

Benthic primary production and nutrient cycling in sediments with benthic microalgae and transient accumulation of macroalgae

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Abstract

Annual rates of sediment denitrification and sediment–water fluxes of oxygen and nutrients were quantified at two shallow locations, Virksund and Ulbjerg, in the Limfjorden, Denmark. The sediment was sandy and colonized mainly by bivalves with a wet weight of 2,508 g m⁻² at Virksund and 572 g m⁻² at Ulbjerg. A benthic microalgal community was present throughout the year, and for 1–2 months in the summer, floating macroalgae partly covered the sediment. Annual budgets for the sediment both including and excluding the activity of macroalgae were calculated. In the absence of macroalgae, the benthic primary production was highest at Ulbjerg, which was autotrophic on an annual basis, whereas Virksund was heterotrophic. When macroalgae were included, both sites were strongly autotrophic on an annual basis. From 13% to 58% of the NH₄⁺ produced by mineralization was retained in the sediment in the absence of macroalgae, primarily because of the assimilation of NH₄⁺ by the microphytobenthic community. Only 25% and 38% of the total NO₃⁻ uptake at Ulbjerg and Virksund, respectively, was denitrified in the absence of macroalgae, whereas in the presence of macroalgae, 12% and 39% of the NO₃⁻ uptake was denitrified at those sites. Nitrate uptake associated with benthic primary production limited denitrification through competition for NO₃⁻. The release of NH₄⁺ from the sediment at Virksund was reduced more than 50%, and at Ulbjerg, release of NH₄⁺ changed to uptake when the macroalgae were included in the annual budget. Nutrient uptake by macroalgae competed with all other nutrient-consuming processes, and the transient occurrence of macroalgae totally changed both the primary productivity and the nutrient budgets for the two sites.

In the marine environment, primary production is remineralized both in the water column and in the sediment, and the relative importance of these two compartments depends very much on the water depth. In Chesapeake Bay, for instance, it was found that at water depths of <5 m, the benthic respiration exceeded the planktonic respiration (Rowe et al. 1975; Kemp et al. 1992). The nutrients that are produced by degradation of organic matter in the sediment can be transported back to the photic zone where it can fuel new primary production (Rowe et al. 1975). The relative importance of this supply of nutrients, the so-called internal loading, compared to the external loading has been found to vary substantially. In a study in the Chesapeake Bay, release of remineralized ammonia from the sediment could account for between 13% and 40% of the photosynthetic nitrogen demand in the water column (Boynton and Kemp 1985), and in the Neuse River estuary, the internal loading of dissolved inorganic nitrogen (DIN) contributed 13% to 21% of the total DIN input to the water column. A compilation of data from 10 different estuaries demonstrated an even more var-

iable relative significance of the internal loading (Fisher et al. 1982): from 0% to 79% of the nitrogen demand and from 0% to 75% of the phosphorus demand was supplied from the sediment.

In shallow coastal environments where the sediment is within the photic zone, the release of nutrients from the sediment to the water column is potentially regulated by nutrient uptake by the microalgae living in the uppermost layer of the sediment or on the sediment surface. The effect of the microphytobenthic community on the nutrient exchange between sediment and water varies between sites as well as diurnally and seasonally (e.g., Nowicki and Nixon 1985; Sundbäck and Granéli 1988; Rizzo 1990; Sundbäck et al. 1991; Rysgaard et al. 1995; Cowan et al. 1996; Sundbäck et al. 2000). The microphytobenthic community also affects denitrification in the sediment, and both positive and negative interactions have been found. A positive interaction was found in situations where NH₄⁺ availability was high, and an increased supply of O₂ in light stimulated nitrification and, therefore, coupled nitrification–denitrification (Risgaard-Petersen et al. 1994). Negative interactions have been found when the availability of DIN was lower than the demand for DIN by microphytobenthic assimilation, nitrification, and denitrification, and the inhibition of denitrification by the microphytobenthic activity was a result of competition for DIN (Henriksen and Kemp 1988; Risgaard-Petersen 2003).

In eutrophied coastal marine waters where sufficient light reaches the sediment, mats of floating macroalgae can develop (Morand and Briand 1996; Valiela et al. 1997). Macroalgae have a high capacity for growth and nutrient uptake (Viaroli et al. 1996a), and because of their position on top of the sediment, they are, like the microphytobenthos, able to control the exchange of nutrients between the sediment

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and the water column (McGlathery et al. 1997; Krause-Jensen et al. 1999). In eutrophied coastal environments in southern Europe, macroalgal mats develop to huge densities, very often followed by a sudden collapse of the whole mat, with sulfidic and anoxic water as the result (Bartoli et al. 1996; Viaroli et al. 1996b; Sfriso and Marcomini 1999). In Danish coastal waters, the macroalgal mats generally do not reach such high densities, and the collapses of macroalgal mats are often only of local importance. A typical situation would be the transient occurrence of a healthy green macroalgal mat of a thickness of 5 to 15 cm for 1–2 months during summer and early fall. Even though the macroalgae reduce the light availability at the sediment surface, the biomass of microphytobenthos is not affected for macroalgal cover lasting up to 6 weeks (Sundbäck et al. 1996).

This study was undertaken to investigate the role of both benthic microalgae and floating macroalgae on benthic primary production, denitrification, and sediment water fluxes of nutrients. The interesting question was whether a transient occurrence of macroalgae could influence these processes on an annual scale. This was investigated at two sites where benthic microalgae were active throughout the year and mats of floating macroalgae were present for 1–2 months during summer. The two sites were selected to give a variation in both macroalgal cover and infauna density.

Materials and methods

Study sites—The study was performed at two field sites, Virksund and Ulbjerg, located in Lovns Broad (Conley et al. 2000) in the Limfjorden, Denmark. Lovns Broad has an average depth of ~5 m. In 1997, the annual mean chlorophyll concentration was $19 \mu\text{g L}^{-1}$, and the annual pelagic primary production was 225 g C m^{-2} (central part of Lovns Broad, data from the county of Viborg). Sampling at Virksund ($56^{\circ}36'18''\text{N}$, $9^{\circ}17'54''\text{E}$) was ~20 m from shore at a water depth of 75 cm. Sampling at Ulbjerg ($56^{\circ}37'35''\text{N}$, $9^{\circ}18'48''\text{E}$) was ~75 m from shore at a water depth of 75 cm. The two sites were situated 2.5 km apart. Salinity was almost identical at the stations and varied from 13 to 25 with an average of 19.5. Two streams discharged into the fjord 300 m north and 300 m south of the sampling station at Ulbjerg, which caused elevated NO_3^- concentrations. The sediment texture was very similar at the sites. A grain size distribution analysis of the upper 10 cm was made using the following size classes: <63, 63–125, 125–250, 250–500, and >500 μm . At Virksund, the distribution between the size classes was 0.1, 22.3, 47.3, 25.2, and 5.1%. At Ulbjerg, the distribution was 0.3, 23.6, 37.2, 37.0, and 1.9%.

Sampling and incubation system—Each of the stations was sampled 11 times from 10 March 1997 to 12 January 1998. At each sampling date, three undisturbed sediment samples from each of the stations were brought back to the laboratory where denitrification and sediment–water fluxes of oxygen and nutrients were measured. On occasions when floating macroalgae were present, three samples without and three with macroalgae were obtained. The sediment was contained in flux chambers measuring $40 \times 20 \times 20 \text{ cm}$ (h \times w \times d) on the inside (Fig. 1A). The lower 10–15 cm was

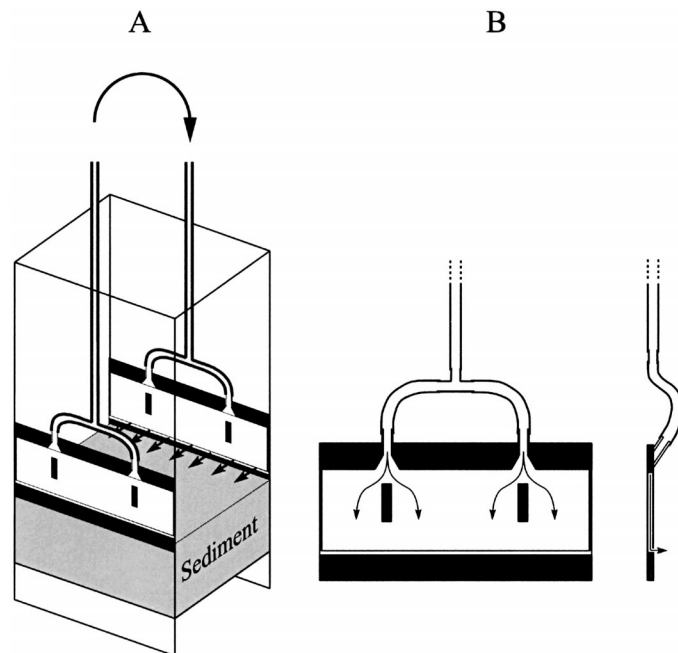


Fig. 1. (A) The flux chamber used for flux and denitrification measurements. (B) Detailed drawing of the diffuser. Water was drawn out through the diffuser on one side and pumped back in through the diffuser on the other side. The flux chamber measured $20 \times 20 \text{ cm}$ on the inside and was 40 cm high. Arrows indicate the direction of the water flow.

filled with sediment. On occasions when macroalgae were present, the sediment was sampled in a spot without macroalgae, and a layer of the macroalgae were subsequently placed on the sediment surface at a density corresponding to that found in situ.

Sampling was done with a hand-held box corer made of a 1-mm-thick steel plate fitting precisely inside the flux chamber. The corer was open at the bottom end and could be opened or closed at the top. After pushing the corer 10 to 15 cm into the sediment, it was closed at the top, dug out with a shovel, and placed carefully on the sediment. A Plexiglas plate ($19 \times 19 \text{ cm}$, 2 mm thick) was then used to cut the sediment across the end of the corer and to prevent the sediment from falling out when transferred to the flux chamber. The Plexiglas plate was held in place when lowering the box corer into the flux chamber with a wooden stick inserted through a hole in the bottom of the flux chamber, which was closed with a rubber stopper when the stick was removed. When removing the corer, a gap of 2 to 3 mm was left between the sediment and flux chamber walls; however, the sediment block expanded horizontally and sunk correspondingly to fill this gap immediately. The water column stayed in place during sampling and there was no resuspension of the sediment. In the laboratory, two water diffusers (Fig. 1B) were placed in each flux chamber (Fig. 1A). The diffuser was a 5-mm-thick sandwich of Plexiglas with a 3-mm-wide slit extending across the diffuser. When water was pumped out through the diffuser, the exit speed of the water was the same throughout the slit. The diffusers were positioned so that the slit was 5 mm above the sediment

surface or, if macroalgae were present, 5 mm above the top of the macroalgal mat. The two diffusers were connected with 8-mm (internal diameter, ID) PVC tubing to a 12-V centrifugal pump drawing water in through one diffuser and pumping it back out through the other. The pump rate was set at 1.5 L min^{-1} , which created a stirring sufficient to distribute an added dye to the whole water column within a couple of minutes. The flow pattern created by the diffusers was investigated by adding dye to the water and following its dissipation visually. The flow rate was found to be the same across the sediment surface except for the 2–3 cm closest to the diffusers, where it was higher.

The two sets of flux chambers, with diffusers and pumps, were placed in two large open polyethylene containers that were filled with site water to 5 cm above the top of the flux chambers. The total volume of water was approximately 85 liters in each container. The temperature in the flux chambers was maintained at in situ level ($\pm 0.5^\circ\text{C}$) throughout the incubation. The water in the container was aerated, which created a strong circulation within the container, and the flux chambers were left open with the stirring turned on until the morning after sampling when the incubations were started. The sediment was illuminated (*see below*) until sunset on the sampling day. The interval between sediment sampling and immersion in the vessels never exceeded 4 h.

Incubations—During the incubations, the water level in the polyethylene vessel was lowered to below the top of the chambers in order to separate each chamber from the rest. Each chamber was closed with a lid floating on the surface of the water to prevent gas exchange with the atmosphere. The lid was a 1-mm-thick Lexan plate with a glass petri dish glued to the lower side for buoyancy and cutouts for the tubing connecting the water diffusers with the pump. The light source was Osram Powerstar HQI-T 400/D metal halide light bulbs. The applied light intensity to the sediment surface was the average daily light intensity for the sampling date, calculated as the average for the last 3 yr and corrected for the extinction coefficient of the water and the water depth. Average daily light intensity was calculated as the average of the hourly light intensities from sunrise to sunset. The extinction coefficient was calculated from simultaneous measurements of light intensities in the field at the time of sampling at both the sediment and the water surface with a Licor LI-192SA underwater quantum sensor and a Licor LI-190SA quantum sensor, respectively. The actual light intensities applied are given in Fig. 2.

Incubations for flux measurements were initiated the day after sampling, and incubations for denitrification measurements were done with the same sediment samples the day after the flux measurements were completed. One hour before the start of the flux and denitrification incubations, the water was exchanged with fresh seawater from the site. The dark incubations were carried out shortly after the light incubation ended, with no exchange of water. In between the light and dark flux incubations, the water in each chamber was bubbled with atmospheric air for 20 min to return the oxygen concentration to atmospheric saturation. The incubation time was the same for both flux and denitrification measurements in both light and dark. It was set to give a

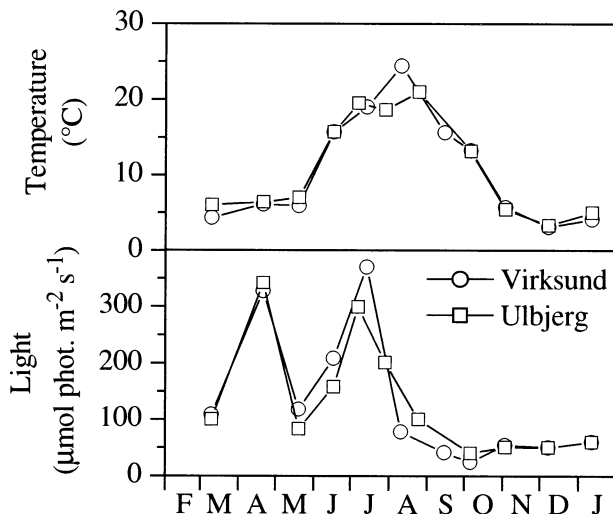


Fig. 2. Light intensity applied in the incubations and in situ temperature.

decrease in oxygen concentration of 10% to 20% of atmospheric saturation during the dark incubation. Incubation times varied from 1 h in the summer to 24 h in the winter.

The sediment–water flux rates of O_2 , $\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , urea, and PO_4^{3-} were measured as the concentration change over time in the water overlying the sediment. The concentrations were measured four times in the light and four times in the dark. Samples for oxygen were taken with a glass syringe with 10-cm Tygon tubing attached. The sample was transferred to a 12-ml gas-tight vial (Exetainer, Labco) and Winkler reagents 1 and 2 were added. Samples for NO_3^- , NO_2^- , NH_4^+ , urea, and PO_4^{3-} were taken with a plastic syringe with a 10-cm Tygon tubing attached. Samples were filtered (GF/C) directly into polypropylene vials and frozen immediately. When sampling with a syringe from the chambers, the tubing attached to the syringe was inserted through the cutouts in the floating lid, and samples could be withdrawn without removing the lid. On one sampling round, $\sim 75 \text{ ml}$ of water was removed from each chamber, typically corresponding to 0.8% of the total volume.

Denitrification rates were measured by the isotope pairing technique (Nielsen 1992). At the start of the incubation, $^{15}\text{NO}_3^-$ was added to the water column of the flux chamber according to the following rule of thumb, which provides good results in most situations: the concentration of $^{15}\text{NO}_3^-$ must be at least 20% of the oxygen concentration, and the labeling of the whole nitrate pool must be at least 30%. The isotopic composition of the nitrate pool was measured after biological reduction to N_2 (Risgaard-Petersen et al. 1993). Samples for $^{29}\text{N}_2$ and $^{30}\text{N}_2$ were taken three times in the light and three times in the dark by a subcoring technique that uses a 35-cm-long steel tube (outside diameter [OD] = 9 mm, wall thickness = 0.5 mm). At each sampling, three subcores containing a 3-cm-long sediment core and the water column above it were taken from each flux chamber. The three subcores were transferred to a cylindrical glass beaker (ID = 2 cm) containing $600 \mu\text{l}$ 50% (w/v) ZnCl_2 to stop bacterial activity. Sediment, water, and ZnCl_2 were gently mixed with a glass spatula and left for 1 min for the coarser

sediment particles to settle before a sample was transferred to a 12-ml gas-tight container (Exetainer). Less than 0.5% of the sediment surface was disturbed at each sampling round.

At the end of the denitrification incubation, three samples for sediment chlorophyll *a* (Chl *a*) were taken as 19-mm (ID) subcores within each chamber. The upper half centimeter of the three subcores from each flux chamber were pooled and frozen immediately (-18°C). The frozen samples were freeze-dried within a couple of days and analyzed for Chl *a* within 1 month. When macroalgae were present, the whole macroalgal mat from each chamber was dried at 90°C to constant weight and weighed.

Fauna density—After completion of the denitrification measurements, the sediment from each flux chamber was sieved through a 0.5-mm sieve. The animals found were assigned to groups, counted, and weighed (wet weight).

Analysis—The samples for isotopic composition of N_2 were analyzed on an isotope ratio mass spectrometer (Rysgaard et al. 1995). Oxygen concentration was determined by Winkler titration (Strickland and Parsons 1972). Concentrations of nitrate and nitrite were determined on a flow injection analyzer (Alpkem FS3000, Perstorp Analytical Environmental) by a standard method (Grasshoff et al. 1983). Ammonium concentration was determined by the salicylate-hypochlorite method (Bower and Holm-Hansen 1980). Phosphate concentration was determined by a standard colorimetric technique (Grasshoff et al. 1983). Urea concentration was determined by the diacetyl monoxime method (Price and Harrison 1987). Ammonium and PO_4^{3-} were analyzed automatically on a robotic sample processor (Tecan RSP-5051, Tecan AG) coupled to a spectrophotometer (Camspec M330, Camspec). Chl *a* was measured colorimetrically after extraction of the freeze-dried surface sediment with 90% acetone (Lorenzen 1967).

Calculations—Rates of O_2 and nutrient fluxes in light and in dark incubations were calculated as the change in concentration over time using linear regression. A positive flux indicates transport from the sediment to the water column. Denitrification was calculated as described by Nielsen (1992) from the production rates of $^{29}\text{N}_2$ and $^{30}\text{N}_2$, which were calculated by linear regression. Diurnal rates of sediment–water fluxes and denitrification were calculated from the rates measured in light and dark and the day length on the day of sampling according to Eq. 1.

$$\text{DR} = (R_l \times H) + [R_d \times (24 - H)] \quad (1)$$

DR is the diurnal rate; R_l and R_d are the rates measured in light and dark, respectively; and H is the day length in hours. Day length was calculated as the time from sunrise to sunset. Average annual rates were calculated according to Eq. 2.

$$\text{AR} = \frac{\text{DR}_1 \times \text{PL}_1 + \text{DR}_2 \times \text{PL}_2 + \dots + \text{DR}_n \times \text{PL}_n}{\text{PL}_1 + \text{PL}_2 + \dots + \text{PL}_n} \quad (2)$$

AR is the annual average rate, DR_n is the diurnal rate for period n , and PL_n is the length of period n in days. Period length was calculated as the number of days from the mid-

point between sampling date n and $n - 1$ and the midpoint between sampling date n and $n + 1$. The start and end dates of the period were chosen so that a whole year was covered and so the length of the first and the last period were approximately equal. Annual averages of concentrations and biomasses were calculated in a similar manner by replacing the diurnal rates in Eq. 2 with the appropriate concentration or biomass. Gross primary production was calculated as the O_2 flux in light minus the O_2 flux in dark. Average annual rates of flux and denitrification were calculated for each site with and without macroalgae according to Eq. 2. The annual rates for microphytobenthos-dominated sediment were calculated from the incubations without macroalgae for all sampling dates. The annual rates that included the transient occurrence of macroalgae were calculated with the rates obtained with macroalgae for the sampling dates when macroalgae were present and the rates obtained without macroalgae for the rest of the year.

Results

Nutrient concentrations and microalgal biomass—At Virksund, there was a clear seasonal variation in the in situ concentrations of nutrients ($P < 0.01$, ANOVA) in the water column and Chl *a* at the sediment surface ($P < 0.01$, ANOVA; Fig. 3). The concentrations of $\text{NO}_3^- + \text{NO}_2^-$ (hereafter denoted NO_3^-) were low ($< 2 \mu\text{mol L}^{-1}$; Fig. 3C) during summer and early autumn and high (up to $110 \mu\text{mol L}^{-1}$) the rest of the year. The concentration of NH_4^+ was $3\text{--}7 \mu\text{mol L}^{-1}$, except in the autumn, when concentrations up to $43 \mu\text{mol L}^{-1}$ were found (Fig. 3E). Phosphate concentrations were high during summer and early autumn and close to the detection limit the rest of the year (Fig. 3G). The benthic microalgal biomass measured as sediment Chl *a* varied from $35 \text{ mg Chl } a \text{ m}^{-2}$ in May and late autumn to $155 \text{ mg Chl } a \text{ m}^{-2}$ in April and January (Fig. 3A). During summer and early autumn, it remained constant around $80 \text{ mg Chl } a \text{ m}^{-2}$.

At Ulbjerg, there also was a seasonal variation in nutrient concentrations ($P < 0.01$, ANOVA). Nitrate concentrations were high in the winter, as they were for Virksund, but did not decline as much during summer (Fig. 3D). Except for one occasion, there was always more than $25 \mu\text{mol L}^{-1}$ NO_3^- . Both NH_4^+ and PO_4^{3-} concentrations were low most of the year and were only high in the late summer and autumn (Fig. 3F,H). The Chl *a* content at the sediment surface was significantly higher at Ulbjerg than at Virksund ($P < 0.01$, ANOVA), and on an annual basis, this difference was 68% (Fig. 3B). The pattern of variation was similar, except that at Ulbjerg, there was a gradual increase from $78 \text{ mg Chl } a \text{ m}^{-2}$ in May to $190 \text{ mg Chl } a \text{ m}^{-2}$ in August, whereas the concentration at Virksund was almost constant. Average concentrations of Chl *a* between the May and October minima were $169 \text{ mg Chl } a \text{ m}^{-2}$ at Ulbjerg or 111% higher than at Virksund.

Sediment without macroalgae—All the measured sediment–water flux rates showed distinct seasonal patterns ($P < 0.01$, ANOVA). The sediment O_2 uptake rates in dark at both stations were highest in summer (Fig. 3A,B). Analysis of variance showed a significant difference between the an-

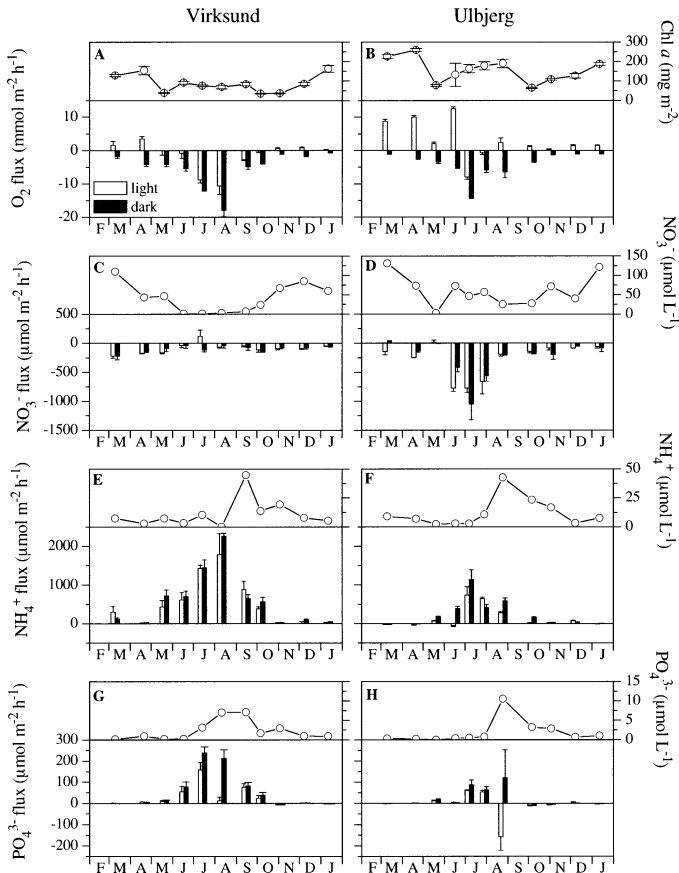


Fig. 3. Sediment water flux rates of (A, B) O_2 , (C, D) NO_3^- , (E, F) NH_4^+ , and (G, H) PO_4^{3-} and the concentrations of (C, D) NO_3^- , (E, F) NH_4^+ , and (G, H) PO_4^{3-} in the water column at the start of the incubations at both Virksund and Ulbjerg. The benthic microalgal biomass as Chl *a* at the two stations is also shown (A, B). Error bars indicate the standard error.

annual average O_2 uptake at the two stations ($P < 0.01$), which was 40% higher at Virksund than at Ulbjerg. At Virksund, the O_2 fluxes in light were positive in spring, late autumn, and winter, whereas during summer and early autumn, the O_2 fluxes in light were negative. At Ulbjerg, the situation was different, with a positive O_2 flux in light most of the year and negative fluxes in light only when O_2 uptake rates in dark were very high (Fig. 3B). Nitrate was generally taken up by the sediment at both stations, but the seasonal patterns were different. The highest uptake rates at Virksund were found in spring and the lowest in summer and winter (Fig. 3C). At Ulbjerg, the pattern was the opposite (Fig. 3D): highest uptake rates of NO_3^- in summer and low rates the rest of the year. The sediment–water fluxes of NH_4^+ (Fig. 3E,F) and PO_4^{3-} (Fig. 3G,H) in dark followed the same pattern as did the O_2 fluxes: peak values in the summer and lowest values during winter. Ammonium and PO_4^{3-} were generally released from the sediment, and the rates in light and dark were generally not different except for a few occasions. The sediment–water flux of urea was very low compared to those of NH_4^+ and NO_3^- (data not shown), and the annual average urea flux was $<3\%$ of the sum of NO_3^- and NH_4^+ fluxes.

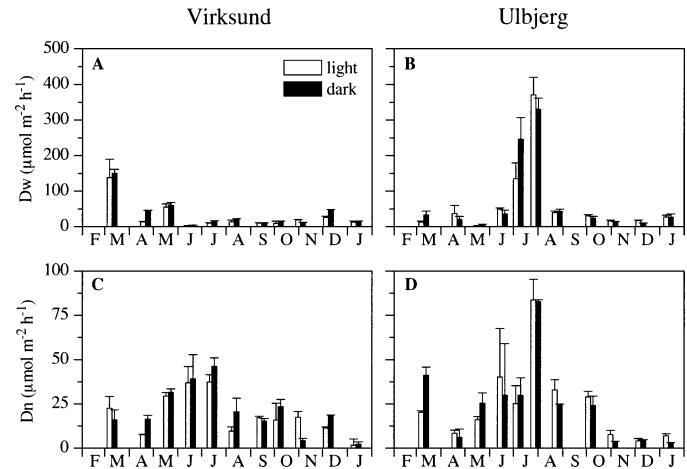


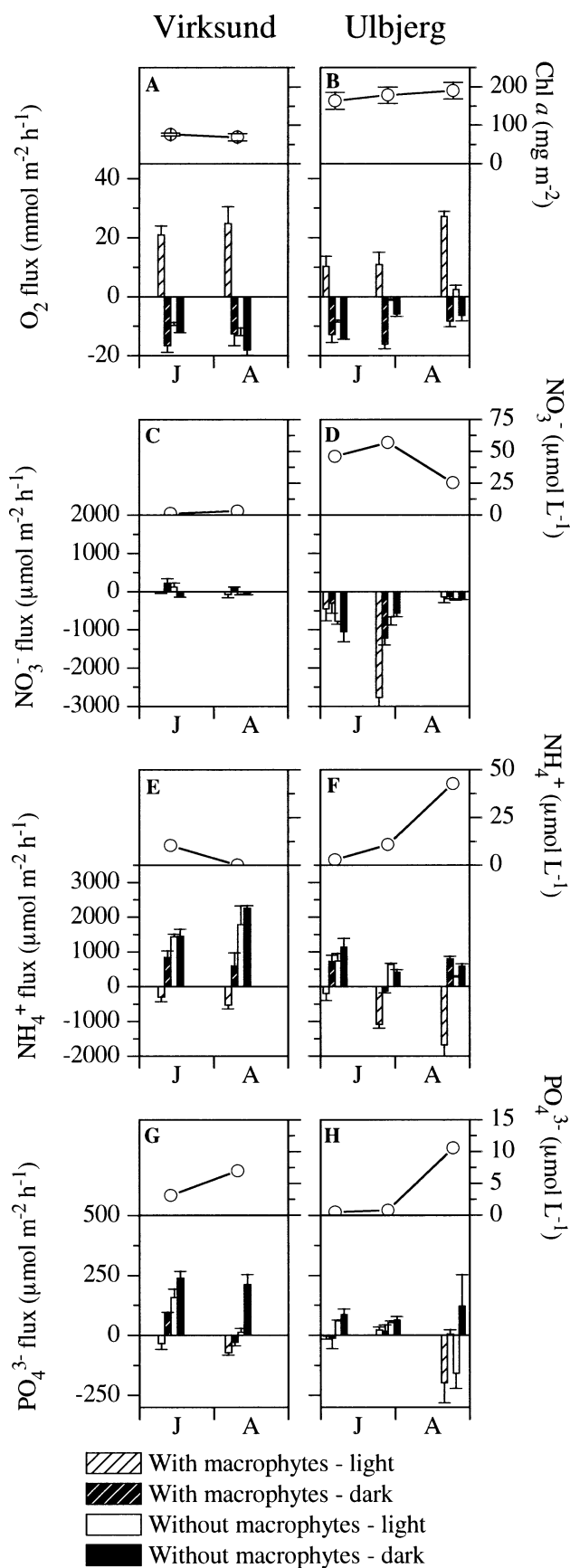
Fig. 4. (A, B) Rates of denitrification of nitrate from the water column (Dw) and (C, D) rates of coupled nitrification–denitrification (Dn) at Virksund and Ulbjerg. Error bars indicate the standard error.

Denitrification of NO_3^- from the water column (Dw) showed very different seasonal variations at the two stations. At Virksund, Dw was highest in the spring and lowest during summer (Fig. 4A), whereas the opposite was the case at Ulbjerg (Fig. 4B). The annual rate of Dw was significantly higher at Ulbjerg than at Virksund ($P < 0.01$, ANOVA), and on an annual basis, this corresponded to 58%. Rates of denitrification of NO_3^- produced in the sediment (Dn) were more similar at the two stations, both with respect to seasonal patterns and actual rates (Fig. 4C,D). The average annual rate of Dn was thus only 25% higher at Ulbjerg than at Virksund, but this difference was not significant ($P > 0.01$, ANOVA). Maximum rates were found in summer at both stations. The rates of Dn showed more differences between light and dark than did Dw, but this was not significant ($P > 0.01$, ANOVA).

Effects of floating macroalgae—Floating macroalgae (*Ulva*, *Chaetomorpha*, or *Enteromorpha*) were present on two sampling dates at Virksund and on three sampling dates at Ulbjerg (Table 1). The mats were between 2.7 and 5.3 cm thick and consisted of green, healthy-looking algae. The algae were lying unattached on the sediment surface. The macroalgae were considered to belong to the sediment community, and the flux rates were measured between the water

Table 1. Genus and biomass of macroalgae and thickness of the macroalgal mat at Virksund and Ulbjerg. Biomass is given in grams dry weight (dw) as an average of the three flux chambers. Standard error is given in brackets.

Site	Date	Genus	Biomass (g dw m ⁻²)	Thickness (cm)
Virksund	14 Jul 97	<i>Ulva</i>	291(14)	3.0
Virksund	11 Aug 97	<i>Enteromorpha</i>	118(9)	2.7
Ulbjerg	7 Jul 97	<i>Enteromorpha</i>	117(9)	5.3
Ulbjerg	29 Jul 97	<i>Chaetomorpha</i>	222(16)	4.7
Ulbjerg	25 Aug 97	<i>Ulva</i>	101(10)	3.0



column and the sediment/macroalgal compartment. There was a clear effect of the macroalgae on most of the flux rates measured at both stations. The macroalgae stimulated oxygen production in light ($P < 0.01$, ANOVA), and there always was a net efflux of O_2 (Fig. 5A,B). In darkness, the O_2 uptake was stimulated by the macroalgae at Ulbjerg ($P < 0.01$, ANOVA), whereas there was no general effect at Virksund ($P > 0.01$, ANOVA).

Water column concentrations of nitrate were very different at the two stations during the period with macroalgae ($P < 0.01$, ANOVA). At Virksund, both fluxes and concentrations were low, and in the presence of macroalgae, there was always an uptake in the light and a release in the dark (Fig. 5C). At Ulbjerg, where the NO_3^- concentrations in the water column were much higher, there was an uptake of NO_3^- in all situations (Fig. 5D). However, the effects of the macroalgae on the NO_3^- fluxes were not significant at either of the stations ($P > 0.01$, ANOVA).

The macroalgae had significant effects on the NH_4^+ fluxes ($P < 0.01$, ANOVA) except at Ulbjerg in the dark (Fig. 5E,F). There was an uptake in the light when macroalgae were present and a release when macroalgae were absent at both stations. At Virksund, there also was a release of NH_4^+ in the dark with macroalgae, but the release was less than without macroalgae ($P < 0.01$, ANOVA).

At Virksund, there was a significant difference between PO_4^{3-} fluxes in the presence and absence of macroalgae ($P < 0.01$, ANOVA; Fig. 5G). In the absence of macroalgae, there was always a release of PO_4^{3-} from the sediment. In the presence of macroalgae, there was an uptake in the light, and the flux in the dark was either negative, as well, or less positive than in the absence of macroalgae. The uptake rate in the presence of macroalgae increased with increasing PO_4^{3-} concentration. At Ulbjerg, the differences in PO_4^{3-} fluxes caused by the macroalgae were not significant ($P > 0.01$, ANOVA; Fig. 5H).

The effects of macroalgae on denitrification differed between the two stations. At Virksund, the rates of both Dw and Dn with macroalgae were generally lower than the corresponding rate without macroalgae (Fig. 6A,C); however, this difference was only significant in the dark ($P < 0.01$, ANOVA). At Ulbjerg, analysis of variance showed a significant effect of the presence of macroalgae on Dw ($P < 0.01$), which was generally lower in the presence of macroalgae (Fig. 6B). There were no effects of macroalgae on Dn at Ulbjerg ($P > 0.01$).

Primary productivity and nutrient budgets—Gross primary production (GPP) was substantially higher in the presence than in the absence of macroalgae at both stations ($P < 0.01$, ANOVA; Fig. 7). The ratio of GPP in the presence

Fig. 5. Sediment water flux rates of (A, B) O_2 , (C, D) NO_3^- , (E, F) NH_4^+ , and (G, H) PO_4^{3-} ; concentrations of (C, D) NO_3^- , (E, F) NH_4^+ , and (G, H) PO_4^{3-} in the water column at the start of the incubations; and the benthic microalgal biomass as Chl *a* for sediment without macroalgae (A, B) at both Virksund and Ulbjerg. Error bars indicate the standard error.

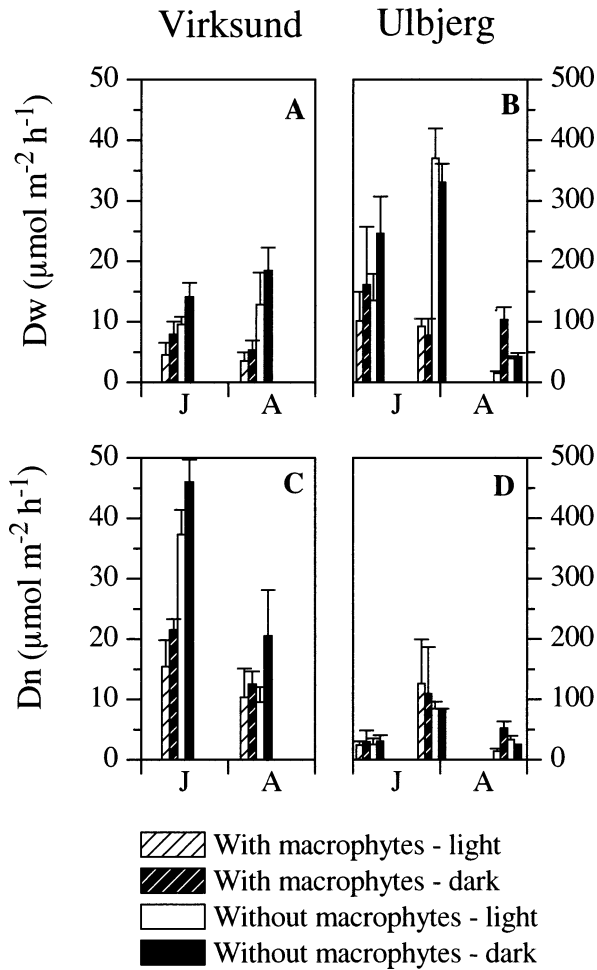


Fig. 6. (A, B) Rates of denitrification of nitrate from the water column (Dw) and (C, D) of coupled nitrification-denitrification (Dn) at Virksund and Ulbjerg. Note the differences in scale between the two stations. Error bars indicate the standard error.

of macrophytes to GPP in the absence of macrophytes ranged from 4 to 12.

The sediment/microalgal community was net heterotrophic on an annual basis at Virksund, but when the activity of the macroalgae was included, the sediment/algae community was net autotrophic (Fig. 8A). The release of NH_4^+ and PO_4^{3-} was 2.3 and 3.8 times higher, respectively, without than with macroalgae, whereas the effects of macroalgae on the annual averages of Dw and Dn and on the flux of NO_3^- were small.

The sediment/microalgal community was net autotrophic even without macroalgae at Ulbjerg, but with macroalgae included, the annual average O_2 flux was more than six times higher (Fig. 8B). There were also clear effects of the macroalgae on the nutrient fluxes, which were either more negative or changed from positive to negative when the activity of the macroalgae was included for both NO_3^- , NH_4^+ , and PO_4^{3-} (Fig. 8B). Denitrification of NO_3^- from the water column was 37% lower with the macroalgae included than without, whereas Dn was 10% higher with the macroalgae included in the annual average.

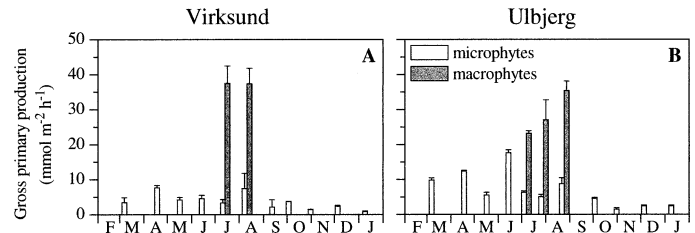


Fig. 7. Gross primary production (GPP) for both Virksund and Ulbjerg calculated as the difference between sediment-water fluxes of O_2 in light and dark. Error bars indicate the standard error.

Fauna—The infauna found at the two stations were mainly bivalves, polychaetes, and oligochaetes. Except for these groups, only a few *Corophium* were found, but always <50 individuals m^{-2} . The annual average biomass of bivalves, polychaetes, and oligochaetes were $2,508 \pm 443$, 149 ± 35 , and 33 ± 9 g fresh weight m^{-2} , respectively, at Virksund and 572 ± 141 , 100 ± 29 , and 2.4 ± 1.9 g fresh weight m^{-2} , respectively, at Ulbjerg. The bivalves were almost exclusively *Mya arenaria* L. and accounted for 93% of the infauna biomass at Virksund and 85% at Ulbjerg. The bivalve biomass was 4.4 times higher at Virksund than at Ulbjerg.

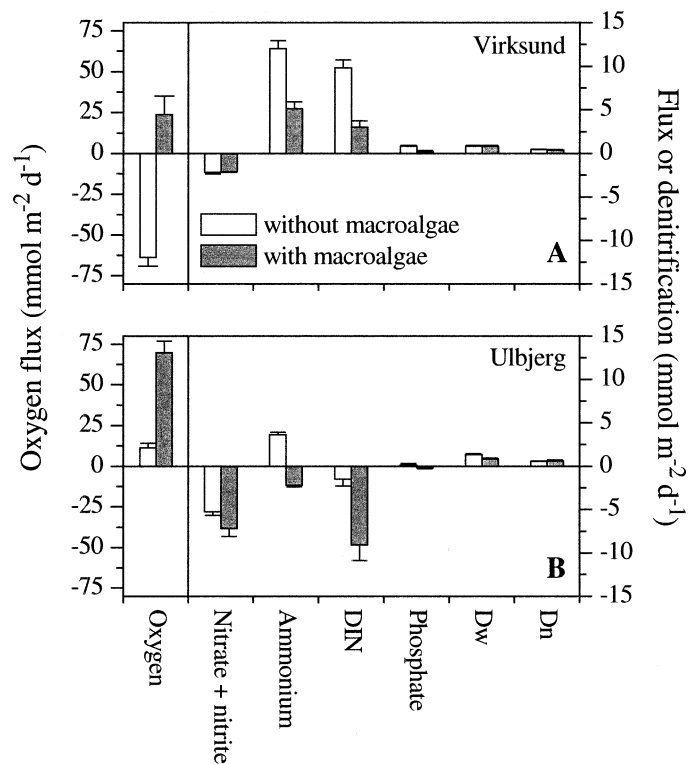


Fig. 8. Annual average rates of denitrification and sediment-water fluxes of oxygen and nutrients for both Virksund and Ulbjerg. Note that oxygen flux is related to the left-hand scale and all the other rates to the right-hand scale. For all rates shown, the difference between the rate with and the rate without macroalgae was significant ($P < 0.01$, t -test), except for Dw at Virksund, where the difference was not significant. Error bars indicate the standard error.

Discussion

The two stations are located in a eutrophied estuary, as evident from the high water column concentrations of inorganic nitrogen and phosphorous (Fig. 3). This leads to a high level of pelagic primary productivity, which in turn supports the relatively high rates of mineralization in the sediments, as indicated by very high oxygen uptake rates in summer (Fig. 3A,B). The two stations are rather similar with respect to water chemistry, except for the higher NO_3^- concentrations during summer at Ulbjerg, which is reflected in the high NO_3^- uptake and high rates of D_w (see below).

Incubation system—The incubation system is novel and proved very suitable for measuring sediment–water fluxes and denitrification on sediments, both with and without macroalgae. In this study, where the effects of macroalgae were investigated, an incubation system was needed where a macroalgal mat could be placed on top of intact sediment, which is not possible in a traditional 5- or 8-cm Plexiglas core tube. Furthermore, the stirring system of the flux chambers was designed to avoid the advective water transport caused by the radial pressure gradient that builds up in flux chambers with a rotating type of stirring. It has been shown that pore-water transport in flux chambers with rotating stirring can be significant and that the magnitude of this transport depends on the permeability of the sediment (Huettel and Gust 1992; Glud et al. 1996). In macroalgal mats, the permeability can be much higher than in sediments, and the advective transport would therefore be dominant. In the present study, the pumping of water through the diffusers created a unidirectional flow across the sediment or algal mat surface, and the stirring was sufficient for added $^{15}\text{NO}_3^-$ to reach the sediment, as indicated by the linear increase in $^{29}\text{N}_2$ and $^{30}\text{N}_2$ after 20–30 min. The exchange of water within a macroalgal mat in the field can be highly variable (Krause-Jensen et al. 1999), and it is very difficult to reproduce the in situ flow in a laboratory setup. It was thus decided to apply a standard flow rate for all incubations, which created sufficient stirring to make the rate measurements possible.

The use of 20- by 20-cm flux chambers, furthermore, has an advantage over the conventional 5- or 8-cm core, in that the activity of large infauna is better represented than in smaller sediment cores (Glud et al. 1994). This was especially important at Virksund because of the high density of large infauna.

Microphytobenthic primary production—Light and temperature were almost identical at the two stations (Fig. 2), but still, gross primary production was 85% higher at Ulbjerg than at Virksund. This difference is probably caused by the large difference in microphytobenthic biomass, which was 1.7 times higher at Ulbjerg than at Virksund. In this shallow-water environment, the microphytobenthos are likely to experience resuspension, during which time they are subject to grazing by the bivalves in the sediment (Sauriau and Kang 2000; Cognie et al. 2001). Because the biomass of bivalves at Virksund was 4.4 times higher than at Ulbjerg, the probability that suspended microphytobenthos would be removed by grazing was much higher than at Ulbjerg, which

would explain the difference in microphytobenthic biomass between the two stations.

The GPP of the benthic microalgae was in the upper range of that found for other microphytobenthic communities. The maximum value recorded at Ulbjerg was $18 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Fig. 7), and the maximum rate reported for benthic microalgae in a literature survey by Krause-Jensen and Sand-Jensen (1998) was $23 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$. It should be noted that the technique applied in the present study—calculating GPP as the difference between light and dark O_2 fluxes in intact sediment cores—probably underestimates the actual GPP because it is based on the assumption that respiration during light exposure is similar to respiration in the dark; this assumption is invalid because the increased O_2 penetration into illuminated sediments can increase respiration beyond that in the dark (Revsbech et al. 1981).

Nitrogen mineralization and fluxes in the absence of macroalgae—The production of NH_4^+ by mineralization in sediments has previously been calculated from the O_2 uptake and the C:N ratio of the organic material being mineralized (Rowe et al. 1975; Nixon et al. 1976; Boynton and Kemp 1985). These authors calculated the theoretical NH_4^+ production, assuming a C:N ratio of 6.6 (Redfield 1958) and compared that to the measured sediment–water flux of DIN. The measured values were generally lower than the theoretical value, and this difference was explained by either denitrification (Boynton and Kemp 1985; Dollar et al. 1991; Banta et al. 1994) or the recycling of a large fraction of the mineralized nitrogen as dissolved organic nitrogen (DON) (Nixon et al. 1976). However, at Virksund and Ulbjerg, the DIN flux was, to a large extent, a flux of NO_3^- from the water column to the sediment, probably caused by algal uptake (see below) and not by sediment mineralization. For that reason alone, the ratio of O_2 uptake to DIN flux would be expected to deviate from the Redfield ratio, and this deviation could not be attributed to either denitrification or production of DON. Instead, the ratio of O_2 uptake in darkness to net NH_4^+ production (NAP) was calculated and compared to the Redfield ratio in order to evaluate the extent to which the NH_4^+ produced by mineralization was retained in the sediment.

The Redfield ratio was used instead of the measured bulk sediment C:N ratio because it is relevant to compare the C:N ratio of the degradable fraction of the organic matter to NAP. The C:N ratio of the bulk sediment would include the refractory fraction of the organic matter and would thus not reflect the ratio between carbon oxidation and ammonium production. The NAP was calculated as the sum of the NH_4^+ efflux and nitrification. Nitrification was calculated as D_n multiplied by two under the assumption that half of the NO_3^- produced by nitrification would diffuse out of the sediment and the other half would become denitrified. This could introduce a small error, but because D_n was only 4% and 11% of the annual NH_4^+ efflux in darkness at Virksund and Ulbjerg, respectively, the small potential error introduced was judged acceptable. The NAP was thus a measure of the total NH_4^+ production in the sediment minus what might be lost to ammonium-consuming processes, other than nitrification, such as assimilation by algae or bacteria.

Table 2. Ratio of O₂ uptake in dark to net NH₄⁺ production (NAP) in light and dark for sediments without the influence of macroalgae. Net NH₄⁺ production was calculated as the sum of the NH₄⁺ flux plus twice the rate of Dn. The ratio was calculated as the slope of the regression for O₂ uptake in darkness as a function of net NH₄⁺ production for all sampling dates. The linearity of the relation is indicated by *r*². Gross NH₄⁺ missing is calculated as the actual measured NH₄⁺ flux relative to the NH₄⁺ flux that would have been if the O₂ flux:NAP ratio had followed the Redfield ratio, assuming a respiratory quotient of 1. *n* = 11 for each of the ratio calculations.

	Virksund		Ulbjerg	
	Dark	Light	Dark	Light
O ₂ flux (dark):NAP	7.6	9.2	10.7	15.9
<i>r</i> ²	0.94	0.88	0.96	0.64
Gross NH ₄ ⁺ missing	13%	28%	38%	58%

O₂ uptake in the dark is a direct measure of carbon mineralization, assuming a respiratory quotient of 1 (Strickland and Parsons 1972). Furthermore, it is assumed that any reduced species formed by anaerobic mineralization of organic matter, such as H₂S formed by sulfate reduction, are completely reoxidized with O₂. O₂ consumption is thus a measure of the total mineralization in the sediment (Trimmer et al. 2000). Considering that the two stations were only 2.5 km apart, it is assumed that the C:N ratio of the organic matter being mineralized was approximately the same at both stations, and it is furthermore assumed that this was close to the Redfield ratio (Nixon et al. 1976; Banta et al. 1994). At Virksund, the ratio of the O₂ flux in dark to NAP in dark was 7.6 (Table 2), which is close to the Redfield ratio of 6.6. This suggests that most of the NH₄⁺ produced in the sediment by mineralization was either released to the water column as NH₄⁺ or was nitrified. At Ulbjerg, on the other hand, the ratio of dark O₂ uptake to NAP in darkness was 10.7 (Table 2), which was 41% higher than at Virksund and 62% higher than the Redfield ratio. This leads to the conclusion that a large fraction (38%; Table 2) of the NH₄⁺ produced by mineralization in the sediment at Ulbjerg must either have been retained in the sediment or have left the sediment in a form different from the nitrogen species included in the NAP.

The close agreement between the ratio of O₂ uptake to NAP in darkness and the Redfield ratio at Virksund suggests that the main end products of nitrogen mineralization were included in the NAP (i.e., NO₂⁻, NO₃⁻, NH₄⁺), with only a minor fraction left for other N compounds such as DON. Assuming that this was also the case at Ulbjerg and given that microphytobenthic primary production was much higher there than at Virksund, it is suggested that assimilation of NH₄⁺ by the benthic microalgal community was the main reason for the deviation of the ratio of O₂ uptake to NAP from the Redfield ratio. This implies that NH₄⁺ must have been assimilated by the benthic microalgae also in darkness, which agrees with previous observations (Petterson and Sahlsten 1990; Cochlan et al. 1991; Rysgaard et al. 1993; Thornton et al. 1999). The ratios of dark O₂ flux to NAP in light at both Virksund and Ulbjerg deviated even more from the Redfield ratio (Table 2), which indicates that more of the

NH₄⁺ produced by mineralization was immobilized in the sediment in light; probably because of a higher assimilation by the microphytobenthic community at the sediment surface. This implies a very tight coupling of mineralization and assimilation in microphytobenthic communities where the nitrogen demand is high (Sundbäck et al. 2000). The lower *r*² values for the relationship between O₂ fluxes in dark and NAP in light indicate that the assimilation of NH₄⁺ in light was much more variable than in dark, which agrees well with the higher variation in O₂ fluxes in light than in dark (Fig. 3A,B).

This method for estimating assimilation of mineralized nitrogen by microphytobenthos is based on the assumption that the mineralization is the same in light and dark. However, because of a higher O₂ penetration in light (data not shown), mineralization was higher in light than in darkness. Consequently the O₂ flux in dark, used as an indicator of mineralization, underestimated the true mineralization in light; therefore, the amount of NH₄⁺ missing was thus also underestimated and the effect of microphytobenthos on the sediment water flux of nitrogen was probably even greater than indicated above.

The conclusion, that NH₄⁺ was more effectively retained in the sediment at Ulbjerg because of assimilation by the microphytobenthic community, is consistent with the three-fold higher NH₄⁺ efflux exhibited by the sediment at Virksund and a much lower benthic photosynthesis in the sediment at Virksund than at Ulbjerg on an annual basis (Fig. 8). By this method, it is possible to estimate the degree to which the microphytobenthos controls the release of mineralized nitrogen from the sediment to the water column. This filter effect has mainly been recognized when DIN fluxes in light and dark were different (e.g., Nowicki and Nixon 1985; Sundbäck 1986; Sundbäck and Granéli 1988; Rizzo 1990; Sundbäck et al. 1991; Rizzo et al. 1992; Rysgaard et al. 1995), but with this technique, the retention of nitrogen in the sediment can be estimated in both light and dark.

From the data presented here, it is evident that estimating denitrification from deviations of the ratio of O₂ flux:NAP or O₂ flux:DIN from the Redfield ratio can be problematic in sediments with an active microphytobenthic community. Even when incubations are performed in the dark, the microphytobenthos can still affect the ratios of carbon mineralization to inorganic nitrogen released by the sediment. Furthermore, the capacity for nutrient uptake by microphytobenthos can persist, even after several days in darkness (Rysgaard et al. 1993).

In the presence of macroalgae, the O₂ flux:NAP technique for estimating N retention in the sediment does not work. Macroalgae can store significant amounts of carbohydrate, which can be respired at night, and carbon oxidation and NH₄⁺ production are thus decoupled. However, the macroalgae retained NH₄⁺ even more effectively than did the microphytobenthos. In light, NH₄⁺ assimilation of the macroalgae created a net uptake of NH₄⁺ from the water column (Fig. 5E,F), indicating that the macroalgae were capable of retaining all of the NH₄⁺ produced in the sediment and, in addition, take up NH₄⁺ from the water column. In the presence of macroalgae in darkness, the efflux of NH₄⁺ from the sediment was smaller than in its absence (Fig. 5E,F). The

macroalgae were thus much more efficient than the microphytobenthos in reducing the release of NH_4^+ from the sediment.

Nitrate assimilation by microphytobenthos and Dw—Nitrate was generally taken up from the water column at both stations, but at very different rates (Figs. 3C,D, 8). Denitrification of NO_3^- from the water column only constituted a relatively small part of the total NO_3^- uptake. On an annual basis, this was 38% for Virksund and 25% for Ulbjerg. Furthermore, it is evident that the small differences in rates of Dw between the two stations cannot explain the large differences in NO_3^- fluxes (Fig. 8). A more likely cause for the large difference between the two stations is that the nitrogen demand for benthic primary production was higher at Ulbjerg and that the NO_3^- concentration there was much higher during summer, when the potential for NO_3^- uptake was high. The higher uptake of NO_3^- in light than dark recorded on some of the sampling dates at Ulbjerg (Fig. 3D) was probably caused by higher NO_3^- assimilation by the benthic microalgae in illuminated sediments. As argued above, NH_4^+ assimilation occurred both in light and darkness, and it is therefore likely that NO_3^- assimilation by microphytobenthos also continued in darkness. Therefore, NO_3^- assimilation might be important even when NO_3^- uptake rates are the same in light and dark. This is supported by the observation that freshwater microphytobenthos assimilated NO_3^- for up to 60 h in darkness (Rysgaard et al. 1993). The uptake of NO_3^- by benthic microalgae from the water column must have been in direct competition with Dw, and it is suggested that this was the main reason that only a relatively low proportion of the NO_3^- uptake was denitrified. This agrees well with the conclusion of Rysgaard et al. (1993) that the uptake of NO_3^- by benthic microalgae was responsible for a large discrepancy between Dw and total sediment NO_3^- uptake.

It should be noted that when applying the isotope pairing technique to sediments with a high benthic primary production, it is critical that the added $^{15}\text{NO}_3^-$ actually reaches the denitrifying zone at a constant rate and that it is not all assimilated by the benthic primary producers. When a linear development of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ over time is recorded, this is the case. All the incubations in this study were carried out as time series, with three samplings in light and three in dark. There was a linear increase in $^{29}\text{N}_2$ and $^{30}\text{N}_2$ concentrations over time in all incubations, indicating that the added $^{15}\text{NO}_3^-$ did reach the denitrifying zone.

Interactions between microphytobenthos and Dn—The differences between light and dark rates of Dn did not reveal a distinct pattern. On some occasions, rates of Dn were found to be lower in light than in darkness (Fig. 4C,D; Virksund in April, August, and December; Ulbjerg in March), coinciding with low NH_4^+ concentrations in the water column (Fig. 3C) and high rates of gross primary production (Fig. 7A). This could indicate that competition for NH_4^+ between benthic primary producers and nitrifiers was limiting nitrification (Henriksen and Kemp 1988) and that this competition was most intense in light. There might also be competition for NH_4^+ in dark because the microphytobenthic

community, as argued above, can assimilate NH_4^+ in the dark.

On other sampling dates, Dn was higher in light than in darkness (Fig. 4; Ulbjerg in August, November, and January). This coincided with higher ammonium concentrations in the water column and relatively low rates of GPP. This stimulation of Dn in light is probably caused by the combination of high NH_4^+ availability and stimulation of nitrification by the increased availability of O_2 in light (Rysgaard et al. 1995). It should be noted that even though Dn was higher in light than dark, it might well have been even higher in the absence of benthic primary producers. This idea is supported by statistical analysis of data from 18 European estuaries (Risgaard-Petersen 2003), which showed that Dn was highest when there was no activity of microphytobenthos. This also agrees with the finding of Henriksen and Kemp (1988) that potential nitrification activity in the upper 4 mm of the sediment was more than 10 times higher when sediment with benthic microalgae had been kept in the dark for 6 weeks than when it had been kept under day/night light cycles.

Phosphate turnover in the absence of macroalgae—The relation between O_2 uptake in the dark and PO_4^{3-} flux was much less linear (not shown) than the relationship for NH_4^+ discussed above, and the ratios of O_2 uptake to net PO_4^{3-} production were not calculated. The main reason for this lack of relationship is probably the dependence of PO_4^{3-} immobilization in the sediment on a number of factors other than mineralization, such as the redox state of the sediment (e.g., Ruttenberg 1992; Jensen et al. 1998; Rozan et al. 2002). The O_2 penetration in darkness into the sediment, and thus the redox state of the sediment, was lower in summer than in winter (data not shown). Therefore, the binding capacity for PO_4^{3-} in the sediment was smaller during summer than winter, which could have led to a release of bound PO_4^{3-} during summer and a binding of PO_4^{3-} during winter; the PO_4^{3-} flux would therefore reflect both mineralization and adsorption/desorption. The PO_4^{3-} flux out of the sediment was generally lower at Ulbjerg than at Virksund (Figs. 3G,H, 8), suggesting that the benthic microalgae possess the same regulatory mechanism for PO_4^{3-} as discussed above for NH_4^+ and that assimilation by the algae reduced the flux of PO_4^{3-} released in the sediment to the overlying water.

Effect of fauna on nutrient fluxes—The larger O_2 uptake in the dark at Virksund than at Ulbjerg (40%, annual average) probably was mainly caused by the differences in biomass of infauna, which was four times higher at Virksund than at Ulbjerg. Thus, the mineralization of both nitrogen and phosphorus was higher at Virksund, and the higher release of both NH_4^+ and PO_4^{3-} was due to a combination of higher production in the sediment and lower assimilation by the benthic microalgae at the sediment surface, as discussed above. Also, the infauna type plays an important role in regulating the balance between mineralization and nutrient release. Bivalves, which made up 93% of the infauna biomass at Virksund, excrete the NH_4^+ produced by mineralization via the siphons directly to the overlying water (Magni et al. 2000). The benthic microalgae therefore did not have the

opportunity to intercept the NH_4^+ transported by the pumping action of the mussels that they had for the NH_4^+ that diffused from the bulk sediment through the sediment surface to the water column. For macroalgal mats lying on the sediment surface, the situation was different. The activity of these mats clearly reduced the efflux of NH_4^+ and PO_4^{3-} at both Virksund and Ulbjerg in light and at Virksund in the dark as well (Fig. 5E,F). Because of their position on top of the sediment, the macroalgae had the opportunity to take up the nutrients whether they were pumped out by the bivalves or they diffused out of the sediment. Floating macroalgae thus have a greater potential for intercepting the nutrient flux from the sediment to the water than do the microphytobenthos.

Macrophyte primary production—The presence of macroalgae even for a couple of months during summer strongly increased the annual benthic primary production, as indicated by the annual average O_2 flux (Fig. 8). At Virksund, the sediment community changed from heterotrophy to autotrophy on an annual basis, whereas at Ulbjerg, the degree of autotrophy greatly increased, as indicated by the stimulation of the annual average O_2 flux by a factor of six (Fig. 8). It is therefore concluded that annual benthic primary productivity in shallow coastal ecosystems can be significantly stimulated by the occurrence of macroalgae, even if this is only a transient event. The maximum GPP of the macroalgae was $37 \text{ mmol m}^{-2} \text{ h}^{-1}$ at Virksund and $35 \text{ mmol m}^{-2} \text{ h}^{-1}$ at Ulbjerg, which was substantially higher than that of the benthic microalgae at both stations (Fig. 7). This is exactly the same rate of macroalgal photosynthesis as found on a British intertidal flat ($37 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$, Trimmer et al. 2000). The GPP of the macroalgae was also quite high compared to other aquatic macrophyte communities; the maximum value found in a literature study was $60 \text{ mmol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Krause-Jensen and Sand-Jensen 1998).

Effects of macroalgae on NO_3^- uptake and denitrification—The partitioning of the NO_3^- uptake between Dw and other NO_3^- sinks differed between the two stations on an annual scale. With the macroalgae included in the annual average, 39% of the NO_3^- uptake from the water column was denitrified at Virksund, whereas this figure was only 12% at Ulbjerg. At Virksund, it was the same as in the absence of macroalgae (38%), whereas at Ulbjerg, it was only about half of that found in the absence of macroalgae (25%). Dw was thus a minor sink for NO_3^- taken up from the water column, and the macroalgae were capable of lowering the significance of this sink. This agrees well with results from a British macroalga-dominated estuary, where Dw was found to be a small sink for NO_3^- (Trimmer et al. 2000). The pattern of the quantitative significance of Dw as a sink for NO_3^- in the present study was mainly caused by the large capacity for NO_3^- uptake by the macroalgae and the high NO_3^- concentrations in the water column at Ulbjerg during the period when the macroalgae were present. The proportion of NO_3^- uptake that was denitrified was lower in the presence than in the absence of macroalgae, indicating that there was competition for NO_3^- between assimilation by the macroalgae and denitrification at Ulbjerg. This is also sup-

ported by a 37% lower rate of Dw at Ulbjerg and a 4% lower rate at Virksund when the macroalgae were included in the annual average.

Therefore, macroalgae have the potential to lower Dw through competition for NO_3^- from the water column. However, whether this potential is realized depends on several factors. The macroalgae form a mat that separates the overlying water from the sediment, and uptake of NO_3^- by the macroalgae can lower denitrification by lowering the NO_3^- concentrations at the sediment surface. The degree to which Dw is lowered depends on the balance between the macroalgal consumption of NO_3^- and the rate at which NO_3^- is transported toward the sediment surface. The advection within the mat here is crucial, and in the flux chambers used in this study, NO_3^- from the water column was apparently transported toward the sediment surface at a rate so high that the mat could not fully intercept the transport. Nitrate would thus reach the sediment surface, but the concentration here would be lower than above the mat. The effect of macroalgae on Dw would thus probably have been even greater at lower stirring rates and vice versa. This is supported by in situ observations of higher flushing of a *Chaetomorpha linum* mat, yielding a much lower accumulation of NH_4^+ and PO_4^{3-} within the mat on windy days than on calm days (Krause-Jensen et al. 1999). Thus, it is not possible from the present data to conclude how macroalgal mats generally affect Dw in the field. In areas where NO_3^- concentrations are high during summer and where Dw would also normally be high, floating macroalgae might significantly interfere with nitrogen removal through denitrification. Nitrogen that would otherwise have been removed by denitrification would instead be stored temporarily in macroalgal biomass and would be released later when the macroalgae are decomposed.

Macroalgae and Dn—Coupled nitrification–denitrification was only affected by the macroalgae in one out of the five samplings with macroalgae. That was in July at Virksund, where Dn in the presence of macroalgae was about half that in its absence. The most likely reason is competition for NH_4^+ between nitrification and assimilation because the NH_4^+ demand in both light and dark was much higher in the presence than in the absence of macroalgae (Fig. 5E). It would therefore seem that macroalgae could affect Dn in the same way as discussed above for microphytobenthos. However, the microalgae can affect both sources of NH_4^+ for nitrification (i.e., NH_4^+ derived from both the sediment and the water column) because of their position within the upper sediment layer where nitrification occurs. The macroalgae, on the other hand, are only able to exert their control of Dn by regulating the availability of NH_4^+ from the water column because of their position on top of the sediment. The potential for macroalgae to lower Dn must therefore be smaller than for microphytobenthos, and the effect of the macroalgae on the annual average Dn was thus only marginal (Fig. 8).

It is well known that macroalgae have a high capacity both for primary production and for nutrient uptake (McGlathery et al. 1997; Valiela et al. 1997). In this study, productivity and nutrient uptake in the macroalgae were found to exceed that of the microalgae by far, and the presence of macroalgae

for just a couple of months during summer completely changed both the nutrient and oxygen budgets of these shallow-water sites. This illustrates the importance of including the macroalgae in estuarine nutrient and oxygen budgets, even if there only is a transient appearance of macroalgae. Denitrification was, on the other hand, only slightly affected by the macroalgae. The microphytobenthos also played a significant role in regulating the nutrient transport between sediment and the water column. By the new approach of comparing the O_2 : NAP ratio to the Redfield ratio, it was calculated that the microphytobenthos retained up to 58% of the NH_4^+ produced in the sediment. The effect was greatest in light but was also significant in darkness. The benthic primary producers were thus able to reduce the release of nutrients to the water column, and therefore reduce the pelagic primary production, ultimately increasing benthic primary production through improved light conditions at the sediment surface.

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