

## Benthic nitrogen fluxes along a depth gradient in a microtidal fjord: The role of denitrification and microphytobenthos

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### Abstract

In littoral sediments, microphytobenthic (MPB) nitrogen assimilation often exceeds nitrogen removal by denitrification, partly because MPB activity suppresses denitrification. Little is known about the balance between these two processes at sublittoral depths. Benthic pigment composition, light and dark oxygen, and nutrient fluxes ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , dissolved organic nitrogen (DON),  $\text{PO}_4^{3-}$ ,  $\text{Si}(\text{OH})_4$ ), as well as denitrification were measured between 1 and 15 m in depth in Gullmar Fjord (Skagerrak) in spring and autumn. The hypothesis was that the assimilation/denitrification ratio would decrease with depth, along with decreasing MPB activity caused by light limitation. MPB photosynthesis occurred along the entire depth gradient, although sediments were net autotrophic only above 5 m. Inorganic nitrogen (DIN) (and silica) flux changed along the depth gradient, the general pattern being sediment uptake at  $\leq 5$  m and efflux at  $\geq 10$  m depth. DON flux ( $\sim 50\%$  of total dissolved nitrogen flux) showed a less clear pattern. Two trends regarding DIN fluxes and denitrification—significant light effects and negative correlations with gross primary productivity—showed that MPB activity influenced nitrogen (N) turnover. Although denitrification increased with depth, rates remained low ( $< 0.4 \text{ mmol N m}^{-2} \text{ d}^{-1}$ ), and MPB assimilation ( $0.2\text{--}3.6 \text{ mmol N m}^{-2} \text{ d}^{-1}$ ) exceeded or equaled denitrification. MPB incorporated  $\sim 35\%$  of the remineralized N along the depth gradient, whereas denitrification removed  $\sim 20\%$ . Thus, the influence of MPB on benthic nitrogen turnover, denitrification included, extends to sublittoral depths. Further, denitrification does not necessarily remove more N in the deeper, heterotrophic part of the photic zone, compared to the littoral, autotrophic zone.

In the coastal zone, sediments are important sites for remineralization of nutrients, and their role as nutrient sources for neritic production is well documented (Cowan and Boynton 1996). Substantial removal of nitrogen (N) by denitrification also occurs in these sediments (Laursen and Seitzinger 2002). In shallow-water sediments that are illuminated, nutrient turnover is complicated by the presence of benthic primary producers. Although denitrification is, along with anaerobic ammonium oxidation (Thamdrup and Dalsgaard 2002), the only known process that removes N permanently from the ecosystem, a potentially important internal sink of N in shallow land-margin areas is temporal retention by benthic primary producers (e.g., Dudley et al. 2001; McGlathery et al. 2001).

In cool microtidal areas, microphytobenthos (MPB) are, in contrast to macroscopic primary producers such as sea-grasses and macroalgal mats, active throughout the year

(e.g., Kristensen 1993, Sundbäck and Miles 2000). Therefore, N incorporation in MPB can be an important N-retaining mechanism on an annual scale (Sundbäck and Miles 2000; Risgaard-Petersen 2003). Efficient N retention by MPB and low denitrification rates seems to be a combination particularly likely in low-nitrogen, northern-climate microtidal areas. Generally, the effect of light and MPB activity on denitrification is complicated. Oxygen production by MPB photosynthesis can stimulate nitrification-coupled denitrification in the sediment by stimulating nitrification (Risgaard-Petersen et al. 1994; An and Joye 2001). However, if nitrogen levels are low, coupled denitrification is instead suppressed by MPB, because they compete with nitrifying bacteria for the limited nitrogen (Rysgaard et al. 1995; Risgaard-Petersen 2003). This latter scenario occurs in cool microtidal areas, especially in sandy sediments, where assimilation by MPB may exceed denitrification by one to two orders of magnitude (Sundbäck and Miles 2000). This is in contrast to what has been found for muddy intertidal sediments, where denitrification often equals or exceeds N assimilation by MPB (Ogilvie et al. 1997; Cabrita and Brotas 2000; Dong et al. 2000). However, the tentative conclusion that predicts a high MPB assimilation/denitrification ratio (A/D) in cool microtidal waters is based on measurements in the littoral zone ( $< 1$  m), and it is not known whether high A/D ratios also apply in the deeper parts of the photic zone.

In clear waters, substantial MPB activity can occur at depths far below 10 m (Cahoon 1999; Nelson et al. 1999; Gillespie et al. 2000; Welker et al. 2002). Not only does

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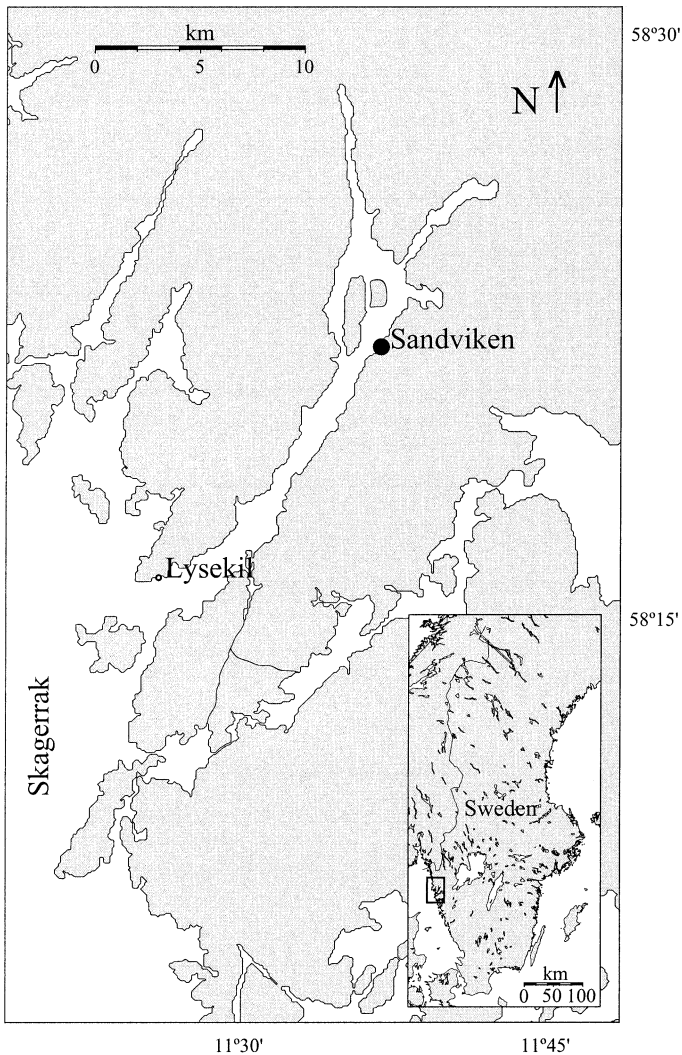


Fig. 1. The study site in Gullmar Fjord, Skagerrak.

MPB influence benthic oxygen exchange at sublittoral and subtidal depths (Granéli and Sundbäck 1986; Glud et al. 2002), but it also has an impact on the sediment–water nutrient fluxes (Sundbäck et al. 1991; Jahnke et al. 2000). Because MPB activity has been found to depress denitrification rates (Risgaard-Petersen 2003 and references therein), it is hypothesized that the importance of denitrification, relative to MPB retention of nitrogen, will gradually increase with depth, as a result of light limitation of benthic photosynthesis.

Our aim was to study the role of MPB and denitrification for nutrient turnover along a depth gradient in a temperate microtidal water body, Gullmar Fjord in the Skagerrak (Fig. 1). Although Gullmar Fjord is a well-studied marine water body, in which research has been ongoing since the late 19th century, the functional role of benthic microbiota for the ecosystem has rarely been studied. Our working hypothesis was that there is a shift in the balance between MPB assimilation and denitrification in the sediment along the depth gradient, so that denitrification becomes more important when nearing the lower parts of the photic zone. Our ap-

proach was to measure nutrient and oxygen fluxes across the sediment–water interface and to compare denitrification in the light versus dark; we also measured the content of photosynthetic pigments in the sediment and concentrations of carbon and nitrogen. These measurements were performed along a depth gradient from 1 to 15 m in both cold (spring) and warm (early autumn) seasons.

## Material and methods

*Site and sampling*—Gullmar Fjord, on the west coast of Sweden, is microtidal (amplitude 20–30 cm) and stratified, with pycnoclines (at 10 to 20 and ~50 m in depth) separating three water layers. A depth gradient from 1 to 15 m was sampled at Sandviken (58°22'N, 11°37'E), a bay located on the east side of the fjord (Fig. 1). This site was chosen because all sampling depths could be easily reached by divers ( $\leq 100$  m from shore) and because a previous study along the same gradient had revealed a vital microbenthic community to a depth of at least 17 m (Wulff et al. in press).

Sediment cores were taken in spring (24 and 26 April) and in autumn (18 and 24 September) in 2001 (two depths could be sampled on the same day). The two seasons were chosen to provide conditions with cold and warm water, respectively, and these dates gave sufficient incident irradiance to support MPB activity below the littoral zone. Cores were taken by SCUBA divers, who used Plexiglas core barrels (see below) at 1, 5, 10, and 15 m depths. The sampling design was hierarchical: At each depth, two stations (Sta. A and Sta. B) were sampled; at each station divers took two to five cores per measuring variable and station. For flux and denitrification measurements, sediment was collected with 24-cm-long Plexiglas tubes (inner diameter [i.d.] 8 cm; 12 cm of overlying water, 5 cores per station, 40 cores for the entire gradient), whereas samples for pigments (3 mm deep) and sediment characteristics (5 mm deep) were taken from smaller corers (i.d. 4.6 or 6.7 cm). Bottom water was collected at each sampling depth for flux incubations in the laboratory. In situ light (PAR) was measured using a LiCOR LI-1400 light meter. Subsamples for pigments and sediment characteristics were taken immediately in the field, whereas cores for flux measurements were kept cool while they were transported to the laboratory in insulated boxes.

*Pigments and algal composition*—For analyses of photosynthetic pigments, two small cores (i.d. 4.7 cm) were sampled from each station. From each core, two samples of the top 3 mm (ca. maximum depth of the sediment photic zone; Köhl et al. 1994) were taken with a cut-off 2-ml (i.d. 8.7 mm) disposable syringe. Samples were immediately frozen in liquid nitrogen ( $-196^{\circ}\text{C}$ ) and transferred to a low-temperature freezer ( $-80^{\circ}\text{C}$ ). These were later analyzed by high-performance liquid chromatography (HPLC) (Wright and Jeffrey 1997) using a diode-array detector (Spectraphysics UV6000LP). The samples were extracted in a 2-ml mixture of 80% acetone and 20% methanol for 36 h at  $-20^{\circ}\text{C}$  and were ultrasonicated for 5 min (continuous cooling with ice). Samples from 1–10 m were diluted by adding 500  $\mu\text{l}$  MeOH to 1 ml of sample, and samples from 15 m were diluted by adding 500  $\mu\text{l}$  to 500  $\mu\text{l}$  of sample. Pigments were identified

and quantified as described in Wright and Jeffrey (1997) using commercial standards (International Agency for  $^{14}\text{C}$  Determination, Hørsholm, Denmark).

To check for dominating species of MPB, thin surface-sediment samples were scanned under an epifluorescence microscope. No attempt was made to count algal cells.

**Flux measurements**—For practical reasons, we used incubations under controlled laboratory conditions following the recommendations of Dalsgaard et al. (2000). Moreover, in a previous study, simultaneous laboratory and in situ incubations yielded similar results (Sundbäck et al. 2003). Oxygen and nutrient fluxes ( $\text{NO}_3^- + \text{NO}_2^-$  (referred to as  $\text{NO}_3^-$ ),  $\text{NH}_4^+$ , DON,  $\text{PO}_4^{3-}$ , and  $\text{Si}(\text{OH})_4$ ), as well as denitrification, were measured at in situ temperature under light and dark conditions. The incubation system was previously described in detail (Sundbäck and Miles 2000). Upon delivery to the laboratory, cores were placed into incubation tanks with circulating ( $20 \text{ L min}^{-1}$ ) unfiltered site water, with stirring (magnets,  $\sim 60 \text{ rpm}$ ) but without lids, and were left overnight in a temperature-controlled room. Before incubation started, cores were preincubated at the experimental light level (see below) for 1 h, and the headwater in the cores was exchanged. Three replicate cores and two blank cores (site water only) were incubated for each station (two stations with three replicates = six replicates per depth). Light and dark incubations were made using the same cores. Oxygen was measured three to four times during the incubation, and the incubation time for nutrient flux (samples taken at the start and end of incubation) was set so that changes in  $\text{O}_2$  concentration did not exceed 20% (between 3 and 6 h, depending on sediment type and temperature). Oxygen samples were taken with 20-ml glass syringes, and nutrient samples with 50-ml disposable syringes through a tube in the transparent Plexiglas lid. Nutrient samples were immediately frozen at  $-80^\circ\text{C}$ . Light intensities used during the light incubations were set to match the daily mean incident radiation for the specific time period (Danish Institute of Agricultural Sciences, Research Center Foulum, Denmark) by applying extinction coefficients from in situ light measurements obtained during sediment sampling. In April, incubation lights (individual halogen bulbs over each core) were adjusted to give approximately 25, 10, 10, and  $5 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  at the sediment surface for the 1-, 5-, 10-, and 15-m depths, respectively. These low light levels were due to high turbidity in the surface layer close to the shore during the spring sampling period (see Results). In September, the incubation irradiances were 120, 40, 22, and  $6 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , respectively.

Oxygen concentrations were measured using Winkler titration. Oxygen flux in the light was used as a measure of net primary production (NPP), and gross primary production (GPP) was calculated by subtracting community respiration in the dark (CR is a negative value) from NPP, assuming for practical reasons that respiration in the light equaled respiration in the dark. Because the irradiance used for the incubations represented a daily mean of the light hours of each month, daily values of NPP and GPP were calculated by multiplying hourly values by the number of daylight hours. Daily CR was calculated by multiplying hourly values by

24. The trophic status (balance between autotrophy and heterotrophy) of the sediment at each depth was assessed by applying the Trophic Oxygen Status Index (TOSI) (Viaroli and Christian 2004), derived from the four-scale Benthic Trophic Status Index (BTSI) (Rizzo et al. 1996, see also Risgaard-Petersen 2003), where a score of 3 is fully autotrophic, 2 is net autotrophic, 1 is net heterotrophic, and 0 is fully heterotrophic. In practice, TOSI is derived graphically by plotting individual core values of NPP against CR (Vi-aroli and Christian 2004).

Nutrients were analyzed on a TRAACS autoanalyzer (Braun-Lubbe). Total dissolved nitrogen (TDN) was measured by oxidation to  $\text{NO}_3^-$  (Valderrama 1981), and DON was calculated by subtracting DIN from TDN. Daily (24-h) rates of nutrient fluxes were calculated by multiplying the hourly rate in light times the number of daylight hours and the hourly rate in the dark times the number of dark hours and adding these two values.

**Calculation of MPB assimilation of N**—Calculation of actual algal assimilation is difficult because of excess uptake, as well as leakage of carbon (C) and N by living cells. We calculated N assimilation by MPB in a two-step calculation. Daily oxygen production was converted to fixed C using a photosynthetic quotient (PQ) of 1.2 (for justification of this quotient, see Kirk 1994; Glud et al. 2002). Fixed C was further converted to assimilated N by assuming a C:N molar ratio of 9, the average found for natural benthic microalgal communities (Sundbäck and Miles 2000; Hillebrand and Kahlert 2002). Assimilation values calculated from NPP ( $A_{\text{NPP}}$ ) provide a conservative measure of the MPB net N demand (Sundbäck and Miles 2000), but most probably underestimate the actual N uptake, since the NPP value is not only affected by algal respiration, but also by the respiration of the whole benthic community (CR). Therefore, we will here base the calculation of microphytobenthic N assimilation on 80% of GPP ( $A_{\text{GPP}80}$ ), assuming that algal respiration is 20% of GPP (Brotas and Catarino 1995). However, for autotrophic sites, we will also show values of  $A_{\text{NPP}}$ , to make it possible to compare with previously published  $A_{\text{NPP}}$  values for littoral sites (Sundbäck and Miles 2000, 2002).

**Denitrification**—Sediment denitrification was measured using the isotope-pairing technique (Nielsen 1992). Although this method was originally developed to discriminate between denitrification supported by  $\text{NO}_3^-$  from the water column ( $D_w$ ) and nitrification-coupled denitrification ( $D_n$ ) in the sediment, for the purpose of this study, only total denitrification rates ( $D_{\text{tot}}$ ) will be considered. Denitrification in light and dark was measured on separate cores (two cores each), since the method involves destructive sampling. The cores were incubated in the same system used for the nutrient fluxes, and samples were treated as described by Dalsgaard et al. (2000). In short,  $^{15}\text{NO}_3^-$  ( $20 \text{ mmol L}^{-1} \text{ Na}^{15}\text{NO}_3$ , 99.6 atom %, Europa Scientific Ltd.) was added to the headspace water to a final concentration of  $\sim 40 \mu\text{mol L}^{-1} \text{ }^{15}\text{NO}_3^-$ . The  $\text{NO}_3^-$  concentration was measured prior to the addition of  $^{15}\text{NO}_3^-$  and at the time that the cores were closed (after  $\sim 0.5 \text{ h}$  of the addition of  $^{15}\text{NO}_3^-$  to allow the label to diffuse into the anoxic layer) in order to calculate the  $^{14}\text{N}$ :

Table 1. Characteristics of the sites during the sampling periods in April and September. Water column characteristics are for water overlying the sediment surface. Shown are mean values. TDN = total dissolved nitrogen.

Depth (in m)	Apr				Sep			
	1	5	10	15	1	5	10	15
Water column								
Salinity	22	30	31	33	30	30	30	30
Temperature (°C)	9	8	8	7	14	14	14	14
TDN ( $\mu\text{mol L}^{-1}$ )	38.9	50.2	28.5	23.9	22.8	26.4	25.6	16.7
$\text{NH}_4^+$ ( $\mu\text{mol L}^{-1}$ )	1.6	1.3	0.7	0.8	0.4	0.3	0.2	<0.1
$\text{NO}_3^- + \text{NO}_2^-$ ( $\mu\text{mol L}^{-1}$ )	9.8	0.82	3.4	9.3	7.0	4.8	1.1	1.2
DON ( $\mu\text{mol L}^{-1}$ )	27.5	48.1	25.4	14.0	15.4	21.3	24.3	15.5
DON as % of TDN	71	96	86	58	68	81	95	93
$\text{PO}_4^{3-}$ ( $\mu\text{mol L}^{-1}$ )	<0.1	<0.1	<0.1	0.9	<0.1	<0.1	0.2	0.3
$\text{Si(OH)}_4$ ( $\mu\text{mol L}^{-1}$ )	22.7	1.7	5.4	8.7	8.6	8.8	4.5	4.2
Sediment (top 5 mm)								
Water content (% weight)	0.33	0.40	0.33	0.24	0.36	0.40	0.37	0.22
C content (% weight)	1.64	1.73	1.58	0.67	0.7	0.9	0.86	0.4
N content (% weight)	0.11	0.16	0.16	0.03	0.06	0.1	0.17	0.06
C:N ratio (molar)	17.4	12.9	11.2	23.8	13.4	10.6	5.9	7.7
% silt (<63 $\mu\text{m}$ )	30.5	18	17.5	17				

$^{15}\text{N}$  ratio in the  $\text{NO}_3^-$  pool. For the extraction of the  $\text{N}_2$  samples, 2 ml  $\text{ZnCl}_2$  (1 g  $\text{ml}^{-1}$ ) was added to the water phase, gently but quickly mixing it with the sediment. Part of the slurry was transferred to a 12.5-ml gas-tight vial. The isotopic ratio in  $\text{N}_2$  was analyzed by mass spectrometry at the National Environmental Research Agency, Silkeborg, Denmark. The rate of denitrification was calculated according to the procedures and assumptions of Nielsen (1992). Daily denitrification was calculated in the same way as daily nutrient fluxes.

*Sediment characteristics*—Sediment water content (top 5 mm) was estimated gravimetrically by drying sediment to a constant weight at 60°C. Solid-phase total C and N were determined on dried samples (top 5 mm) using a Carlo Erba CHN elemental analyzer. Before analysis, samples for organic C content were treated with HCl to remove carbonates.

*Statistical analysis*—Spatial variation was analyzed using nested analysis of variance (ANOVA), with depth and station (nested within depths) as factors. When no statistical differences for a variable were found between stations within a depth, results will be shown only for depths. Differences between light and dark fluxes were tested using a two-way ANOVA, with treatment (L/D) and depth or station as main factors. Homogeneity of variances was checked using Cochran's test, and heterogeneous data were transformed according to Underwood (1997). When there was a significant interaction between factors, pairwise comparison of the means was done with Student–Newman–Keul's (SNK) test. Differences were accepted as significant when  $P < 0.05$ . Whenever the words “significant” or “significantly” appear in the text in connection with ANOVA or SNK, they denote a probability level of  $P < 0.05$ . The Pearson correlation analysis was used to look for correlations between variables, with  $r$  and  $P$  levels given in the text.

## Results

*Field conditions*—Although the mean daily incident radiation during daylight hours in April and September was similar (300–400  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), underwater light conditions at the two shallowest sites were different in April and September. As a result of a turbid layer of surface water in April—caused by runoff—the extinction coefficient ( $K_d$ ) was much higher close to the shore, ranging from  $>2 \text{ m}^{-1}$  at 1 m to  $<0.3 \text{ m}^{-1}$  at 15 m. However, light at all sampling depths was above the 1% level when compared to surface light. This resulted in small differences in the in situ (and hence incubation) light conditions along the depth gradient in April. In September, the water was clearer, with  $K_d$  varying from 1.2 to 0.28  $\text{m}^{-1}$  along the gradient.

Water column and sediment characteristics are shown in Table 1. Vertical differences in temperature (7–9°C) and salinity (22–33), as well as in nutrient concentrations, reflect the stratified water column in April (see Nutrient flux). In September, the temperature (14°C) and salinity (30) were the same at all depths as a result of a mixed water column.

The main sediment type (analyzed only in spring) along the depth gradient was silty sand, with the highest proportion of silt at 1 m (Table 1). The lower sediment water content at 15 m reflects a sandier sediment. Both C (0.4–1.7%) and N (0.03–0.17%) contents at the 1-, 5-, and 10-m sites were significantly higher in spring than in the autumn (Table 1). The particulate C:N molar ratio varied between 5.9 and 23.8 and was generally higher in spring (average 16.4) than in the autumn (average 9.4), the lowest ratios occurring at 10–15 m in the autumn. Sieving sediment after incubations showed the presence of cockles (*Cerastoderma* sp.) and polychaetes (*Nereis* sp.) at 1 and 5 m, while some specimens of *Cardioechinus* were present at the deeper sites.

*Microbenthic community*—In April, a community of small (<10  $\mu\text{m}$ ) and middle-sized (20–40  $\mu\text{m}$ ) pennate benthic

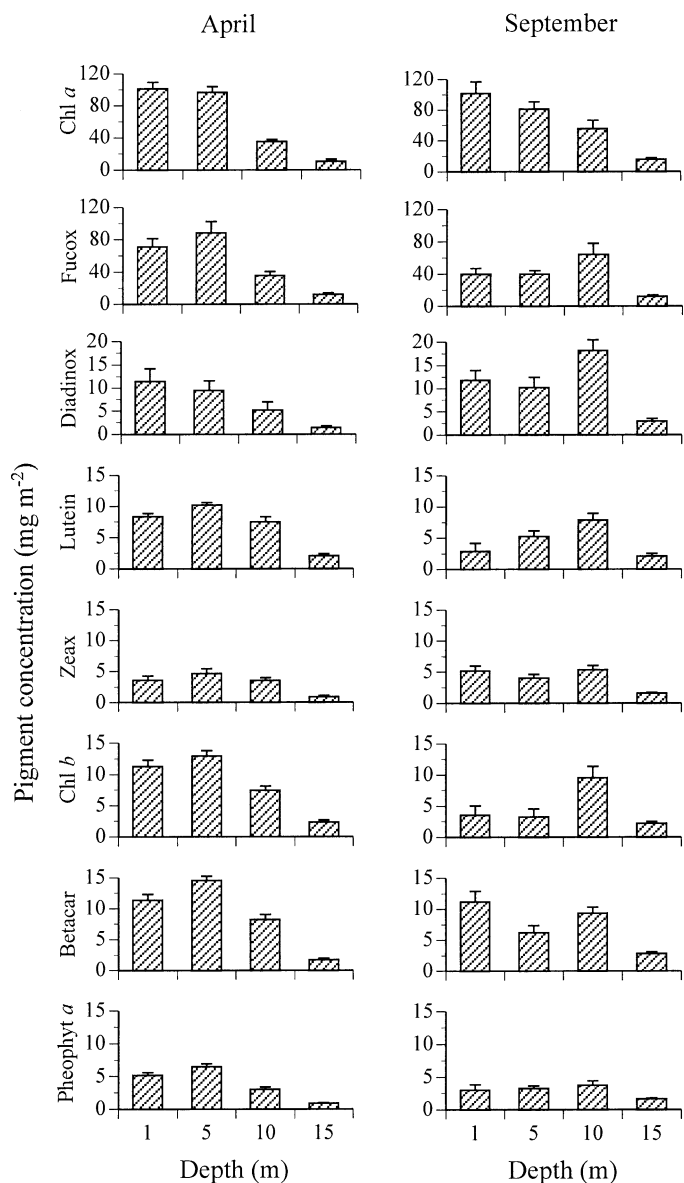


Fig. 2. Pigment concentrations ( $\text{mg m}^{-2}$ ) in the top 3 mm of sediment at four depths in Gullmar Fjord in April and September. Shown are means + SE,  $n = 8$ .

diatoms dominated at all depths (e.g., *Navicula* and *Nitzschia* spp.), together with *Surirella* sp. Deposited, but vital, specimens of the planktonic centric diatom *Skeletonema costatum* were common in April, particularly at 10 m. In September, well-developed microalgal mats occurred at all depths with the exception of 15 m. At 1 and 5 m, these mats consisted of a mixture of cyanobacteria (*Oscillatoria* sp.) and large-sized diatoms such as *Amphora decussata* and sigmoid species (mainly *Gyrosigma balticum*), which dominated the diatom biomass. At 10 m, there was a cohesive pure diatom mat dominated by *Pleurosigma* cf. *clevei*, *Pleurosigma formosum*, and *A. decussata*. At 15 m, there was no visible mat, but the biomass was dominated by species similar to those noted at 10 m.

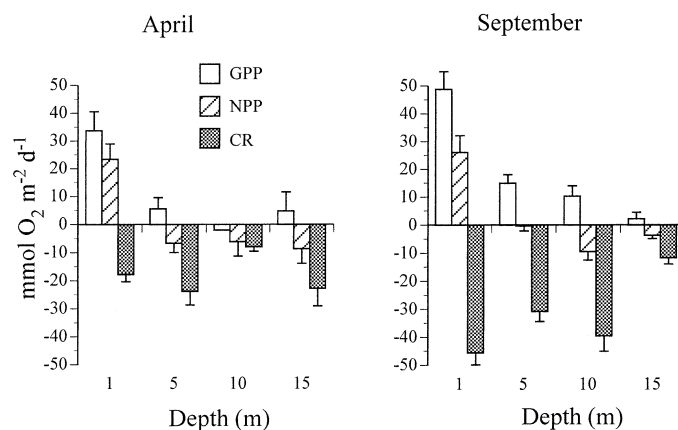


Fig. 3. Daily oxygen flux (mean + SE,  $n = 6$ ) between sediment and water at four depths in Gullmar Fjord in April and September. GPP = gross primary production, NPP = net primary production, CR = community respiration.

**Photosynthetic pigments**—Chlorophyll *a* (Chl *a*) content, which is here used as a proxy for MPB biomass, decreased with depth, but was within the same range ( $11\text{--}102 \text{ mg m}^{-2}$ ) in both April and September (Fig. 2). The only significant difference between the two seasons was a higher content of Chl *a* at 10 m in September. Additional photosynthetic pigments were found—the diatom pigments fucoxanthin (fucox) and diadinoxanthin (diadinox), the green algal pigments chlorophyll *b* (Chl *b*) and lutein, zeaxanthin (zeax, a marker pigment for both green algae and cyanobacteria), the chlorophyll breakdown product pheophytin *a* (pheophyt *a*), and betacarotene (betacar, common for all algal groups) (Fig. 2). As for Chl *a*, all other pigments had the lowest concentrations at 15 m (Fig. 2). Chromatograms revealed that diadinox from 10 and 15 m was subject to interference from unidentified chlorophyll breakdown products, and thus these values should be treated with caution. In April, the highest concentrations for all pigments except diadinox and Chl *a* were found at 5 m in depth (Fig. 2). In September, however, the highest concentrations were found as deep as 10 m for all pigments except Chl *a* and betacar (Fig. 2). In April, zeax correlated significantly and positively with lutein ( $r = 0.99$ ,  $P < 0.001$ ) and Chl *b* ( $r = 0.95$ ,  $P < 0.001$ ), indicating that zeax originated from green algae. In September, however, zeax indicated a cyanobacterial rather than green-algal source, as it correlated significantly with Chl *a* ( $r = 0.77$ ,  $P < 0.05$ ) but not with lutein ( $r = 0.62$ ,  $P > 0.1$ ) or Chl *b* ( $r = 0.64$ ,  $P > 0.1$ ). Fucox correlated well with Chl *a* in April ( $r = 0.97$ ,  $P < 0.001$ ) but not in September ( $r = 0.47$ ,  $P > 0.1$ ), when it correlated best with pheophyt *a* ( $r = 0.97$ ,  $P < 0.001$ ). These varying correlations indicate different degradation rates of pigments in spring and autumn.

**Oxygen flux and primary productivity**—Benthic oxygen production occurred at all depths (except at 10 m in April). However, a positive NPP was found only for the 1-m site (Fig. 3). Along the depth gradient, daily GPP varied between 49 and  $0 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$  (equivalent to  $490\text{--}0 \text{ mg C m}^{-2} \text{ d}^{-1}$ , PQ 1.2), while NPP varied between 26 and  $-9.3 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ . At 5 and 10 m, GPP was significantly higher in

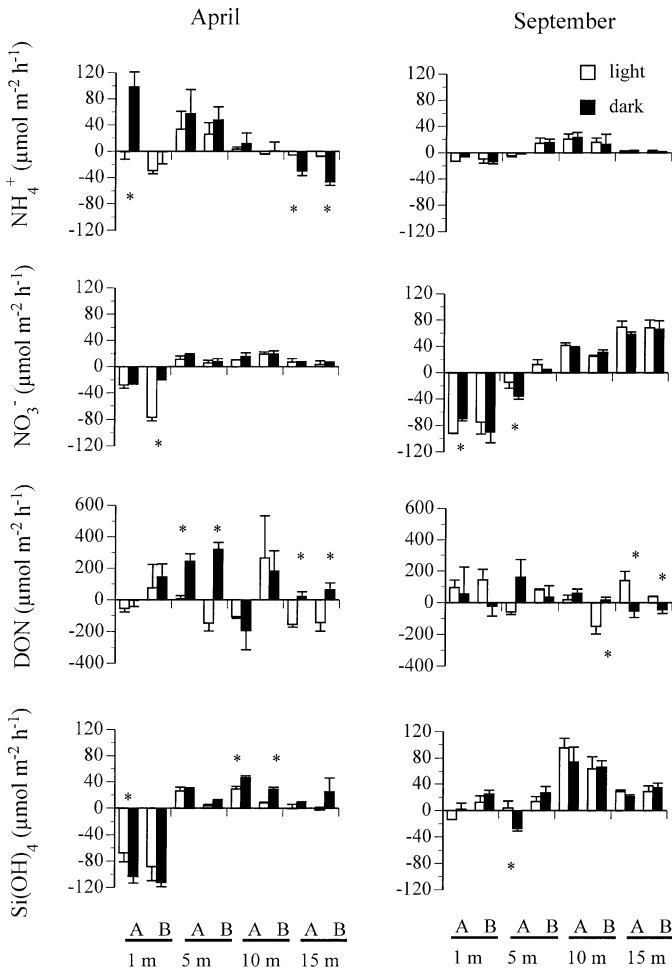


Fig. 4. Hourly nutrient fluxes in light and dark at four depths in Gullmar Fjord in April and September. Rates for two replicate stations per depth are shown (mean + SE,  $n = 3$ ). \* Denotes a significant light/dark difference.

September than in April. Daily (24-h) CR ( $8\text{--}45 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) was significantly higher in autumn than in spring (except at 15 m), but showed no evident pattern along the depth gradient (Fig. 3). A significantly lower CR was found at 10 m in April and at 15 m in September.

Applying the trophic status index, TOSI, showed that the 1-m site was highly autotrophic (BTSI 3) in both April and September, that the 5-m site was net autotrophic to net heterotrophic (BTSI 2-1), and that the 10-m site was net heterotrophic (BTSI 1). The sediment at 15 m varied between a net autotrophic and a fully heterotrophic state (BTSI 2-0). Thus, no depth could be described as fully heterotrophic (BTSI 0).

**Nutrient flux**—Water column concentrations: The pool of TDN in the bottom water was dominated by DON (range  $14\text{--}48 \text{ } \mu\text{mol L}^{-1}$ ), making up  $\sim 60\text{--}95\%$  of the TDN (Table 1).  $\text{NH}_4^+$  concentrations were always  $< 2 \text{ } \mu\text{mol L}^{-1}$  and were lower in the autumn (Table 1).  $\text{NO}_3^-$  concentrations were highest ( $9\text{--}10 \text{ } \mu\text{mol L}^{-1}$ ) at the 1-m and 15-m sites in April but were significantly lower at the middle depths, reflecting

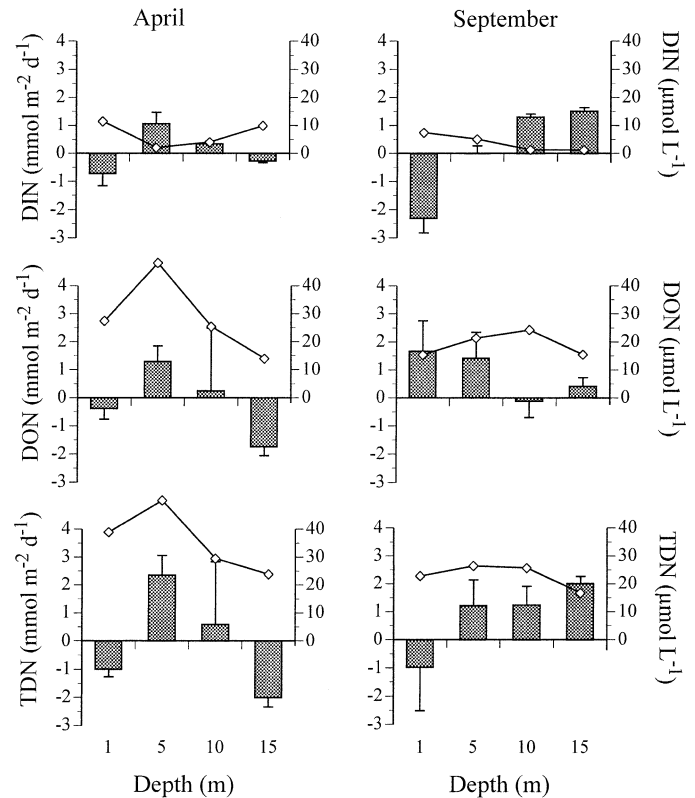


Fig. 5. Daily fluxes of dissolved inorganic (DIN), organic (DON), and total nitrogen (TDN) (mean + SE,  $n = 6$ ) at four depths in Gullmar Fjord in April and September. Curves show the concentration of DIN, DON, and TDN in the overlying water (mean,  $n = 3$ ).

a stratified water column. In September,  $\text{NO}_3^-$  concentrations decreased with depth, from 7 to  $\sim 1 \text{ } \mu\text{mol L}^{-1}$ . Concentrations of  $\text{Si(OH)}_4$  ( $1.7\text{--}22.7 \text{ } \mu\text{mol L}^{-1}$ ) were highest in the surface water in April.  $\text{PO}_4^{3-}$  concentrations were often below detection ( $< 0.1 \text{ } \mu\text{mol L}^{-1}$ ).

**General patterns of sediment–water fluxes:** Because there were some statistically significant differences between stations within a depth, hourly nutrient fluxes are shown separately for each station (Fig. 4). The general trends were (1) Both light and dark fluxes of inorganic nitrogen (DIN) and silica changed along the depth gradient, the most common pattern being uptake at shallow (autotrophic) sites and efflux at deeper (heterotrophic) sites (exception  $\text{NH}_4^+$  in spring); (2) DIN flux was dominated by  $\text{NH}_4^+$  in spring and by  $\text{NO}_3^-$  in autumn; (3) DON flux equaled or exceeded DIN flux (cf. Fig. 5); and (4) Significant light effects on flux rates were observed, although the pattern was not always consistent (Fig. 4).

**Light/dark fluxes:** Fluxes of  $\text{NH}_4^+$ , particularly in the dark, were higher in spring ( $-46$  to  $100 \text{ } \mu\text{mol m}^{-2} \text{ h}^{-1}$ ) than in autumn ( $-13$  to  $23 \text{ } \mu\text{mol m}^{-2} \text{ h}^{-1}$ ) (Fig. 4). Moreover, in spring,  $\text{NH}_4^+$  fluxes deviated from the general trend for DIN flux along the depth gradient— $\text{NH}_4^+$  was mainly released in shallow water (with some exceptions in light), but was taken up at 15 m. In autumn,  $\text{NH}_4^+$  fluxes were lower but followed the general trend for DIN flux, with uptake at shallow sites

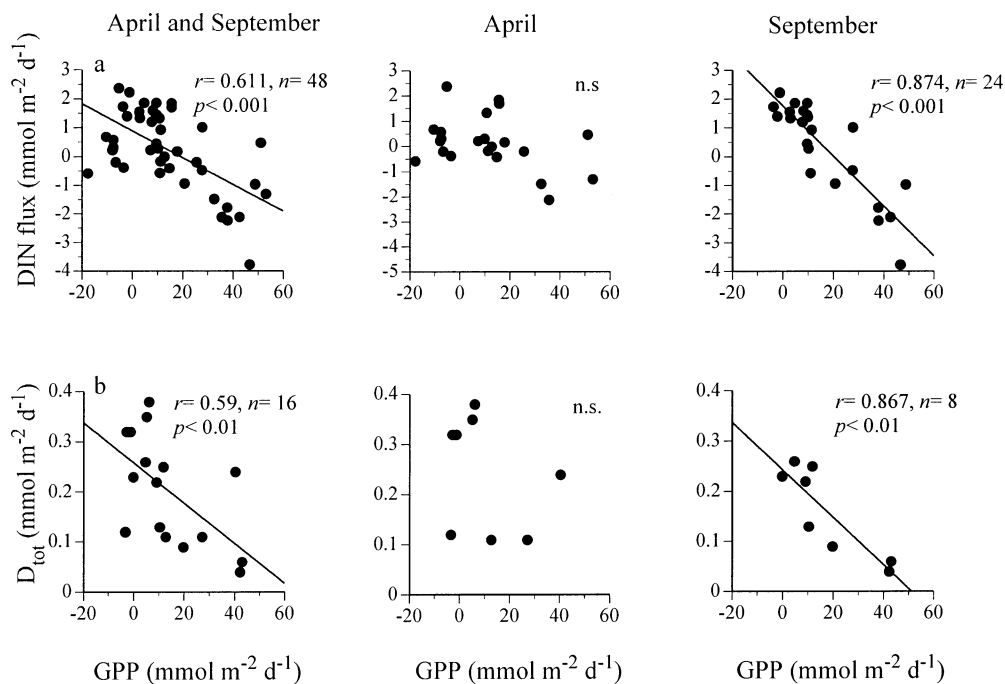


Fig. 6. Daily fluxes of (a) DIN and total (b) denitrification versus daily gross primary productivity (GPP), plotted for both sampling periods, as well as separately for April and September.

and efflux at deeper sites. In April, effect of light showed a trend—there was either higher uptake or lower efflux of  $\text{NH}_4^+$  in light (except at 15 m)—but L/D differences were, in most cases, not statistically significant (Fig. 4).

During both seasons, light and dark fluxes of  $\text{NO}_3^-$  followed the pattern of net uptake at shallow depths and release at deeper sites. This pattern was particularly clear in September, when  $\text{NO}_3^-$  fluxes were higher ( $-92$  to  $70 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) than in April ( $-76$  to  $20 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). Significant light effects on  $\text{NO}_3^-$  fluxes were found at 1–5 m in depth (Fig. 4).

Light and dark fluxes of DON ( $-192$  and  $322 \mu\text{mol m}^{-2} \text{h}^{-1}$ , respectively) either equaled or exceeded DIN fluxes. Both efflux and uptake of DON occurred; however, they did so with no clear pattern along the depth gradient (Fig. 4). Interestingly, there were significant light effects on DON flux at deeper sites, although this L/D pattern was not always consistent.

The general pattern observed for  $\text{NO}_3^-$  was also evident for silica, particularly in April, when  $65$ – $110 \mu\text{mol m}^{-2} \text{h}^{-1}$  of  $\text{Si}(\text{OH})_4$  were taken up at the 1-m site (Fig. 4). In September, there was an overall release of  $\text{Si}(\text{OH})_4$ , with a tendency of increasing rates along the depth gradient. Significant L/D differences for  $\text{Si}(\text{OH})_4$  were occasionally observed.

Sediment as a sink and source of dissolved N: Whereas comparing light and dark fluxes can tell us whether N flux is regulated by MPB, comparing *daily* fluxes gives us a hint about whether sediments function as sinks or sources of dissolved N (Fig. 5). Somewhat deviating patterns were found in April and September. In spring, daily DIN ( $-0.7$  to  $1.1 \text{ mmol m}^{-2} \text{d}^{-1}$ ) and DON ( $-1.7$  to  $1.3 \text{ mmol m}^{-2} \text{d}^{-1}$ ) contributed equally to the TDN flux and showed the same depth

pattern—the sediments at 1 m and 15 m were sinks, whereas the middle-depth sediments functioned as sources (Fig. 5). In autumn, the pattern for DIN was opposite that of DON—the shallowest sites were sinks of DIN (uptake  $\sim 2.3 \text{ mmol m}^{-2} \text{d}^{-1}$ ) and sources of DON ( $1.4$ – $1.7 \text{ mmol m}^{-2} \text{d}^{-1}$ ). Deeper sediments were clearly sources of DIN ( $1.3$ – $1.5 \text{ mmol m}^{-2} \text{d}^{-1}$ ) in the autumn, whereas no or little DON was released at these depths. Daily DIN flux showed a significant negative correlation with GPP (Fig. 6a), while no significant correlation was found between DON flux and GPP. In terms of TDN (Fig. 5), the sediment was a net sink at 1 m and a source deeper down, with one exception (15 m in spring).

**Denitrification**— $D_{\text{tot}}$  rates, in light and dark, ranged from  $0.4$  to  $23 \mu\text{mol N m}^{-2} \text{h}^{-1}$  (Fig. 7a). In April, rates were significantly higher at 5 and 10 m both in light and dark, while in September there was a steady significant increase with depth. In September, rates were overall significantly lower in light, whereas in spring, significant light effects were seen only at 10 and 15 m. Daily rates of  $D_{\text{tot}}$  ( $0.04$ – $0.36 \text{ mmol N m}^{-2} \text{d}^{-1}$ ) were higher in spring than in the autumn, except for the 15-m site (Fig. 7b). Daily  $D_{\text{tot}}$  showed a significant negative correlation with daily GPP (Fig. 6b).

**Microphytobenthic assimilation of N**—Calculated MPB assimilation based on 80% GPP ( $A_{\text{GPP80}}$ ) varied between  $0.2$  and  $2.5 \text{ mmol N m}^{-2} \text{d}^{-1}$  in spring and between  $0.2$  and  $3.6 \text{ mmol N m}^{-2} \text{d}^{-1}$  in autumn (Fig. 8). The highest values were found for 1-m depths, rates being generally higher in September than in April (no oxygen production was found in April at 10 m). When adopting the more conservative approach (i.e., basing calculations of N assimilation on NPP), a positive  $A_{\text{NPP}}$  value was found only for the 1-m station,

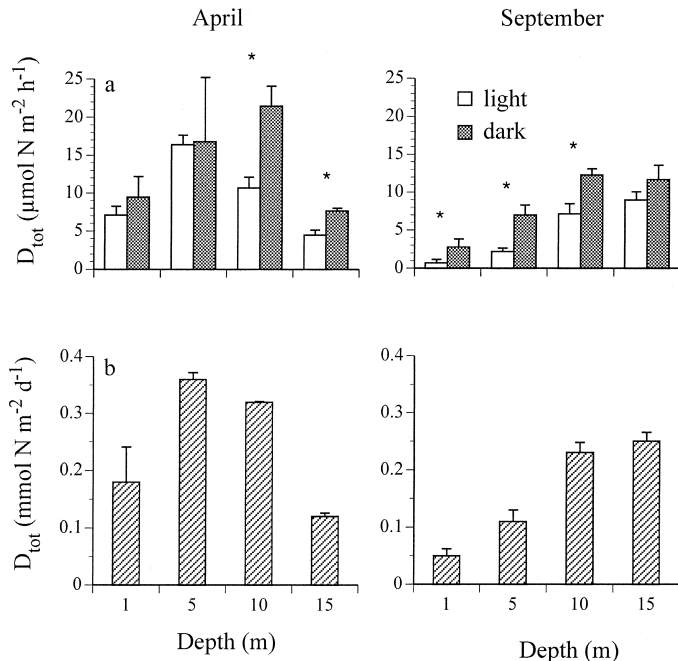


Fig. 7. (a) Denitrification ( $D_{tot} = D_w + D_n$ ) in light and dark and (b) total daily denitrification at four depths in Gullmar Fjord (mean + SE,  $n = 4$ ). \* Denotes a significant L/D difference.

being  $2.2 \text{ mmol N m}^{-2} \text{d}^{-1}$  in spring and  $2.4 \text{ mmol N m}^{-2} \text{d}^{-1}$  in autumn.

## Discussion

Our study is one of the few that has considered simultaneously both microphytobenthic activity and denitrification at sublittoral or subtidal depths (>10 m) in temperate areas. However, the study also has its limitations, as it includes only one transect that was studied during only two seasons. The choice of the two sampling periods was, however, based on a previous 12-month study in the Kattegat (Sundbäck and Miles 2000), which indicated that April and September were periods when both significant MPB activity and denitrification could be expected to occur. This premise also agrees with previous observations of two annual denitrification peaks in similar waters (e.g., Jørgensen 1989; Dalsgaard et al. 1999). Although only one transect was studied, hierarchical sampling (with two stations at each depth) provided information about the scale of spatial variation, showing that the main variation existed between depths. Thus, although our study certainly represents only two snapshots, it provides us with introductory information on the pattern of some key processes in the N turnover along a depth gradient in the photic zone of the benthos.

**Microphytobenthic community and pigments**—Vital communities of benthic microalgae existed down to at least 15 m in depth, although their biomass decreased with depth. This agrees with the findings of a previous study along the same gradient (Wulff et al. in press). Diatom mats consisting of large sigmoid diatoms, such as those found in September

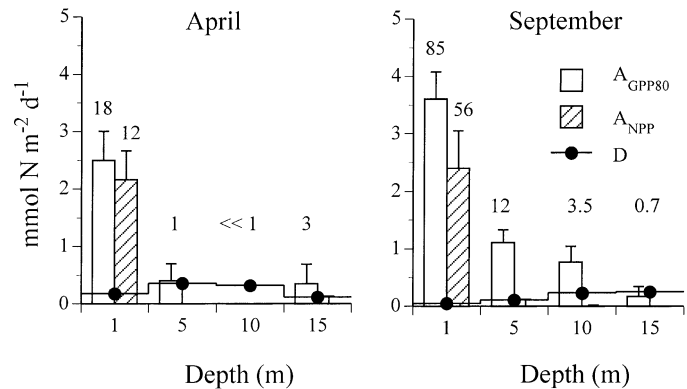


Fig. 8. Balance between daily N assimilation by microphytobenthos (mean + SE,  $n = 6$ ) calculated from 80% GPP ( $A_{GPP80}$ ) and NPP ( $A_{NPP}$ ), assuming a PQ of 1.2 and a C:N ratio of 9, and measured daily denitrification ( $D$ , mean + SE,  $n = 4$ ) at four depths in Gullmar Fjord in April and September. Numbers above bars show the  $A_{GPP80}/D$  ratio.

in Gullmar Fjord, have also been found in subtidal sediments (6–20 m) in New Zealand (Gillespie et al. 2000) and in the Adriatic Sea (14–66 m; Totti 2003). Photosynthesis/irradiance (P/I) curves of such subtidal diatom communities have shown a fast acclimation to low, as well as quickly changing, in situ light levels (Light and Beardall 2000; Glud et al. 2002).

Pigment concentrations and ratios were within the same range observed previously at the same location (Wulff et al. in press). In previous studies of diatom-dominated MPB communities, the fucox to Chl *a* ratio was  $\sim 0.4$  at <1 m depth (Sundbäck et al. 1997; Wulff et al. 1999). A similar ratio was found in Gullmar Fjord at 1 and 5 m in April, but a significantly higher ratio (0.7–0.9) was observed in September. This might be due to a faster degradation of Chl *a*, compared to fucox, in September (higher mineralization rates), which is also supported by a good correlation between fucox and pheophytin *a*. In addition to diatoms, other algal groups contain fucox; however, the microscopic check verified the dominance of diatoms. Moreover, the fucox peak at the 10-m depth in September coincided with the observed pure diatom mat consisting of large sigmoid diatoms. Low, but consistent, concentrations of typical green algal pigments (Chl *b*, lutein, and zeax) indicate the presence of green flagellates or euglenophytes, particularly in spring. These algae may have been easily missed in the microscope, as no detailed analysis of species composition was made. Part of the zeax pool can also be related to cyanobacteria, which were observed, particularly in the autumn.

**Autotrophy and heterotrophy along the depth gradient**—The sediment system was highly autotrophic (BTSI 3) at 1 m and mostly net heterotrophic from 5 m downwards (cf. Fig. 3). Even though light penetration during the September sampling was higher than in April, higher CR rates (except at 15 m) still resulted in net heterotrophy (BTSI 1) below 5 m in September. The significantly higher GPP at 10 m in September (Fig. 3), when compared with April values, accords with significantly higher content of Chl *a* as well as

other pigments at 10 m in September (Fig. 2). Since we took no samples between 1 and 5 m, we do not know at which depth the autotrophic–heterotrophic transition occurred. Positive NPP at one of the two 5-m stations in September hints that the transition zone was closer to 5 m than 1 m, but it is reasonable to expect that the transition depth varies with season. Most likely the transition point occurs more deeply during summer, with high insolation and a clear water column. The fact that in situ  $^{14}\text{C}$ -uptake by MPB can be higher at 4 m in depth than at 0.5 m (Sundbäck et al. 1996) also indicates that sediments can be autotrophic at depths of  $>1$  m in similar waters. In the microtidal Neuse River estuary (North Carolina), the autotrophic–heterotrophic transition depth varied between 1 and 4 m (Rizzo et al. 1992).

How do our primary production values compare with those from other sublittoral and subtidal areas? If we assume NPP to be 80% of GPP and adopt a PQ of 1.2, we can roughly compare our values with those measured as  $^{14}\text{C}$  uptake.  $\text{GPP}_{80}$  rates for the 1- to 5-m sites ( $44\text{--}490\text{ mg C m}^{-2}\text{ d}^{-1}$ ) are within the range of those reported for littoral and intertidal sediments in temperate areas (Underwood and Kromkamp 1999). However, it is more interesting to compare our values for 10- and 15-m depths ( $\text{GPP}_{80}$   $2\text{--}8\text{ mmol O}_2\text{ m}^{-2}\text{ d}^{-1} = 20\text{--}80\text{ mg C m}^{-2}\text{ d}^{-1}$ ) with those for similar depths in other temperate areas. Our values agree with those measured at 9–16 m in Laholm Bay, Kattegat, during April and September ( $\sim 10\text{--}80\text{ mg C m}^{-2}\text{ d}^{-1}$ ; Sundbäck and Jönsson 1988) and for sandy mid-Atlantic shelf sediments at 11–15 m in May and September ( $1\text{--}4\text{ mmol O}_2\text{ m}^{-2}\text{ d}^{-1}$ ; Laursen and Seitzinger 2002). On the other hand, our values are often one order of magnitude lower than those reported for northeast Greenland sediments at 10–20 m ( $367\text{--}172\text{ mg C m}^{-2}\text{ d}^{-1}$ ) during the Arctic summer (Glud et al. 2002) and for a New Zealand bay at 19–20 m in autumn ( $200\text{ mg C m}^{-2}\text{ d}^{-1}$ ; Gillespie et al. 2000), as well as those measured for sandy shelf sediments in North Carolina and Georgia at 14–40 m ( $250\text{--}400\text{ mg C m}^{-2}\text{ d}^{-1}$ ) (Cahoon and Cooke 1992; Jahnke et al. 2000). Whether these differences are due to differences in microphytobenthic biomass (Chl *a*) we do not know, since we used HPLC, while most of the cited studies used spectrophotometry.

*Pattern of DIN flux*—The observed general pattern of DIN flux—uptake at the shallow sites and efflux at the deeper sites—supports the idea that autotrophic sediments tend to function as N sinks and heterotrophic sediments function as N sources (Eyre and Ferguson 2002; Sundbäck and Miles 2002; Tyler et al. 2003). That the sediment at 1 m is a sink for DIN is in agreement with a number of studies from shallow-water sediments (e.g., Cerco and Seitzinger 1997; Sundbäck and Miles 2000; Anderson et al. 2003). The fact that the autotrophic state of the sediment is due to MPB photosynthesis makes MPB a key controlling factor in N flux at the sediment–water interface (see further discussion below).

The observed pattern of  $\text{NO}_3^-$  flux along the depth gradient reflects the changing balance between microphytobenthic and bacterial influence on the N flux. In shallow water,  $\text{NO}_3^-$  is taken up by MPB; with increasing depth, nitrification increases as a result of reduced competition between MPB and nitrifiers (cf. Risgaard-Petersen 2003).

Through the concept of coupled denitrification, this is in concordance with the trend of significantly increasing denitrification with depth. The lower efflux (or even uptake) of DIN (particularly of  $\text{NH}_4^+$ ) at 15 m in spring may reflect hydrodynamics, with less accumulation of organic matter, probably because of higher water flow at the deepest site. This interpretation is supported by the observation of a sandier sediment at 15 m (compared to other depths), with significantly lower C and N contents.

Although DIN fluxes in spring and autumn were within the same order of magnitude, there were some differences between the two seasons. Significantly higher  $\text{NO}_3^-$  efflux at 15 m in September reflects more favorable conditions for nitrification in autumn, although the reasons for this are not obvious. Another seasonal difference was that  $\text{NH}_4^+$  dominated the DIN flux in spring, while  $\text{NO}_3^-$  dominated in autumn. Higher  $\text{NH}_4^+$  dark efflux at 1–5 m in April may be due to a combination of (1) stimulated  $\text{NH}_4^+$  production by deposited organic matter in spring (e.g., phytoplankton; Jensen et al. 1990) and (2) increased solute transport by shallow-dwelling bivalves. Regarding the first, higher C and N contents (top 5 mm) at 1–10 m in spring, when compared with content values in autumn, support this interpretation. Hydrodynamic conditions may also have led to more deposition in the inner, shallower part of the bay (cf. higher proportion of silt at 1 m; Table 1). Some of the highest DON effluxes in the dark were also found in spring and can reflect hydrolysis of fresh organic material at the sediment surface (see DON flux). An alternative method of  $\text{NH}_4^+$  production could be via dissimilatory nitrate reduction to ammonium (DNRA). However, as DNRA has been found to be typical of sulphide-rich sediments (An and Gardner 2002), this pathway was less probable in our shallow-water sediment. As for the second, the presence of cockles (*Cerastoderma*) at 1 and 5 m may have also enhanced  $\text{NH}_4^+$  efflux (e.g., Swanberg 1991). Generally, animal activity increases solute transport across the sediment–water interface (e.g., Christensen et al. 2000 and references therein). A further mechanism that can enhance sediment–water fluxes, particularly in permeable sandy sediments, is advection (Huettel et al. 2003). In our laboratory incubations, with no oscillating boundary flows, this transport mechanism was probably not functioning. Because sediments at our study sites had rather high silt content ( $>17\%$ ) and well-developed diatom mats, we believe that these sediments were dominated by diffusion rather than advection.

The general magnitude of daily DIN fluxes ( $-0.3$  to  $1.5\text{ mmol N m}^{-2}\text{ d}^{-1}$ ) at 10 and 15 m in Gullmar Fjord were within the range of those measured in July at 15 m in Laholm Bay (Kattegat) (Sundbäck et al. 1991). They also agree in magnitude with DIN fluxes ( $0.17\text{--}2.2\text{ mmol m}^{-2}\text{ d}^{-1}$ ) measured in situ in May and September at 11–15 m in sandy mid-Atlantic shelf sediments (Laursen and Seitzinger 2002).

*DON flux*—Along with the growing awareness of the importance of DON in marine nutrient cycling (Bronk and Ward 2000), fluxes of DON have started to gain more attention in shallow-water ecosystems (Lomstein et al. 1998; Tyler et al. 2003). DON accounted for a large portion ( $\geq 50\%$ ) of the daily TDN flux (Fig. 5), which agrees with previous observations in shallow-water sediments (Lomstein et al.

1998; Anderson et al. 2003). Large fluxes of DON are considered to reflect hydrolysis of freshly produced organic material at the surface of the sediment (Blackburn and Blackburn 1993), a situation that can be very likely in the presence of microalgal mats, such as those existing in Gullmar Fjord.

When compared with DIN, the pattern of DON flux along the depth gradient was erratic, displaying large variation even within the same station. For instance, in contrast to daily DIN fluxes, no significant correlation was found between daily GPP and DON fluxes. The lack of a distinct pattern in DON fluxes has been shown to be typical of shallow coastal sediments (Tyler et al. 2003). However, occasional uptake of DON, particularly at the shallow depths in spring, together with some significant light effects, points to DON uptake by MPB. Dissolved free amino acids (DFAA), which can constitute up to 25% of the sediment DON (Gulberg et al. 2002), are taken up by benthic microalgae (Admiraal and Peletier 1979; Nilsson and Sundbäck 1996), and the addition of DFAA can significantly stimulate the growth of natural MPB communities (Linares unpubl. data). This emphasizes the potential role of DON as an alternative N source to sustain MPB growth under conditions with low DIN concentrations (cf. Lomstein et al. 1998).

**Denitrification**—As predicted, denitrification increased with depth, most clearly during autumn. This pattern agrees with that of increasing nitrification with depth, which should result in stimulated  $D_n$ . Risgaard-Petersen's (2003) analysis of 18 littoral and intertidal sites in European estuaries indeed showed that  $D_n$  rates are lower in autotrophic sediments (median value  $4.2 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) compared to heterotrophic (median value  $20.3 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) sediments. Our mean  $D_n$  values (including light and dark rates) of  $2.3 \mu\text{mol m}^{-2} \text{h}^{-1}$  for the autotrophic 1-m site and  $9.3 \mu\text{mol m}^{-2} \text{h}^{-1}$  (not shown) for the deeper heterotrophic sites fit this pattern. Lower denitrification in autotrophic sediments is due to MPB-induced N limitation of nitrifying bacterial populations. This makes MPB a major controlling factor in coupled denitrification.

Despite increased denitrification with depth, rates remained rather low ( $D_{\text{tot}} < 20 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ), being comparable to previously measured rates (isotope-pairing) in shallow-water sediments of other microtidal northern waters ( $1.6\text{--}34 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ; see table 3 in Sundbäck and Miles 2000). The highest daily value in Gullmar Fjord ( $\sim 0.4 \text{ mmol N m}^{-2} \text{d}^{-1}$ ) roughly equals the mean value for a number of Danish shallow fjords ( $\sim 0.6 \text{ mmol N m}^{-2} \text{d}^{-1}$ , recalculated from annual values given by Dalsgaard et al. (1999) and Nielsen et al. (2001)). The denitrification rates at 10–15 m in Gullmar Fjord were only  $\sim 20\text{--}30\%$  of those measured in situ by the  $\text{N}_2/\text{Ar}$  method ( $1.2\text{--}2 \text{ mmol m}^{-2} \text{d}^{-1}$ ) in sandy mid-Atlantic shelf sediments at 11–15 m in May and September (Laursen and Seitzinger 2002), and they were within the lower range of rates for other temperate continental shelves ( $0.1\text{--}3.2 \text{ mmol N m}^{-2} \text{d}^{-1}$ ) measured by a variety of techniques (see table 2 in Laursen and Seitzinger 2002). Denitrification (particularly  $D_n$ ) is positively correlated with  $\text{O}_2$  consumption ( $r = 0.655$ ,  $P < 0.01$  in Gullmar Fjord), which in turn links to the availability of mineralized  $\text{NH}_4^+$  (Laursen and Seitzinger 2002; Risgaard-Petersen 2003 and references

therein). The rather low D rates found at greater depths in Gullmar Fjord, despite the increasing trend with increasing depth, could, particularly in April, be due to limited mineralization—hinted at by low CR values, little or no efflux of  $\text{NH}_4^+$ , and low sediment N content. A significant linear, positive correlation between  $D_{\text{tot}}$  and the C ( $r = 0.567$ ,  $P < 0.05$ ) and N ( $r = 0.560$ ,  $P < 0.05$ ) contents of the sediment also support this interpretation.

**Influence of MPB on the pattern of N turnover**—One of our main hypotheses was that the regulating role of MPB on benthic N turnover, typically found for littoral and intertidal zones, would decrease with depth. The distribution of Chl *a* and GPP along the depth gradient certainly supported this assumption, as did the observed pattern of sediment–water DIN flux. Although sediment uptake of DIN can reflect denitrification and bacterial immobilization of N (Anderson et al. 2003), we believe that the N uptake typical for the shallowest autotrophic site was mediated primarily by MPB. This conclusion is supported by a concomitant uptake of silica, which can only result from uptake by diatoms (cf. Sundbäck et al. 1991; Sigmon and Cahoon 1997). Despite the fact that DIN flux changed direction below 5 m, we found that MPB could still affect the N turnover at the deeper sites as well. However, because we did not measure the actual N uptake by MPB, we rely on indirect evidence—light effects on fluxes, correlations between variables, and calculations of the N demand of MPB.

Light effects on N flux pointed to the role of MPB as a regulator of sediment–water nutrient exchange, although these differences were not as consistent, as was previously noted for littoral and intertidal zones (Henriksen et al. 1980; Reay et al. 1995; Sundbäck and Miles 2002 and references therein). Interestingly, there were more significant L/D differences for denitrification than for DIN fluxes, showing that MPB can indeed suppress denitrification even at low light levels. Considering that insolation and water transparency during summer is usually higher, the influence of MPB can be expected to be even greater during summer.

Further support for the regulating role of MPB can be gained by plotting GPP against DIN fluxes and denitrification (Fig. 6). The lower number of *n* in the  $D_{\text{tot}}$  plot is due to the fact that mean values have been used, as denitrification and GPP could not be measured in the same cores. When values for both seasons were plotted together, a significant negative correlation with GPP was found for both DIN and  $D_{\text{tot}}$  (Fig. 6). Plotting the two seasons separately revealed no significant correlation in spring, but a better (more significant) correlation in autumn (Fig. 6). This discrepancy may be related to the differences in water column stratification between the two sampling periods, resulting in different light and nutrient conditions.

**Balance between MPB assimilation and denitrification**—We hypothesized that there would be a point of transition along the depth gradient at which denitrification would override MPB incorporation of N. Despite net heterotrophic conditions at depths  $\geq 5$  m, this did not happen, and at most, the two processes were roughly balanced (Fig. 8). The assimilation/denitrification ratio (A/D; Sundbäck and Miles

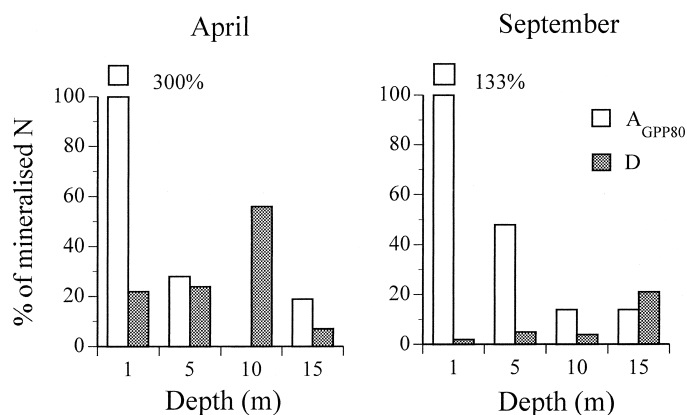


Fig. 9. Percentage of remineralized N consumed by MPB assimilation (calculated from 80% GPP) and denitrification at four depths in Gullmar Ford in April and September.

2000) for the different depths varied from 0.7 to 85 (once no GPP was measured) (Fig. 8). Interestingly, in September, calculated MPB assimilation of N at 5–10 m was still 3.5–12 times greater than N removal by denitrification. However, basing MPB nitrogen demand on 80% of GPP may to some extent overestimate MPB assimilation, because DOC and DON release from the cells is not taken into account (Midelburg et al. 2000). If we instead apply  $A_{NPP}$ , which on the other hand probably underestimates algal assimilation,  $A$  exceeds  $D$  only at 1 m ( $A/D$  12 and 56, Fig. 8). These ratios agree well with those found for littoral sediments (also based on  $A_{NPP}$ ) on the west coast of Sweden (Sundbäck and Miles 2000, 2002), supporting the previous conclusion that, in shallow autotrophic sediments, the rate of MPB incorporation of N far exceeds that of denitrification. This also fits into the model predicting that, at a low nitrogen load (usually implying a clear water column), N turnover in shallow-water sediments is dominated by benthic microalgae (Christensen 1998).

Another approach for comparing MPB assimilation with denitrification and assessing their relative importance for benthic N turnover is to calculate the proportion of the remineralized N that is used by these two processes. N-remineralization rates can be roughly estimated from benthic oxygen consumption and sediment C:N ratios (Trimmer et al. 2000). Assuming a mean respiratory quotient (RQ) of 1.25 (Glud et al. 2002), we calculate an N remineralization of 0.6–5.4 (average 1.9)  $\text{mmol N m}^{-2} \text{d}^{-1}$ . These values are ~50% of those found for a shallow temperate lagoon (Virginia) by using the  $^{15}\text{N}$ -technique (Anderson et al. 2003). In Gullmar Fjord, MPB assimilated between 0 and 133% (average 37% for both spring and autumn) of the mineralized N along the depth gradient (excluding one extreme value of >300%) (Fig. 9). Dalsgaard (2003) found that 13–58% of the  $\text{NH}_4^+$  produced by mineralization was retained in the sediment in Limfjorden (Denmark), primarily because of assimilation by the MPB community. At 1 m in Gullmar Fjord,  $A_{GPP80}$  exceeded estimated N mineralization. Anderson et al. (2003) found similar results, and their interpretation was that dark  $\text{NH}_4^+$  uptake by MPB may be an important sink for mineralized N. In Gullmar Fjord, denitrification used ~30%

(range 7–56%) of the remineralized N during spring and ~10% (range 2–21%) during autumn, the percentage increasing with depth (Fig. 9). The mean percentage (20%) falls within the range of those calculated for two shallow bays in the same area (10–20%; Sundbäck and Miles 2002) as well as those found in the Great Ouse estuary (8–26%; Nedwell and Trimmer 1996).

Thus, in terms of actual amounts, denitrification does not remove significantly more N in the deeper (heterotrophic) parts of the photic zone than at the littoral (autotrophic) sites. In terms of amounts relative to mineralized N, however, denitrification can remove 20–50% of this N at  $\geq 10$  m in depth. On the other hand, although N assimilation into MPB decreases with depth, MPB can still incorporate a substantial proportion of the remineralized N at depths  $\geq 10$  m (in our study 15–20%). Remaining, however, is the evaluation of the importance of alternative N-removal processes in the shallow coastal zone, such as anaerobic ammonium oxidation (Thamdrup and Dalsgaard 2002).

In summary, our study corroborates previous findings that MPB dominates the nitrogen turnover in shallow-water (<5 m) autotrophic sediments, particularly in clear waters with a relatively low N load (Christensen 1998; Sundbäck and Miles 2000). However, also at sublittoral depths (>5 m depth), MPB can exert a significant influence on sediment-water N fluxes and denitrification, provided that light reaches the sediment surface. Fluxes of DIN (and silica) can be expected to change along the depth gradient, with a general pattern of sediment uptake at shallow autotrophic sites and increasing efflux deeper down, along with increasing heterotrophy of the sediment system. But local variation in light penetration, deposition of organic matter, and faunal activity can change this general pattern. DON fluxes, which often equal or exceed DIN flux, may also be influenced by MPB, but this needs to be verified in further experiments. The importance of denitrification will increase with depth relative to benthic algal N assimilation. This shift in balance is partly related to increased nitrification with depth, which is a result of decreased competition from MPB (cf. Risgaard-Petersen 2003). Still, algal assimilation may equal or exceed denitrification even in the lower part of the photic zone, and denitrification does not necessarily remove substantially more nitrogen in the deeper parts of the photic zone than it does in the littoral, autotrophic zone.

Although our study was made in a microtidal northern water body, the findings should apply to any water body with clear enough water to allow MPB activity at sublittoral or subtidal depths. On an ecosystem scale, the regulating role of MPB should be particularly important in shallow (microtidal) areas, where a large portion of the benthos is within the photic zone (e.g., in the Adriatic Sea [Blackford 2002] and the Kattegat [Granéli and Sundbäck 1986; Sundbäck et al. 1991]). Similar conditions also exist on continental shelf sediments off macrotidal coasts (e.g., Jahnke et al. 2000) and on subtidal sediments in clear-water tropical areas. Therefore, when studying benthic nutrient turnover in such areas, the role of MPB must be taken into account. And this implies that in these studies, light versus dark measurements must be used, since not only the sediment–water nutrient

fluxes, but also denitrification, can be influenced by MPB also at subtidal and sublittoral depths.

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