1} h^{-1}) (Fig. 1). The bacterial C uptake in the eelgrass sediment incubation was not sufficient to sustain the C demand (Fig. 1F). However, similar to the experiment in April, a significant ($p < 0.05$) net DOC flux from the eelgrass sediment (22 ± 4 μmol l^{-1} h^{-1}) was observed (Fig. 1F). Despite this input of DOC, and a net efflux of DON from the eelgrass sediment (Fig. 1H), the bacterial C and N uptake (DFAA + DCAA + urea) at this station was not significantly different from the bare sediment (Fig. 1E–H). As in the April experiments, a divergence between uptake and demand of N was observed.

The effect of the diatom sediment was more complex than described above for the bare sediment and the eelgrass sediment (Table 3, Fig. 2). During the day, a significant ($p < 0.05$) positive effect on the DFAA and DCAA uptake rates and a stimulation of the bacterial production were found (Table 3). Urea turnover, on the other hand, was significantly reduced ($p < 0.05$). During the night, DCAA turnover was stimulated ($p < 0.05$) by the sediment, however, both DFAA uptake and bacterial production were reduced significantly ($p < 0.01$) relative to the controls without sediment contact (Table 3).
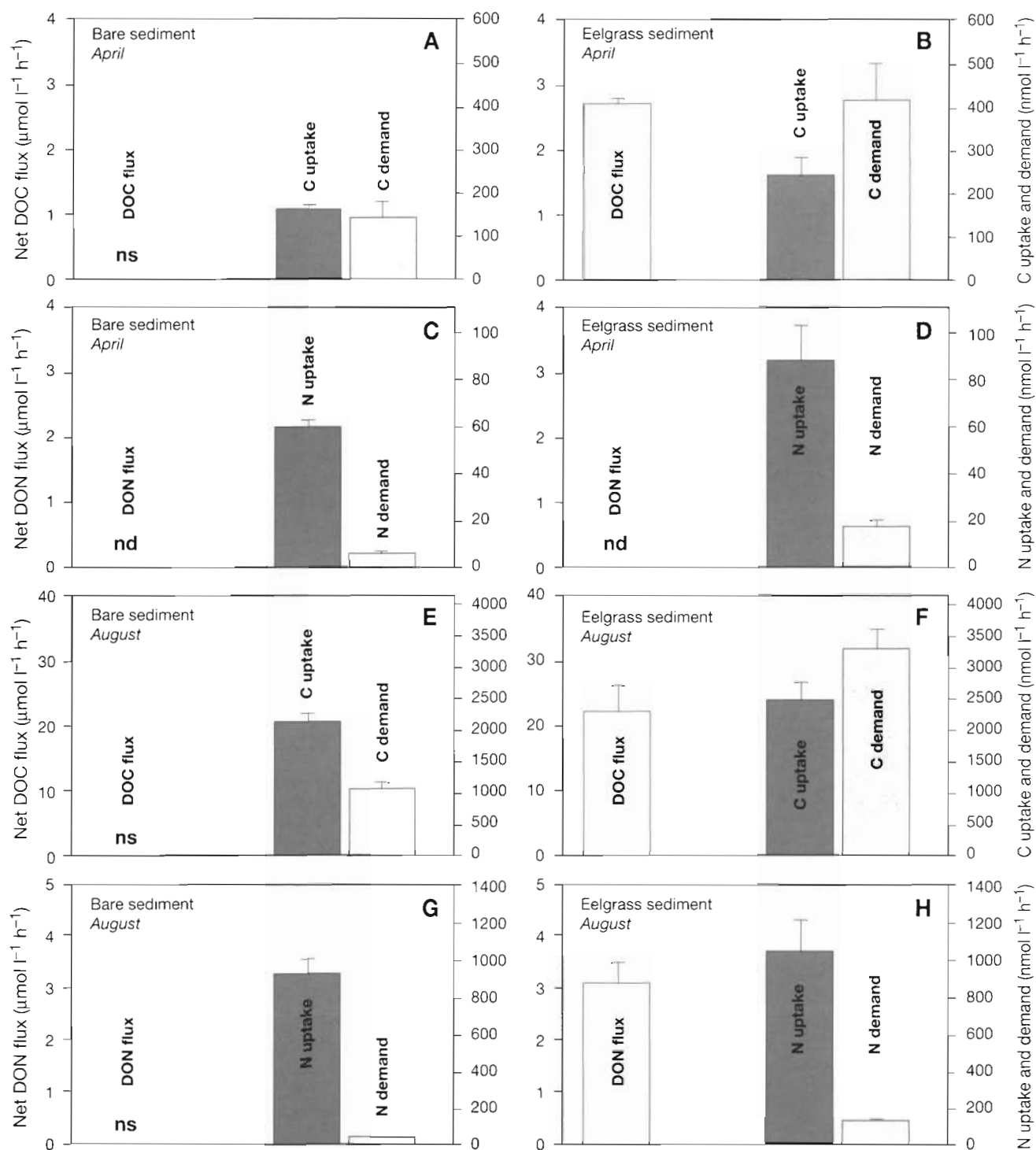
Fig. 1. Bacterial C and N uptake and demand and net fluxes of DOC and DON in the bare sediment and eelgrass sediment chambers from the April and August experiments. Rates are given as averages of the day and night values. Bacterial uptake of C and N was calculated as the sum of the uptake of DFAA, DCAA and urea, assuming an average C and N content of 3.1 C atoms and 1.1 N atoms per amino acid. Bacterial C and N demand was calculated on the basis of cell production, using the measured cell biovolumes and the estimated carbon conversion factor. In addition, a growth efficiency of 20% (Middelboe et al. 1992) and a C:N ratio of 5 (Kroer 1994) were applied. Fluxes in DOC and DON between the sediment and water column were calculated by subtracting concentration changes in the controls from concentration changes in the water phase of the sediment chambers. (A–D) April experiments. (E–H) August experiments. Notice the different scales on the y-axes for flux rates and the rates of bacterial uptake and demand. Bars indicate + SD. ns: not significant; nd: not determined

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Table 2. Bacterioplankton production and turnover of DFAA, DCAA and urea, and the effect of sediment on the bacterial activities in the bare sediment and the eelgrass sediment in the April experiment (± SD). The effect of sediment on the pelagic bacterial turnover of DFAA, DCAA and urea was assessed by subtracting the change in bacterial activity in the control bags from the change in activity in the sediment chambers during the incubation. Only significant effects are shown. Statistical significance (2 sample t-test): $^*p < 0.05$, $^{**}p < 0.01$; ns: not significant; nd: not determined

| | DFAA uptake (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | DCAA uptake (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | Urea hydrolysis (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | Bacterial production (10^6 cells l^{-1} h^{-1}) | Effect of sediment | |
								(10^6 cells l^{-1} h^{-1})	(nmol C l^{-1} h^{-1})[a]
Bare sediment									
Day	25 ± 2	ns	4.7 ± 1.3	ns	0.21 ± 0.05	ns	9.4 ± 1.0	ns	ns
Night	73 ± 1	5.6 ± 0.1**	3.2 ± 0.9	ns	0.94 ± 0.14	ns	23 ± 4	ns	ns
Eelgrass sediment									
Day	93 ± 4	ns	nd	nd	1.32 ± 0.16	ns	42 ± 2	ns	ns
Night	63 ± 13	ns	nd	nd	0.50 ± 0.10	ns	53 ± 10	3.8 ± 1.4*	34 ± 13*

[a]Effect on bacterial C demand was calculated on basis of the measured cell biovolumes and the estimated carbon conversion factor, using a bacterial growth efficiency of 20% (Middelboe et al. 1992)

The bacterial C and N demand in the diatom sediment were 2800 nmol C l^{-1} h^{-1} and 112 nmol N l^{-1} h^{-1}, respectively, during the day (Fig. 2A, C), i.e. significantly higher than for the bare sediment (p < 0.05). Similar to the eelgrass sediment, the C uptake could not support the C demand during the day, however, the high C demand coincided with the occurrence of a net flux of DOC from the sediment (19 µmol l^{-1} h^{-1}) (Fig. 2A).

Although a turnover of DFAA and DCAA was demonstrated using radioisotopes and Leu-MCA, no significant net fluxes of DFAA or DCAA across the sediment were found at any of the sediment types (not shown). However, a significant efflux of ammonium was observed in the experiments with the diatom sediment (Fig. 3). During the day, concentrations increased by 2.3 µM in the sediment chamber but

decreased by 1.2 µM in the control, resulting in a net flux of ammonium from the sediment of 0.89 µmol NH_4^+ l^{-1} h^{-1} (Fig. 3A). During the night, the increase in ammonium concentration in the sediment chamber was 6.2 µM and the decrease in the control 1.2 µM (Fig. 3B), giving a net ammonium flux of 2.1 µmol NH_4^+ l^{-1} h^{-1}. There were no significant fluxes of nitrate or urea in these experiments (Fig. 3A, B). The net DON release in the diatom sediment incubation was 2.1 to 3.5 times higher than the net DIN release (Fig. 3).

Diel differences in pelagic bacterial C and N turnover

In the bare sediment experiments, the bacterial production was significantly higher (p < 0.05) at night than

Table 3. Bacterioplankton production and turnover of DFAA, DCAA and urea, and the effect of sediment on the bacterial activities in the bare sediment, the eelgrass sediment, and the diatom sediment in the August experiment (± SD). The effect of sediment on the pelagic bacterial turnover of DFAA, DCAA and urea was assessed by subtracting the change in bacterial activity in the control bags from the change in activity in the sediment chambers during the incubation. Only significant effects are shown. Statistical significance (2 sample t-test): $^*p < 0.05$, $^{**}p < 0.01$; ns: not significant

| | DFAA uptake (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | DCAA uptake (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | Urea hydrolysis (nmol l^{-1} h^{-1}) | Effect of sediment (nmol l^{-1} h^{-1}) | Bacterial production (10^6 cells l^{-1} h^{-1}) | Effect of sediment | |
								(10^6 cells l^{-1} h^{-1})	(nmol C l^{-1} h^{-1})[a]
Bare sediment									
Day	292 ± 19	ns	301 ± 31	22 ± 12*	9.5 ± 0.1	ns	87 ± 4	ns	ns
Night	438 ± 29	ns	330 ± 11	ns	166 ± 350	ns	150 ± 15	ns	ns
Eelgrass sediment									
Day	375 ± 46	ns	462 ± 17	84 ± 12**	141 ± 59	ns	339 ± 16	27 ± 6*	240 ± 53*
Night	447 ± 11	58 ± 7*	297 ± 79	ns	36 ± 22	ns	404 ± 37	79 ± 19*	688 ± 166*
Diatom sediment									
Day	303 ± 45	58 ± 12*	89 ± 30	16 ± 8*	8.1 ± 1.7	−6.7 ± 2.6	319 ± 37	23 ± 11*	197 ± 94*
Night	181 ± 23	−122 ± 9**	82 ± 13	12 ± 5*	5.8 ± 0.9	ns	124 ± 6	−11 ± 2**	−95 ± 17**

[a]Effect on bacterial C demand was calculated from the carbon conversion factor obtained in the April experiment, using a bacterial growth efficiency of 20% (Middelboe et al. 1992)
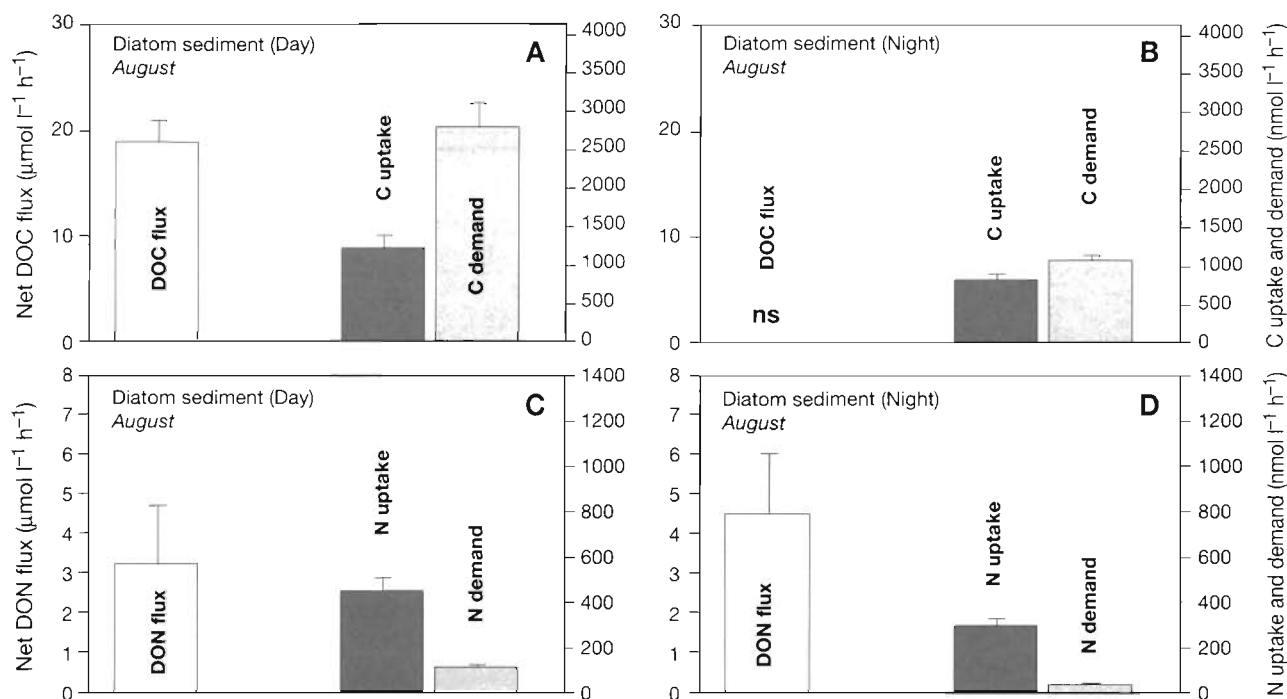
Fig. 2. Bacterial C and N uptake and demand and net fluxes of DOC and DON in the diatom sediment chambers in the August experiment. Values from both day and night experiments are presented. Uptake, demand and flux were calculated as described in the legend for Fig. 2. (A, C) Day experiment. (B, D) Night experiment. Notice the different scales on the y-axes for flux rates and the rates of baterial uptake and demand. Bars indicate + SD. ns: not significant

during the day in both April and August (Tables 2 & 3). This enhanced production at night was in both seasons associated with significantly higher uptake of DFAA (p < 0.05; Tables 2 & 3). Also, urea turnover was higher at night on bare sediment (p < 0.01; Tables 2 & 3). The eelgrass sediment experiments, on the other hand, did not reveal significant diel variations in the measured microbial activities (p > 0.05; Tables 2 & 3).

In the diatom sediment experiment, diel variation in bacterial DFAA and DCAA uptake in the sediment chambers was significantly enhanced relative to the control in the day experiment (p < 0.05) (Table 3). Urea uptake, on the other hand, was significantly reduced (p < 0.05). The bacterial C and N demand was about 3 times higher in the daytime than during the night (p < 0.05; Fig. 2). Accordingly, DFAA uptake was lowest at night (Table 3).

DISCUSSION

Effects of sediment on pelagic C and N concentrations and turnover

This study shows that the sediments may have a significant impact on nutrient turnover in the overlying water. In the eelgrass and diatom sediment experi-

ments (day expt), the bacterioplankton production was significantly affected by the sediment contact, and the production was significantly higher compared to the bare sediment (Tables 2 & 3).

The stimulation of bacterial production was, in all experiments, coupled with a net influx of DOC from the sediments. However, the increased production was not associated with an increased uptake of amino acids, nor was the sum of the uptake of C and N from DFAA, DCAA and urea significantly lower at the bare sediment than at the other sediments (Figs. 2 & 3). This suggested that the enhanced bacterial production was sustained by an input of labile DOM from the sediment and that this DOM had a low content of amino acids and urea.

The hypothesis that the bacterial production was sustained by an input of DOM other than amino acids and urea was supported by the relatively low contribution of DFAA and DCAA as C sources for the pelagic bacteria in the eelgrass and diatom sediment incubations compared to the bare sediment (Figs. 1 & 2). In the eelgrass and diatom sediments, DFAA and DCAA uptake could sustain 58% of the bacterial C demand in April (eelgrass sediment only) and 43 to 75% of the demand in August (Figs. 1 & 2). In the bare sediment, however, DFAA and DCAA contributed 114 and 204% to pelagic bacterial C demand in April and August,
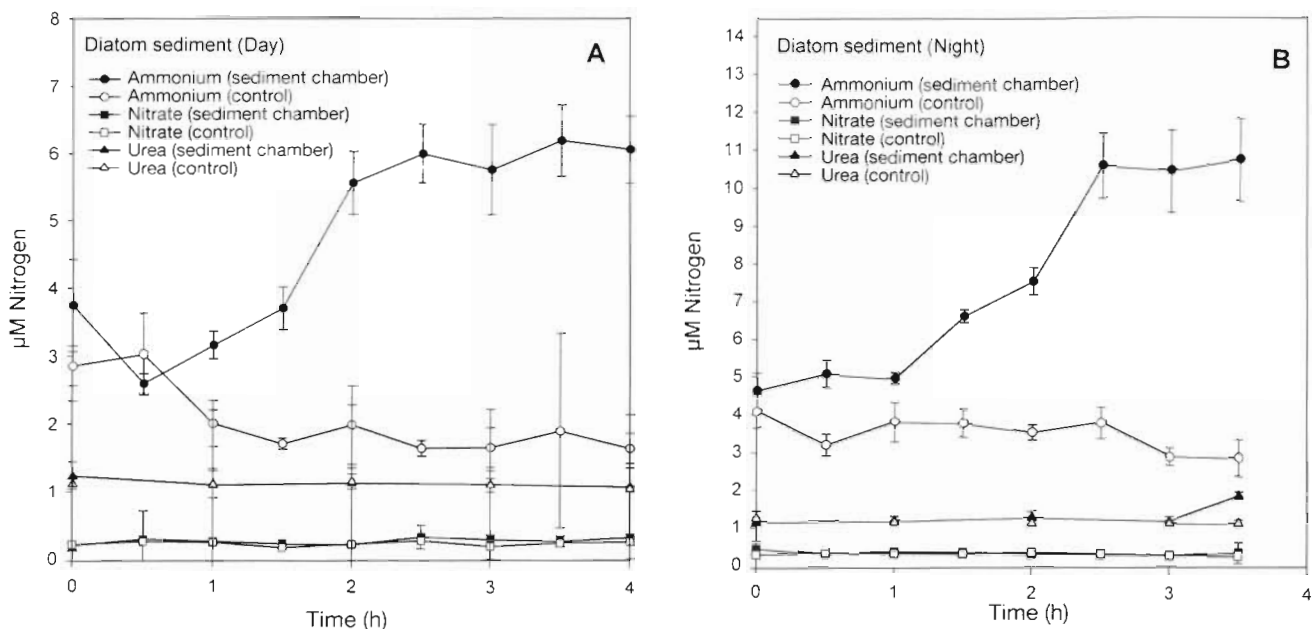
Fig. 3. Time course of the concentration of NH_4^+, NO_3^-, and urea in the sediment chambers and the controls during the (A) day and (B) night incubations at the diatom sediment station. Bars indicate ± SD

respectively (Fig. 1). It should be noted that the contribution of DCAA to bacterial C and N demand was based on bacterial hydrolysis of a model peptide (Leu-MCA) and was not a direct measure of bacterial DCAA uptake. The method assumed all the measured DCAA to be potential substrates for the extracellular aminopeptidase. Since only a part of the measured DCAA may constitute a substrate for bacterial extracellular hydrolysis (Keil & Kirchman 1993), rates of DCAA hydrolysis based on Leu-MCA are probably overestimated. On the other hand, the aminopeptidases as detected by Leu-MCA may not fully reflect total bacterial protease activity (Middelboe et al. 1995). Overall, Middelboe et al. (1995) suggested that the Leu-MCA hydrolysis approach may underestimate total DCAA hydrolysis.

The effect of the DOC release on the bacterial carbon demand (Tables 2 & 3) indicated that only a few percent of the DOC was taken up within the incubation period (2 to 7 h). Previous studies have demonstrated leaching of DOM from submerged macrophytes (Pakulski 1986, Turner 1993). Pakulski (1986) found that *Spartina alterniflora* in a salt marsh released significant amounts of monosaccharides, corresponding to 1–2% of the total DOC release from the plants. Based on the composition of the DOM and the rate of its release, the authors suggested that the losses of labile DOM from the macrophytes constitute an important substrate source for the pelagic bacteria (Pakulski 1986, Turner 1993). Similarly, microalgae have been shown to release low molecular weight

DOC, which was readily taken up by bacteria (Jensen 1983). These studies, thus, support our hypothesis that DOC, possibly as carbohydrates, was released from the benthic flora and subsequently taken up by pelagic bacteria.

In both April and August, bacterial N uptake (DFAA+DCAA+urea) exceeded their estimated N demand (Figs. 1 & 2). If ammonium was also used by the bacteria in the experiments as an additional N source (Kirchman et al. 1989, Middelboe et al. 1995) the measured N uptake may underestimate the bacterial N uptake. Consequently, the excess uptake of N relative to the bacterial demand may be even larger than indicated in Figs. 1 & 2. This apparent excess bacterial N uptake may indicate that bacteria acted as net mineralizers of N in the incubations. However, the rather invariable concentrations of inorganic N found in the experiments imply that, if the bacterioplankton mineralized the excess uptake of DON, the produced inorganic N must have been taken up immediately by the phytoplankton. The low pools of ammonium (April and August) and nitrate (August) may support a high demand for DIN by photosynthetic organisms. Another explanation for the unbalanced N budgets may be a bacterial release of methylamines and urea as suggested by Kroer et al. (1994). However, urea concentrations did not change significantly in the experiments. Phytoplankton is known to utilize urea as a source of N (Antia et al. 1991), and we cannot exclude that a portion of the measured urea uptake was due to phytoplankton. However, since urea-N was a minor

portion of the N uptake, an excess uptake of N would still occur.

Since bacterial carbon and nitrogen demand was higher at the eelgrass and diatom sediments, the bacteria retained 2 to 4 times more nitrogen in their biomass than at the bare sediment. Consequently, input of DOC from eelgrass and benthic algae may reduce the bacterial mineralization of DON and cause a temporary immobilization of nitrogen in pelagic bacterial biomass.

The portion of the DOM efflux that accumulated in the water column after the incubations had a C:N ratio of 5.9 to 6.1 (Figs. 1 & 2). Since there was no measurable net flux of DFAA, DCAA and urea in any of the experiments, the part of the DON flux that accumulated in the water must have consisted of other compounds than amino acids and urea. Assuming that stimulation of the bacterial DFAA and DCAA uptake (Tables 2 & 3) was caused by an amino acid input from the sediment, the released DFAA and DCAA constituted 5 to 6 % of the total DON flux (Figs. 1 & 2). Thus, only a small fraction of the dissolved organic material released from the sediment in this experiment seemed readily available for the pelagic bacteria, although it had a high nitrogen content. However, the relatively small input of labile substrate had a significant influence on the pelagic bacterial production. Previous studies of the composition of interstitial nitrogen pools in seagrass beds (Boon 1986) support this observation. Boon (1986) found that DON constituted the majority (75 to 85 %) of the interstitial N, and that primary amines only comprised 5 to 10 % of the total DON.

The significantly higher bacterial carbon demand and the direct positive effect of the eelgrass sediment and the diatom sediment (day expt) on the bacterial growth (Figs. 1 & 2, Tables 2 & 3) suggest that these 2 sediment types did influence pelagic bacterial turnover of carbon and nitrogen. On the other hand, the bare sediment seemed only to have minor effects on the pelagic nutrient turnover. Therefore, large horizontal variations in pelagic C and N metabolism may occur in shallow environments with variable eelgrass cover and sediment structure.

Diel variations

Significant diel variations in the pelagic C and N turnover were found only for the bare sediment and the diatom sediment (Tables 2 & 3, Fig. 2).

The increased bacterial production in the bare sediment incubations at night coincided with a significantly higher DFAA uptake. In fact, the increase in DFAA uptake from day to night could account for 70 to 100 % of the increase in bacterial C production, assum-

ing a carbon content of 3.1 C atoms per amino acid. This suggested that DFAA constituted the dominant carbon source for the bacteria at the bare sediment.

For the diatom sediment, bacterial production was highest during the day (Table 3) and, similar to the bare sediment, the higher bacterial production coincided with a higher DFAA uptake. In this case, however, DFAA uptake could only sustain about 20 % of the increase in C demand. This underlined the hypothesis that DOC input other than DFAA from the sediment in the day experiment was the primary cause for the increased C demand (Table 3, Fig. 2). Thus, diel variations in bacterial production may be ascribed to changes in the input of labile DOC and amino acids from the sediment.

The higher net efflux of ammonium during the night incubations in the diatom sediment was probably due to an increased ammonium release from the sediment, as the ammonium uptake in the control incubations was similar during day and night (Fig. 3). This enhanced efflux of ammonium at night may be due to an increased mineralization in the sediment or to a reduced uptake of ammonium by benthic microalgae.

Conclusion

Our data show that exchange of organic matter in the sediment-water interface may have a strong influence on the pelagic bacterial production and on the composition of the substrates utilized by the bacterioplankton. Furthermore, the effects of the sediment on pelagic C and N turnover appeared to be influenced by the presence and activity of benthic microalgae and macrophytes. We suggest that release of biodegradable DOM from these organisms stimulates the bacterioplankton production, resulting in a temporary immobilization of N in pelagic bacterial biomass. Finally, our data confirm that DON may be the dominant form of nitrogen released from sediments.

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