

# Seasonal cycles of $O_2$ , $NO_3^-$ and $SO_4^{2-}$ reduction in estuarine sediments: the significance of an $NO_3^-$ reduction maximum in spring

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**ABSTRACT:** Rates of oxygen uptake, denitrification, nitrate reduction to ammonia, and sulfate reduction were measured in a Danish estuary over 1.5 yr. In sediments near the sea entrance,  $O_2$  and  $SO_4^{2-}$  reduction dominated and the relative contributions of the 4 processes were 65, 3, 5 and 27 %, respectively, of the total electron flow. In sediments near the river outlet, sulfate became limiting while nitrate was more abundant. This shifted the relative contributions towards nitrate reduction: 44, 4, 33 and 19 %, respectively. At low salinities, depth of the sulfate reduction zone (4 to 10 cm), but not maximum reduction rate, was limited by low sulfate concentrations in the overlying water. The nitrate zone varied from 0.5 to 5 cm depth over the year. Oxygen uptake and sulfate reduction varied seasonally in accordance with temperature. Reduction of nitrate to  $N_2$  and to  $NH_4^+$ , as well as emission of  $N_2O$  to the atmosphere, showed a short maximum in spring and were relatively constant throughout the rest of the year. The spring maximum coincided with a rapid water-temperature increase and a high influx of nitrate from the main river. Annual emission of  $N_2O$  corresponded to only 1 to 5 % of the measured denitrification. Annual loss of combined nitrogen by denitrification in sediments corresponded to 5 % of nitrate influx from the river.

## INTRODUCTION

Estuaries play an important role in regulating the flow of nitrogen, phosphorus and other elements from rivers into the sea (e.g. Wollast 1983). Due to the generally high productivity and shallow water depth of estuaries, a large part of the mineralization of the organic material takes place within the sediments. The exchange of nutrients between sediment and water can therefore significantly affect primary production within the estuary as well as lateral transport of the elements out to coastal waters.

Availability of combined nitrogen seems to be a main limiting factor for algal growth in coastal marine ecosystems (e.g. Boynton et al. 1982). Available nitrogen is lost through reduction of  $NO_3^-$  to  $N_2$  by denitrifying bacteria or via formation and emission of  $N_2O$ . Nitrous oxide may appear as an intermediate product of both nitrification and denitrification, and these processes are primarily bound to the sediments. This leads to a mutual regulation of nutrient cycling in the estuarine sediment and water. Nitrogen-limited primary production in the water column is usually the

main source of organic flux to the sediment. This organic flux will in turn determine regeneration and transport of nutrients from the sediment to the water.

There does not seem to be a simple relation between the flux of organic nitrogen to the estuarine sediment, and the partitioning between regenerated combined nitrogen and gaseous nitrogen,  $N_2$  and  $N_2O$ . Parameters such as temperature, chemical zonations in the sediment, bioturbation, and regulation by other mineralization processes may all play a significant role. Comparative studies of  $O_2$ ,  $NO_3^-$ , and  $SO_4^{2-}$  respiration in Danish fjord sediments have previously shown that nitrate had little quantitative effect on the overall mineralization of organic matter (Fenchel & Blackburn 1979, Sørensen et al. 1979). This, however, did not preclude a large influence of nitrate reduction on the nitrogen balance.

The purpose of the present field study was to analyze the relative contributions of  $O_2$ ,  $NO_3^-$ , and  $SO_4^{2-}$  reduction to the mineralization of organic matter in estuarine sediments and to show how these contributions changed from the river outlet to the sea. Another purpose was to estimate the role of nitrogen loss by

Table 1. Sediment characteristics of 5 investigated sites. Porosity is stated as % volume of porewater, organic content as % dry weight

Station	Description	Porosity		% dry org.	
		0 to 1 cm	9 to 10 cm	0 to 1 cm	9 to 10 cm
1.	'Marine', fine and medium sand	40 %	39 %	1.2	1.1
2.	'Marine', silt, sheltered bay	84 %	58 %	8.9	3.3
3.	'Marine', silt and sand, exposed	69 %	46 %	2.6	1.7
4.	'Fresh', silt, near reed swamp	82 %	66 %	10.2	4.8
5.	'Fresh', silt, near river outlet	84 %	48 %	12.1	4.0

denitrification in relation to the riverine influx of  $\text{NO}_3^-$  to the estuary. Concurrent measurements of  $\text{N}_2\text{O}$  emission were made for comparison with the  $\text{N}_2$  losses by denitrification. Due to the strong seasonality of the processes, their rates were measured at regular intervals over 1.5 yr.

### MATERIALS AND METHODS

The study was carried out in Norsminde Fjord, a shallow estuary of 1.9 km<sup>2</sup> situated on the east coast of Jutland, Denmark. Mean water depth is only 1 m. The estuary is connected to the sea through a narrow entrance to the east and receives freshwater mostly from Odder River to the west. The catchment area of the river is 101 km<sup>2</sup>. It receives drainage water mostly from agricultural areas as well as sewage from the town of Odder. Salinities in the eastern ('marine') part of Norsminde Fjord varied from 8 to 23‰ and in the western ('fresh') part from 0.5 to 8‰. The tidal amplitude is 10 to 25 cm. Extreme high waters are prevented by sluices at the sea-entrance. The estuary is surrounded by farmland and is moderately eutrophic with an annual primary productivity of plankton of 200 g C m<sup>-2</sup>.

A general description of Norsminde Fjord, its hydrography and ecology is given by Muus (1967). Different aspects of the C, N, and S cycles in sediments of the estuary have been published previously (e.g. Sørensen 1978, 1984, Sørensen et al. 1979, Hansen et al. 1981, Iversen & Blackburn 1981, Troelsen & Jørgensen 1982, Howarth & Jørgensen 1984). (In some of these papers the estuary was called 'Kysing Fjord' which, however, comprises only the southeastern part).

Five localities were studied at 1 to 2 mo intervals from January 1983 to June 1984. Three of these, Stations 1 to 3, were situated in the high-salinity part and 2, Sta. 4 and 5, in the low-salinity part of the estuary. The 5 localities and their sediments are characterized in Table 1.

**Process rates.** The whole seasonal study comprised 500 measurements of oxygen uptake rates, 100 measurements of denitrification and total nitrate reduction, and 600 measurements of sulfate reduction in undis-

turbed sediment cores. Measurements of oxygen uptake and sulfate reduction were done at all 5 stations while measurements of nitrate reduction, denitrification and  $\text{N}_2\text{O}$  emission were done at Sta. 1 and 4 only. All rate measurements were done on sediment cores collected by hand in Plexiglas tubes and brought back to the laboratory. The cores were submerged in aerated and circulating water from the sampling locality in order to maintain the natural distribution and turnover of the electron acceptors. The cores were kept in the dark at *in situ* temperature until analysis within 1 to 2 d. No detectable changes in the  $\text{O}_2$ ,  $\text{NO}_3^-$  or  $\text{SO}_4^{2-}$  distributions took place during this pre-incubation. Rate measurements were done also in the dark at *in situ* temperature.

Oxygen uptake rates were measured in four 5 cm wide cores both with and without magnetic stirring as described previously (Jørgensen 1977). Each uptake rate was calculated as the mean of these 8 measurements. Sulfate reduction rates were measured with <sup>35</sup>S tracer by the core injection technique (Jørgensen 1978). Three parallel cores were injected at 1 cm intervals down to 10 cm and incubated for 4 h. Sediment segments from the same depths in the 3 cores were pooled before analysis of radioactivity in free and acid volatile sulfide. The radioactivity of other reduced sulfur pools was not analyzed here but was included in an earlier study (Howarth & Jørgensen 1984).

Denitrification rates were measured by the acetylene inhibition technique of Sørensen (1978) as modified by Andersen et al. (1984). Twelve parallel sediment cores per station were sampled in 4 cm wide tubes and stoppered with 50 ml of seawater and 30 ml of air space above the sediment. Continuous magnetic stirring of the water provided a natural flux of oxygen and nitrate across the sediment-water interface. In 6 of the cores, multiple injections of  $\text{C}_2\text{H}_2$ -saturated dist. water were made to obtain a final 10%  $\text{C}_2\text{H}_2$  saturation in both sediment and water. A parallel series of 6 cores was injected with  $\text{N}_2$ -purged water for control. The accretion of  $\text{N}_2\text{O}$  in the cores was assayed after 1 to 5 h of incubation.

$\text{N}_2\text{O}$  was analyzed in 1 cm segments of sediment as well as in the overlying water and headspace. Sedi-

ment and water samples were transferred to 60 ml vials with 5 ml of 1M KCl and equilibrated with the gas phase by shaking. Gas samples of 0.3 ml were injected into a gas chromatograph (Packard 427) equipped with a <sup>63</sup>Ni Electron Capture Detector. Gases were separated on a 2 m × 3.2 mm Porapak Q column at 60 °C.

Measurement of total nitrate reduction rates was based on the recent observation that acetylene is also an inhibitor of nitrification (Walter et al. 1979). Thus, in the C<sub>2</sub>H<sub>2</sub>-injected cores there was no NH<sub>4</sub><sup>+</sup> oxidation and the rates of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> depletion were therefore a measure of their rate of consumption. Due to the high ammonium concentration in the sediments, assimilatory nitrate reduction is expectedly inhibited (cf. Payne 1973), and nitrate and nitrite are reduced partly by denitrification to N<sub>2</sub> and partly by dissimilatory reduction to NH<sub>4</sub><sup>+</sup>. Nitrate and nitrite were therefore assayed in the same samples that were used for N<sub>2</sub>O determination. After N<sub>2</sub>O analysis, the samples were centrifuged at 2000 × g and the supernatant assayed colorimetrically on an autoanalyzer (Chemlab) after the method of Armstrong et al. (1967). The difference between the total rate of NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> disappearance and the rate of denitrification was taken as a measure of the rate of NO<sub>3</sub><sup>-</sup> reduction to NH<sub>4</sub><sup>+</sup>.

**Other determinations.** Water samples of 120 ml were collected in the fjord at Sta. 1 and 4 for N<sub>2</sub>O analysis. Twenty ml was replaced by pure N<sub>2</sub> and, after shaking and temperature equilibration in the laboratory, N<sub>2</sub>O was analyzed in gas samples of the headspace. Other water samples were taken for determination of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> as described above as well as for sulfate and salinity. Sulfate was measured gravimetrically or photometrically after barium precipitation (Amer. Publ. Health Ass. 1971, Tabatabai 1974).

Porewater samples for the measurement of sulfate were obtained by pressure filtration under N<sub>2</sub> through 0.45 μm filters. The filtrate was collected directly in 10% Zn-acetate to trap the sulfide. Sulfate analysis was done as in water samples. Other porewater samples were obtained for the analysis of nitrate and nitrite. Sediment porosity and dry organic matter were measured by drying known volumes of sediment to constant weight at 105 °C and igniting at 450 °C for 12 h, respectively.

Emission rates of N<sub>2</sub>O across the water-air interface were estimated from N<sub>2</sub>O concentrations in the fjord water by use of the stagnant film model (Broecker & Peng 1974):

$$F = D \times (C_s - \alpha C_a) / z$$

where  $F$  = flux from water to atmosphere;  $D$  = molecular diffusion coefficient of N<sub>2</sub>O in the water;  $C_s$  and  $C_a$  = concentrations of N<sub>2</sub>O in water and atmosphere;  $\alpha$  = Bunsen solubility coefficient for N<sub>2</sub>O;  $z$  =

thickness of stagnant film. Values of  $D$  and  $\alpha$  were obtained from Broecker & Peng (1974) and Weiss & Price (1980), respectively; they were corrected to the *in situ* salinity and temperature. The atmospheric N<sub>2</sub>O concentration, about 0.3 ppm, was measured in air samples from the field. A stagnant film thickness,  $z$ , of 300 μm was chosen to be representative of the average field conditions with moderate wind speeds in the sheltered estuary (cf. Broecker & Peng 1974, Emerson 1975, Jørgensen & Okholm-Hansen in press).

Emission rates of N<sub>2</sub>O from the sediment were measured directly on cores as described by Jensen et al. (1984). Water and air volumes of 25 ml each were maintained over the sediment and the water was stirred to facilitate a natural gas exchange. N<sub>2</sub>O was analyzed at intervals in both water and headspace and from the linear increase the emission rate was calculated.

## RESULTS

### O<sub>2</sub> uptake and SO<sub>4</sub><sup>2-</sup> reduction

Sulfate reduction rates followed seasonal temperature variations at all stations. At the eastern stations, 1 to 3, in the marine part of Norsminde Fjord, the sulfate reduction zone extended far below 10 cm depth in the sediment during the whole year. At the 2 western stations, 4 and 5, near the freshwater outlet, low sulfate concentration in the water limited depth of sulfate reduction zone (Fig. 1). The salinity in the water varied from 0.5 to 8‰ and the sulfate concentrations varied accordingly from 0.4 to 6 mmol l<sup>-1</sup>. The highest concentrations were found during summer when run-off was minimal, and the lowest were found in winter and spring. In spite of the lower concentration, sulfate penetrated deeper into the sediment during winter due to low sulfate reduction activity. The sulfate zone was 8 to 10 cm deep from October to April. From May through September the higher temperature stimulated sulfate reduction and sulfate was depleted already at 4 to 5 cm depth. Within the sulfate zone, however, the lower concentrations did not seem to limit reduction rates strongly. The maximum rates of 600 to 800 nmol SO<sub>4</sub><sup>2-</sup> cm<sup>-3</sup> d<sup>-1</sup> at Sta. 5 were as high as the maximum rates at the marine stations.

Comparison of the oxygen uptake rates and the rates of sulfate reduction calculated for 1 m<sup>2</sup> of sediment showed that they both varied in proportion to temperature with a maximum in July-August and a minimum in January-February (Fig. 2). Temperature extremes were 20 °C in summer and 2 °C in winter. The temperature coefficient ( $Q_{10}$  = the factor of rate increase per 10 C° temperature increase) was 2.7 for oxygen uptake

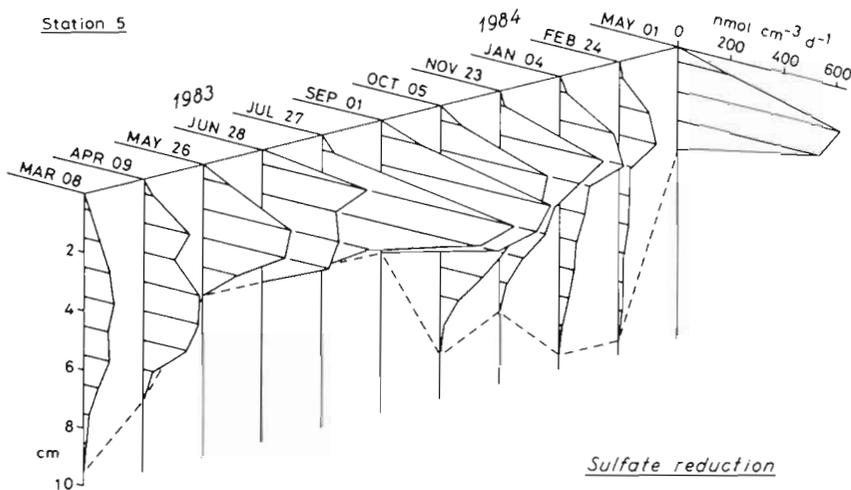


Fig. 1. Seasonal variation of sulfate reduction rates in sediments of Sta. 5. Broken line: lower boundary of sulfate zone

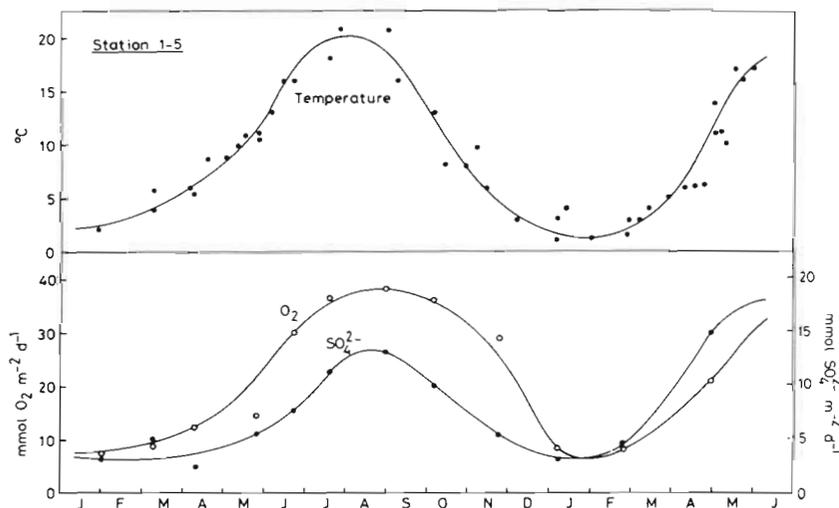


Fig. 2. Mean rates of oxygen uptake and sulfate reduction per unit area at 5 stations in Norsminde Fjord. Sulfate reduction was integrated down to 10 cm depth. Process rates follow temperature variations in the overlying water

and 2.1 for sulfate reduction. The lower temperature effect for sulfate reduction was probably due to the narrow reduction zone during summer which limited the areal reduction rates.

### Nitrate reduction

Seasonal variations in total nitrate reduction and denitrification were regulated by nitrate availability as well as by temperature. The influx of nitrate to the estuary from the main freshwater source, Odder River, was high during winter and spring, but low during summer (Fig. 3, top). The nitrate level in the river water fluctuated between 310 and 1130  $\mu\text{mol l}^{-1}$  with no clear seasonal trend. The freshwater discharge reached a maximum of 15 to 30  $\times 10^4 \text{ m}^3 \text{ d}^{-1}$  in winter and spring and was only 2 to 8  $\times 10^4 \text{ m}^3 \text{ d}^{-1}$  during summer (unpubl. data from Det Danske Hedeselskab and Aarhus Amts Vandvaesen). There was a significant difference in nitrate influx between the 2 yr. The

spring of 1983 had an unusually high rainfall, especially during May, which resulted in an extensive leaching of nutrients from the surrounding agricultural areas. High nitrate influx as well as low biological activity during winter resulted in high nitrate levels in the estuary and a relatively deep penetration of nitrate into the sediment (Fig. 3).

At Sta. 1, the gradients showed that nitrate in winter was taken up by sediments from the overlying water. As daylight intensity and water temperature increased during spring, nitrate uptake of both phytoplankton and sediments was enhanced and the water concentration fell below 10  $\mu\text{mol l}^{-1}$ . During summer, the nitrate zone was only about 1 cm thick and a slight maximum of nitrate indicated that nitrate was produced by nitrification in the sediment and was now released to the water. The sudden pulse of nitrate influx to the estuary in May 1983 had a dramatic effect in the sediment where a nitrate penetration to 5 cm would otherwise not occur late in spring.

At Sta. 4 near the river outlet, nitrate in the water

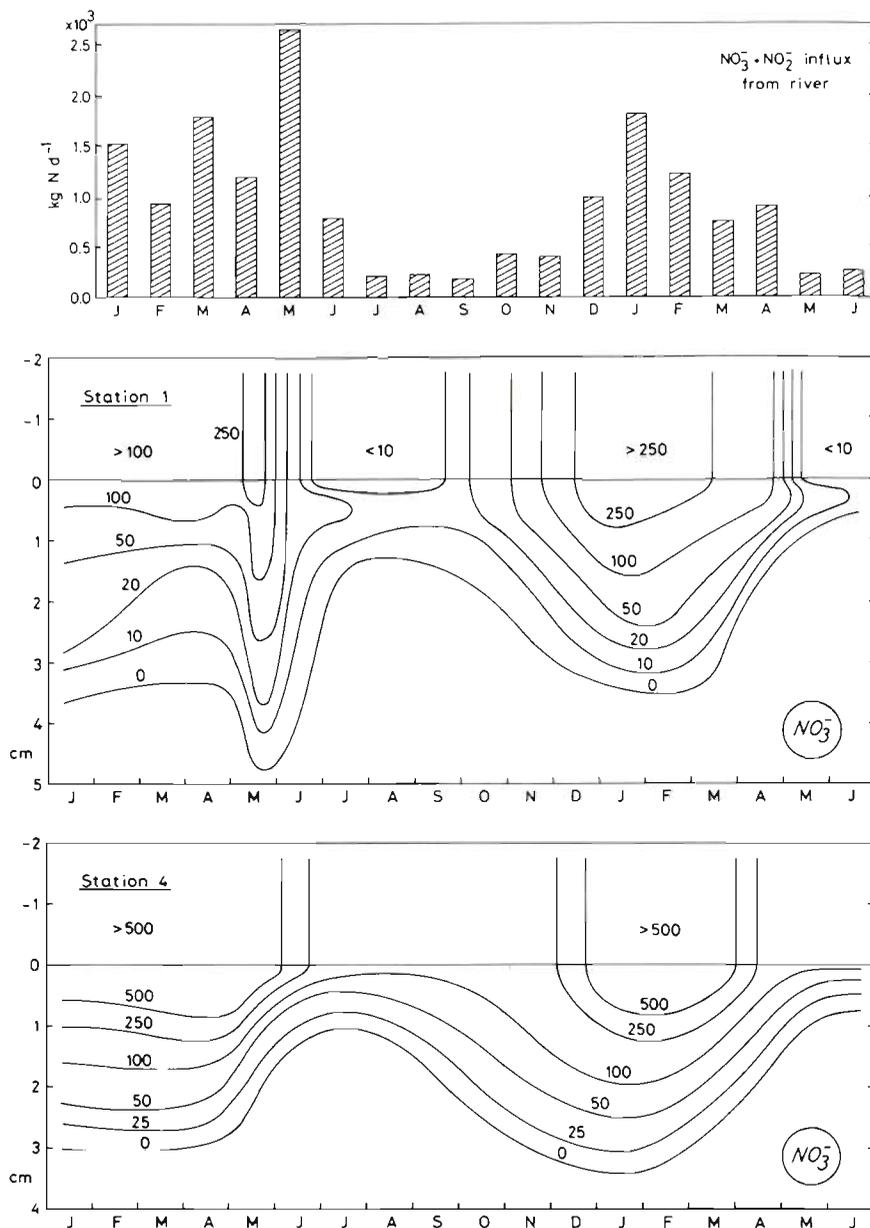


Fig. 3. Top: monthly influx of nitrate and nitrite from Odder River to Norsminde Fjord. Middle and bottom: isopleths of nitrate distribution in the sediments at Sta. 1 and 4. Numbers on the isopleths indicate μmol nitrate l<sup>-1</sup> water or porewater

varied from 600 to 900 μmol l<sup>-1</sup> in winter and 100 to 250 in summer (Fig. 3). Thus, nitrate was present throughout the year at relatively high concentration and the level in the uppermost cm of the sediment was mostly >50 μmol l<sup>-1</sup>, even during summer. The nitrate gradient indicated uptake from water to sediment at all seasons.

Rates of denitrification and of total nitrate reduction at the 2 stations, 1 and 4, are shown in Fig. 4 together with rates of oxygen uptake and sulfate reduction. The seasonal pattern of activities shows that while oxygen and sulfate consumption followed temperature, nitrate consumption was relatively constant throughout the year with sudden peaks during spring. It is especially the result of the nitrate pulse in May 1983 which is

notable. There was a large difference in the rates of total nitrate reduction at the 2 stations. Reduction rates were 10-fold higher near the river outlet than near the sea entrance due to the high nitrate levels. At Sta. 1 the lowest reduction rates were found during summer when nitrate availability was highly limiting. Denitrification was <50% of the total nitrate reduction at both stations.

### N<sub>2</sub>O emission

Measurements of N<sub>2</sub>O concentrations in the estuary showed that the water was always supersaturated with N<sub>2</sub>O relative to the atmosphere. N<sub>2</sub>O concentrations in the western part of the estuary near the river outlet

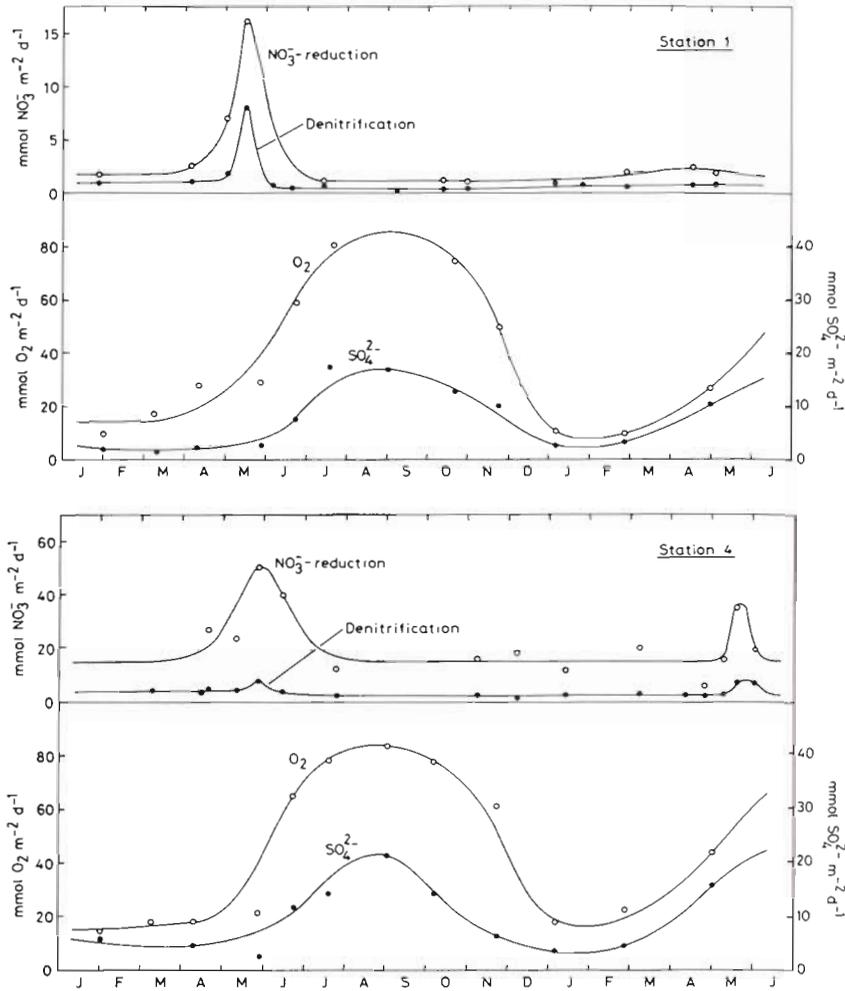


Fig. 4. Sta. 1 und 4: seasonal variation in areal rates of total nitrate reduction, denitrification, oxygen uptake and sulfate reduction in the sediment

varied from 0.03 to 0.49  $\mu\text{mol l}^{-1}$  (mean 0.142) over the year with highest values during spring. In the eastern part,  $\text{N}_2\text{O}$  concentrations varied from 0.01 to 0.05  $\mu\text{mol l}^{-1}$  (mean 0.031). Calculated emission rates across the

water-air interface showed the strong effect of the nitrate pulse in spring 1983 and the maxima in nitrate reduction (Fig. 5). Emission rates from sediments to water were similar to or less than the water-air flux.

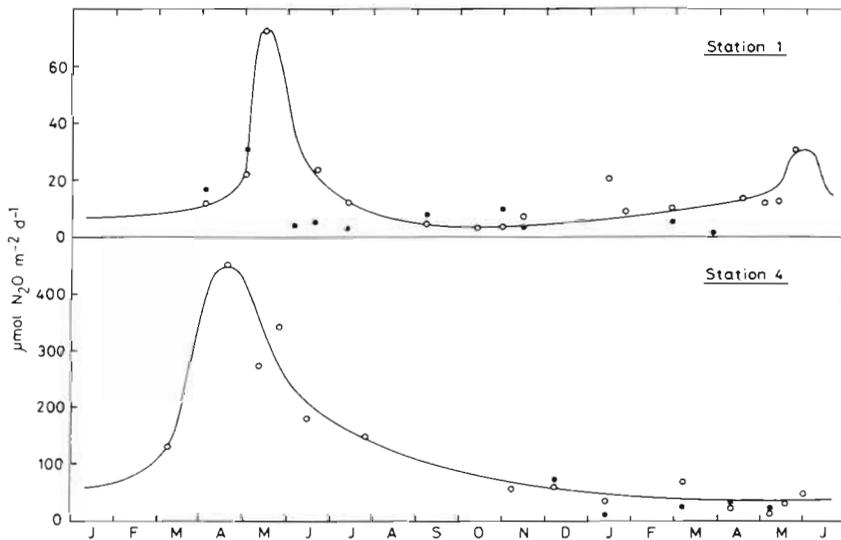


Fig. 5. Flux of  $\text{N}_2\text{O}$  across the water-air interface (open circles) and across the sediment-water interface (filled circles) at Sta. 1 and 4

The lower sediment flux may indicate that a part of the N<sub>2</sub>O comes from the river water or it may reflect differences and uncertainties in the 2 approaches used. N<sub>2</sub>O emission rates from the western part of the estuary at Sta. 4 were 10-fold higher than from the eastern, more marine part at Sta. 1.

## DISCUSSION

Zonation of the 3 processes studied – oxygen, nitrate and sulfate reduction – was determined by the dynamic balance between their consumption rate in the sediment and the diffusional flux from the overlying water. Depths of the 3 zones during summer were: O<sub>2</sub> = 0.5 to 1.5 mm, NO<sub>3</sub><sup>-</sup> = 0.5 to 1 cm, and SO<sub>4</sub><sup>2-</sup> = 4 to >100 cm (O<sub>2</sub> data from Howarth & Jørgensen 1984, Jørgensen & Okholm-Hansen in press). The depth of the sulfate zone depended mostly on salinity. We believe that the salinity gradient and thus the trend in mineralization processes between the stations in Norsminde Fjord are characteristic also of other estuaries where the main source of nitrate is from rivers and of sulfate is from the ocean.

The variations of the 3 processes were highly different in their seasonal pattern (Fig. 2 & 4). Oxygen and sulfate followed the temperature: nitrate did not. This shows the importance of detailed, seasonal studies, especially for nitrate reduction, before calculations are made of annual budgets.

Our seasonal studies of oxygen, nitrate, and sulfate reduction at low and high salinity in Norsminde Fjord can further be used to compare the contribution of the 3 electron acceptors to mineralization of organic matter in the sediments. For this purpose, rate measurements have been recalculated as weighted, yearly averages for each station and presented as daily rates per m<sup>2</sup>.

### O<sub>2</sub> uptake and SO<sub>4</sub><sup>2-</sup> reduction

Data for oxygen uptake and sulfate reduction are summarized in Table 2. Oxygen uptake rates showed little variation between the 5 localities investigated in spite of the very different sediment types. The more exposed localities of Sta. 1 and 3 had sandy sediments with an organic content 5 to 10-fold lower than the more sheltered, silty sediments. Calculated turnover of the total organic matter in sand was thus faster than in the more fine-grained sediments. A similar trend was found in another Danish fjord, Limfjorden (Jørgensen 1977).

Results for sulfate reduction were quite comparable to data from other coastal marine environments. Thus,

a sulfate reduction equivalent to about 50 % of the oxygen uptake was also found in other organic-rich sediments and marshes (Jørgensen 1982, Howes et al. 1984, Howarth 1984). Because of extensive reoxidation of produced sulfide by oxygen in such sediments, close to half of the measured oxygen uptake may be diverted to this process (Jørgensen 1977). A recent study in Norsminde Fjord near Sta. 2 has shown that the present sulfate reduction rates may even be underestimated by

Table 2. Rates of sediment oxygen uptake and sulfate reduction in mmol m<sup>-2</sup> d<sup>-1</sup> of 5 investigated sites. Mean values over 1 year

Process	Station					Mean
	1	2	3	4	5	
Oxygen uptake	36.6	39.5	40.9	43.0	40.6	40.1
Sulfate reduction	7.5	23.9	19.8	9.1	10.6	14.2

20 to 30 % because the tracer measurements did not include the label incorporated into pyrite and elemental sulfur (Howarth & Jørgensen 1984).

Sulfate reduction varied 3-fold between stations, with lowest values in the sand of Sta. 1 and in the sediments near the river mouth. The lower sulfate reduction at Sta. 1 was possibly due to the less reducing conditions in this sediment where dense populations of polychaete worms and crustaceans ventilated their burrows. At Sta. 4 and 5, the low salinity and sulfate concentration in the water limited the depth of the sulfate reduction zone to 4 to 10 cm. Especially during summer a significant part of the anaerobic metabolism would therefore be expected to take place below the sulfate zone. The terminal process in this metabolism is methanogenesis which was not analyzed. In the eastern, marine, part of the estuary methane formation was not important in the upper 10 to 15 cm of the sediment due to the high sulfate levels (Iversen & Blackburn 1981).

Such gradual transition from sulfate reduction to methanogenesis with depth in marine sediments has been observed also in other coastal environments (e.g. Crill & Martens 1983, Iversen & Jørgensen in press). Although there are indications that sulfate may become limiting for the reduction rate already at mmolar concentrations (Westrich 1983), our recent results indicate a much lower half-saturation level of ≤100 μM (Ingvorsen & Jørgensen 1982, Jørgensen & Ingvorsen in prep.). We therefore conclude that areal reduction rates were limited by total flux of sulfate into sediment which in combination with the low sulfate concentration in the water determined the depth of the sulfate reduction zone.

### NO<sub>3</sub><sup>-</sup> reduction

In Table 3 we have compared the data for nitrate reduction with those for oxygen and sulfate reduction at Sta. 1 (high salinity) and at Sta. 4 (low salinity). The mean process rates over 1 yr have been recalculated to the equivalent amount of organic carbon (mmol CH<sub>2</sub>O) which would be oxidized by the process. In this way their contributions to the electron flow in the sediments become directly comparable (cf. Jørgensen 1980). The conversion factors are given in Table 3 together with the percentage contribution of each process to the total electron acceptor reduction. It should be noted that diurnal variations in the distribution and reduction of oxygen and nitrate may be significant in estuarine sediments (Andersen et al. 1984). As our measurements were made only in the dark, this may have biased the comparison of the electron acceptors somewhat.

There was a significant difference between the sediments at high and at low salinity. At the higher salinity, oxygen uptake accounted for 2/3 of the total reduction rate while sulfate was 1/4. Nitrate reduction was only 8% of which 1/3 was due to denitrification and 2/3 to a dissimilatory reduction to NH<sub>4</sub><sup>+</sup>. The most important difference to the sediment at lower salinity was a 10-fold higher nitrate reduction, mostly due to a high rate of reduction to NH<sub>4</sub><sup>+</sup>.

The very high rates of nitrate reduction to NH<sub>4</sub><sup>+</sup> at Sta. 4 make nitrate comparable to both sulfate and oxygen as an electron acceptor. The dissimilatory reduction of nitrate to ammonia is, however, not always a respiratory process, since the nitrate may also function as an electron sink in bacterial fermentations. For example in clostridia, this process may be a mechanism for the removal of excess reducing power (Hasan & Hall 1975). In other cases, however, the pathway seems to be a true respiration. This has now been demonstrated in several strains of *Desulfovibrio*, which may start to reduce nitrate when sulfate is depleted (Steenkamp & Peck 1981). An assimilatory

Table 3. Process rates in meq m<sup>-2</sup> d<sup>-1</sup> (see text) in sediments of Norsminde Fjord. Mean values over 1 yr for a seawater dominated (Sta. 1) and a freshwater dominated (Sta. 4) site. The relative contribution of each electron acceptor is shown in parentheses. The applied conversion factors for mmol to mequivalent recalculation are also shown

Process	meq/mmol	Sta. 1	Sta. 4
O <sub>2</sub> uptake	1/1	36.6 (65 %)	43.0 (44 %)
NO <sub>3</sub> <sup>-</sup> reduction	-	4.7 (8 %)	35.5 (37 %)
NO <sub>3</sub> <sup>-</sup> N <sub>2</sub>	5/4	1.5 (3 %)	4.3 (4 %)
NO <sub>3</sub> <sup>-</sup> NH <sub>4</sub> <sup>+</sup>	2/1	3.2 (5 %)	31.2 (33 %)
SO <sub>4</sub> <sup>2-</sup> reduction	2/1	15.0 (27 %)	18.2 (19 %)
Total	-	56.3 (100 %)	96.7 (100 %)

reduction of nitrate is not expected to be significant, as the process in bacteria is generally inhibited by ammonium. There is a high concentration and net release of ammonium from coastal sediments due to the relatively high N/C ratio of the organic matter near the sediment surface (Blackburn & Henriksen 1983). There is still not enough data available to evaluate whether the high stimulation of nitrate reduction to ammonium, which we have observed, is a general phenomenon in the transition from seawater to freshwater in estuaries.

For both stations, measured rates of denitrification were found to be mostly a function of nitrate availability when calculated per unit area. The source of nitrate to the sediment seemed to be mostly from the overlying water during winter and early spring according to the gradients. During summer, the slight nitrate maximum at 0 to 1 cm depth at Sta. 1 indicated that nitrification would now be the main source and that nitrate was released to the water rather than taken up.

The rather insignificant role of denitrification in mineralization of organic matter was also observed previously in this fjord (Sørensen et al. 1979) as well as in Limfjorden and Kattegat between the Baltic and the North Sea (Blackburn & Henriksen 1983). The observed denitrification rate is of similar magnitude as that found in other coastal sediments from which rates of about 0.5 to 2 mmol m<sup>-2</sup> d<sup>-1</sup> have been reported (Billen 1978, Seitzinger et al. 1984, Jenkins & Kemp 1984).

Due to the low nitrate concentration in the sediments during summer, however, rates may have been underestimated in this period. Since acetylene blocks nitrification as well as denitrification (Walter et al. 1979, Blackmer et al. 1980) the small pool of nitrate may be depleted during incubation. Furthermore, there is recent evidence that the acetylene blockage of denitrification may become less efficient at low nitrate concentrations (Kaspar et al. 1981, Oremland et al. 1984).

### Denitrification and N<sub>2</sub>O emission

Formation of N<sub>2</sub> by denitrification and of N<sub>2</sub>O by denitrification or nitrification result in a loss of combined nitrogen from the estuarine ecosystem. The estuary may thereby play a role as a buffer against the discharge of nutrients from the river into the sea. The coastal waters from the Baltic to the North Sea are susceptible to eutrophication due to excessive nutrient discharge and have in recent years shown increasing symptoms of anoxia in bottom water.

Mean rates of N<sub>2</sub>O emission over 1 yr from water surface to atmosphere were 0.014 and 0.150 mmol m<sup>-2</sup> d<sup>-1</sup> at Sta. 1 and 4, respectively. These are insignificant

ant nitrogen loses compared to the measured rates of N<sub>2</sub> formation of 1.2 and 3.4 mmol m<sup>-2</sup> d<sup>-1</sup>. Mean nitrogen influx to the fjord can be calculated on the basis of data in Fig. 3 and a total fjord area of 1.9 km<sup>2</sup>. The influx is equivalent to 35 mmol m<sup>-2</sup> d<sup>-1</sup>, i.e. 10 to 30 times higher than denitrification. The maximum influx correlates seasonally with the maximum rate of denitrification. Thus, only about 5% of nitrate from the main river is lost within the estuary by denitrification in the sediment. We do not have sufficient data to evaluate the sink of the main part of the nitrate, but it is likely that most is taken up by plankton algae. The annual mean nitrate concentration in the water was reduced 5-fold from 540 to 105 µM between the western (fresh) and the eastern (marine) ends of the estuary. A part of the assimilated nitrogen is trapped in the estuary, which presently has very high sedimentation rates, and much of the organic nitrogen may also leave the estuary as plankton or suspended detritus.

Emission rates of N<sub>2</sub>O showed that the inner part of the estuary was the most important source with rates 10-fold higher than the outer part. Although N<sub>2</sub>O formation seems insignificant for the overall nitrogen budget, both in Norsminde Fjord and in other estuaries (e.g. Seitzinger et al. 1983), its natural release may be important for the tropospheric nitrogen cycle (McElroy & McConnell 1971, Hahn & Crutzen 1982). Our data are equal to or higher than other rates of N<sub>2</sub>O emission from sediments or water in estuaries (McElroy et al. 1978, Seitzinger et al. 1983, Jenkins & Kemp 1984, Jensen et al. 1984). The high rates may be due to the relatively high influx of nitrate from the river and to the shallow water which facilitates gas transfer from the sediments to the atmosphere.

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