

# Nitrogen cycling in North Sea sediments: interaction of denitrification and nitrification in offshore and coastal areas\*

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**ABSTRACT:** Benthic nitrogen cycling was studied in different areas of the southeastern North Sea in August 1991 and February 1992. Main attention was focused on the coupling between nitrification and denitrification. Additionally, extensive measurements of sediment-water exchange of inorganic nitrogen compounds and of the penetration of oxygen into the sediment were made. Denitrification rates ranged from 0 to  $8 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ , while nitrification rates ranged between 5 and  $26 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ . Although the oxic layer of the sediment was thinner in August, nitrification was higher during that period, indicating the importance of ammonium availability rather than oxygen penetration. Except in the depositional area in the German Bight, denitrification was completely dependent on nitrification. In general, however, the coupling between the 2 processes was incomplete. On average, 3 and 25% of the produced nitrate was denitrified in August and February respectively. The bulk of the produced nitrate diffused upward (along the steepest concentration gradient) to the nitrate-depleted overlying water and was unavailable for denitrification, particularly in August. It is postulated that the coupling efficiency is indirectly influenced by the nitrate concentration in the overlying water.

## INTRODUCTION

It is generally accepted that coastal sediments can be an important site for cycling of organic matter (Blackburn 1988, Seitzinger 1988). Bacteria mediate a wide variety of diagenetic processes. The resulting gradients of solutes cause a part of the mineralized compounds to be released to the water column or to become adsorbed and buried in deeper sediment layers. Particularly benthic nitrogen processes have received substantial attention, because the release of nitrogen compounds from the sediments can contribute to the requirements of pelagic primary production (Nixon 1981, Klump & Martens 1981). Although

fundamental knowledge concerning the responsible processes is available uncertainty still exists on the quantitative interactions.

The driving force for inorganic benthic nitrogen cycling is the degradation of organic matter which is deposited at the sediment surface. Ammonium, mineralized from organic matter, can be released from the sediment, can be adsorbed onto sediment particles or can take part in several microbial processes (Rosenfeld 1981, Blackburn & Henriksen 1983). Nitrification occurs in the upper oxygenated sediment layer, where nitrifying bacteria oxidize ammonium to nitrate. As with ammonium, nitrate can diffuse to the overlying water or to deeper anoxic sediment layers where it can be reduced by denitrifying microorganisms (Koike & Sørensen 1988, Seitzinger 1988). The products of denitrification, nitrogen gas and nitrous oxide, can be released to the water column and subsequently to the

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atmosphere. A tight coupling of nitrification and denitrification can therefore eliminate substantial amounts of nitrogen from the marine ecosystem. Apart from nitrification in sediments, the overlying water can also be an important source of nitrate for denitrification, particularly in estuaries and coastal waters which receive high loads of nutrients via rivers and land runoff (Nishio et al. 1983).

An efficient coupling of denitrification and nitrification has been demonstrated in several studies. Jenkins & Kemp (1984) found that more than 99% of the produced nitrate was subsequently denitrified (Patuxent River, Chesapeake Bay, USA). Seitzinger (1987) reported that in Ochlockonee Bay (Florida, USA) more than 80% of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  was denitrified. Weaker couplings between both processes were reported by Japanese workers (Nishio et al. 1983). In their investigation area (Odawa Bay) only 30% of nitrate produced by nitrification was denitrified. Enoksson (1987) suggested that an increased flux of organic matter towards the sediment can suppress nitrification due to the reduction of the thickness of the oxic layer. If nitrification is the prevailing source for nitrate then denitrification can also be reduced. The same sugges-

tion was made by Kemp et al. (1990). These authors concluded that eutrophication can be a self-accelerating process, once nitrification is inhibited by low oxygen concentrations.

Most of these studies were carried out in sheltered and shallow bays, fjords and estuaries. Many of these areas receive considerable inputs of nutrients and organic loads. Only a few of the investigations have been executed on continental shelf sediments. In this study results of 2 North Sea cruises performed in August 1991 and February 1992 are presented. Our primary concern was to evaluate the factors which influence denitrification and nitrification rates in different types of sediments in the North Sea.

## MATERIAL AND METHODS

**Study sites and sampling.** The study was part of the BELS project (Benthic Links and Sinks in North Sea Nutrient Cycling). Seven stations in the southeastern North Sea were selected for this investigation (Fig. 1). The stations are located within the main route of residual currents and organic matter transport. Both depositional and erosional areas were included.

An extensive hydrographical description of the area is given by Otto et al. (1990). North Atlantic seawater enters the North Sea by the opening between the northern UK and Norway and through the Straits of Dover. The counterclockwise circulation of water masses results in a residual transport of water and suspended matter from the British and Dutch coasts in the south towards the Norwegian Channel in the north. The southern part of the North Sea is generally well mixed throughout the year. Thermal stratification occurs in the deeper, central and northern parts during summer. The depths of the selected stations were between 19 and 50 m (Table 1). Three stations (Stns 12, 13 & 14) are located in the German Bight. These stations are influenced by the discharges of the Elbe and Weser rivers. Restricted water exchange and deposition of mud that contains organic matter in parts of this area (ca  $1 \text{ cm yr}^{-1}$ ; Eisma 1987) sometimes cause oxygen depletion of bottom waters (Hickel et al. 1989).

Some general features of the stations are given in Table 1. Temperature differences of the bottom waters were related to the depth of the stations. The shallowest stations (Stns 12, 13, 14 & 16) show pronounced differences up to  $14^\circ\text{C}$  between August and February, whereas seasonal differences varied from  $3$  to  $6^\circ\text{C}$  at deeper stations (Stns 5, 6 & 7). Salinity ranged from 33 to 35‰ in August. Elevated river runoff caused lowered salinity (31.3 to 33.5‰) at Stns 12, 13 & 14 in February. Oxygen concentrations were mostly near saturation values in both periods. Relatively low con-

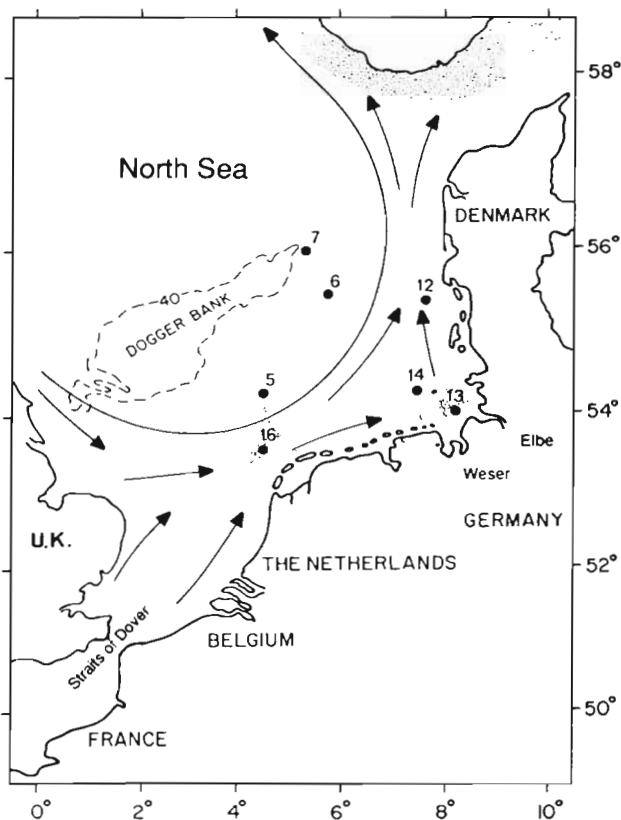


Fig. 1 The North Sea showing visited stations. Main transport routes of water and suspended matter are indicated. Shaded areas indicate main deposition regions

centrations were only found at Stns 5, 13 & 14 (74 to 82% of saturation) in August. Some basic sediment characteristics are given in Table 1. According to the Udden-Wentworth size scale (Pettijohn & Potter 1972) very fine sand prevailed at Stns 5, 6, 14 & 16. Coarser sand was found at Stns 7 & 12. Silty sediments were found in the deposition area of the Helgoland Bight (Stn 13). This area is also characterized by higher organic carbon and nitrogen contents of the sediment. While on average contents range around 0.24% for organic carbon and 0.03% for organic nitrogen, the contents at Stn 13 were significantly higher (1.3% org. C and 0.15% org. N).

Sediment cores were collected on board RV 'Pelagia' with a cylindrical box corer (i.d. 31 cm) developed at the Netherlands Institute for Sea Research (NIOZ). This box corer encloses a 30 to 50 cm sediment column together with 15 to 25 l of overlying bottom water. Any disturbance of the sediment-water interface and contamination by water from the upper water column was prevented by a closing lid on the upper end of the box corer. Subsamples were taken from the box core with acrylic liners and were closed with rubber stoppers. Only cores without any visible surface disturbance were used for further measurements.

**Oxygen profiles.** Two subcores (5.4 cm i.d., 20 cm length) were used to determine oxygen profiles. All measurements were carried out in a thermostated water bath immediately after box core retrieval. The overlying water was not stirred during the measurements. Profiles were measured with Clark type micro-electrodes (Diamond Corp., type 737) provided with an internal reference electrode. Average tip diameter was 100  $\mu\text{m}$ . Typical output at 100%  $\text{O}_2$  saturation at ambient conditions (15°C, 33‰ S) was 400 to 500 pA. The stirring effect, defined as the difference of the signal in stagnant and vigorously stirred water was less than 2%. The zero signal in anoxic sediment ranged from 8 to 15 pA. The electrodes were calibrated after determining the oxygen concentration of bottom water by an automated Winkler titration in triplicate. A linear relationship between oxygen concentration and output (pA) was assumed (Revsbech & Jørgensen 1986). The electrode was inserted into the sediment in steps of 0.25 to 0.5 mm using a micromanipulator. At least 2 profiles were made in each core.

**Profiles of nitrogen and carbon.** Vertical profiles of ammonium, nitrite and nitrate were obtained by slicing 10 to 15 subcores (3.1 cm i.d., 25 cm length) under oxygen-free conditions. The cores were sectioned after adjusting the sediment surface to the upper end of the plexiglas tube. Subsequently, the piston located at the lower end of the sediment column was pushed upwards in the plexiglas tube by putting plastic disks of varying thickness (according

Table 1. Numbers, location, depth and some basic characteristics of the sampling stations

Station	No.	Geographic position		Water depth (m)	Bottom temperature (°C)		Porosity (v/v)	Org. C (%)	Org. N (%)	C/N ratio	Median grain size ( $\mu\text{m}$ )	Sediment classification (see text)
		N	E		August	February						
Oystergrounds	5	54° 25'	4° 04'	49	10.2	6.4	0.38	0.12	0.02	6.0	100	Very fine sand
Weiss Bank	6	55° 17'	6° 00'	49	12.2	5.8	0.45	0.16	0.02	8.0	95	Very fine sand
Tail End	7	56° 00'	4° 38'	50	9.5	6.1	0.41	0.16	0.03	5.3	155	Fine sand
Esbjerg	12	55° 12'	7° 38'	25	17.7	4.8	0.39	0.06	0.01	6.0	175	Fine sand
Helgoland Bight	13	54° 05'	8° 09'	19	18.7	4.4	0.66	1.28	0.15	8.5	25	Medium silt
Elbe Rinne	14	54° 14'	7° 20'	39	16.5	5.4	0.48	0.46	0.05	9.2	90	Very fine sand
Fristan Front	16	53° 42'	4° 32'	39	17.4	6.3	0.53	0.46	0.05	9.2	70	Very fine sand

to the required slicing interval) under the piston. In this way the sediment was extruded out of the plexi-glas tube in intervals of 0–4, 4–10, 10–15, 15–20, 20–30, 30–40, 40–60, 60–80 and 80–100 mm. Slices of each depth were pooled in centrifuge tubes (Saager et al. 1990) provided with a built-in filter (cellulose-acetate, 0.45  $\mu\text{m}$ , Sartorius) and centrifuged for 10 min at  $1700 \times g$ . All procedures took place at *in situ* temperature. Nitrate and nitrite were measured on a Technicon TRAACS-800 autoanalyzer according to Strickland & Parsons (1972). Ammonium was measured following the modified phenol-hypochlorite method after Helder & de Vries (1979).

Eight additional cores (5 cm i.d., 25 cm length) were sliced into 0.5 (0 to 1 cm) and 1 (1 to 10 cm) cm slices, which were pooled and frozen ( $-20^{\circ}\text{C}$ ). Subsamples were analyzed for contents of organic carbon and nitrogen on a Carlo Erba NA 1500-2 elemental analyzer according to Verardo et al. (1990). Porosity was determined in additional subsamples by drying for 48 h ( $60^{\circ}\text{C}$ ). The porosity was calculated from the weight before and after drying assuming a specific sediment weight of  $2.65 \text{ kg dm}^{-3}$ .

**Inorganic nitrogen fluxes across the sediment-water interface.** Nutrient fluxes of ammonium, nitrite and nitrate were determined using 3 whole core techniques, differing from each other with respect to core diameter, incubation time and sampling intervals (Table 2). Subsamples of overlying water were filtered (cellulose-acetate, 0.45  $\mu\text{m}$ ) and analyzed immediately. The fluxes were calculated from the change in concentration in the overlying water during the incubation (linear regression) and multiplying the obtained rate with the specific volume/area ratio of the core. All data were treated statistically by means of the nonparametric Wilcoxon test.

**Denitrification.** Rates of denitrification were measured by a modified acetylene block technique (Sørensen 1978). Acetylene blocks the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (see Fig. 2, Step 5). The water phase of 12 replicate sediment cores (5.4 cm i.d., 20 cm length) was adjusted

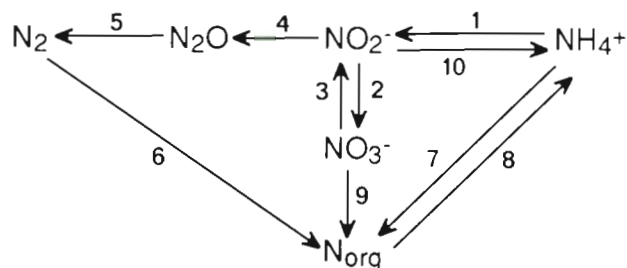


Fig. 2. The benthic nitrogen cycle in marine sediments. Numbers indicate steps of different pathways. Step 1: first step of nitrification inhibited by nitrapyrine (Methods 1 & 4), acetylene (Method 2) and absence of oxygen (Method 3); Step 2: second step of nitrification; Steps 3 & 4: first and second step of denitrification; Step 5: third step of denitrification, inhibited by acetylene; Step 6: nitrogen fixation; Step 7: ammonium assimilation; Step 8: ammonification; Step 9: assimilative nitrate reduction; Step 3 in combination with Step 10: dissimilative nitrate reduction

to a volume of 100 ml. The headspace, serving as oxygen reservoir, was adjusted to 15 ml. The cores were sealed with gastight polyacrylate lids, provided with a sample port and a Teflon-coated magnetic stirrer. The stirrer was placed underneath the lids and mixed the water phase to a rate just below sediment resuspension. To obtain a final concentration of 10% (v/v) acetylene, 10 ml of the water column was replaced by acetylene-saturated water from the sampling location. Then 200  $\mu\text{l}$  of acetylene-saturated North Atlantic surface water ( $\text{NH}_4^+ < 0.2 \mu\text{M}$ ,  $\text{NO}_3^- < 0.1 \mu\text{M}$ ,  $\text{NO}_2^- < 0.1 \mu\text{M}$ , diluted to a salinity of 33‰) was injected from 2 different directions into the sediment through silicon-filled side ports at 0.5 cm intervals. Eight cores were treated in this way, the remaining 4 cores served as controls. All cores were incubated in the dark in a thermostated water bath at *in situ* temperature.

Duplicate cores were analysed successively after 1, 2, 3 and 4 h. The control cores were analysed after 1 and 4 h. Both sets were treated by the following extraction procedure: 1 ml headspace gas of the cores was withdrawn with a gas-tight syringe (Hamilton) and stored in a pre-evacuated Venoject tube (Terumo, Belgium) for later analysis. Overlying water (10 ml) was put into a 30 ml glass vial with 20 ml gas phase and shaken for at least 10 min to achieve equilibrium between the fluid and the gas phase. A 1 ml sample of this gas phase was taken with a gas-tight syringe and stored in a pre-evacuated Venoject tube.

The remaining overlying water and the upper 3 to 5 cm of the sediment column

Table 2. Differences in equipment used to estimate ammonium and nitrate fluxes

	Bell jars	Method Flux cores	Denitrification cores
No. of cores station <sup>-1</sup>	2–4	5	8–12
Inner diameter (cm)	31	10	5.4
Incubation time (h)	4–6	6	1–4
Sampling interval (h)	0.5	0.5	0.5
Stirring device	Magnetic stirrer	Bubbling air	Magnetic stirrer



were put into a glass extraction vial (300 ml) provided with 10 ml gas phase and a sample port. Nitrous oxide reduction was stopped by adding 1% (v/v) formaline (Rudolph et al. 1991). The vials were shaken for 30 min. Previous investigations showed that this time is necessary to achieve complete equilibrium of nitrous oxide between the sediment slurry and the gas phase. Again, 1 ml of this gas phase was stored in a pre-evacuated Venoject tube.

Nitrous oxide ( $\text{N}_2\text{O}$ ) was analyzed on a Packard 438a gas chromatograph equipped with an electron capture detector (ECD). The detection, inlet and oven temperature were 310, 110 and 50°C respectively. The  $\text{N}_2\text{O}$  peak was separated on a Hayesep S column (2 mm  $\times$  2 m, 80 to 100 mesh). Acetylene contamination of the ECD detector was prevented by using a split system which was activated after  $\text{N}_2\text{O}$  had passed the detector. Interfering water vapour and  $\text{CO}_2$  were absorbed in a 5 cm-long pre-column containing 0.5 cm<sup>3</sup> Carbo-sorp (Hammond) and 0.5 cm<sup>3</sup> Drierite (BDH) which was mounted before the main column. The concentration of  $\text{N}_2\text{O}$  in water was calculated using solubility coefficients of  $\text{N}_2\text{O}$  in seawater (Weiss & Price 1980). The denitrification rate was calculated from the increase of nitrous oxide in sediment pore-water, overlying water and head-space of the cores.

**Nitrification.** Nitrification was estimated from 4 approaches. The methods were compared applying the nonparametric Wilcoxon test.

**Nitrapyrine inhibition (Method 1):** Direct measurements using the nitrapyrine (N-serve) technique (Henriksen 1980) were performed with 6 to 8 cores (i.d. 3.1 cm, 25 cm length) from 1 box core. The volume of the overlying water was adjusted to 70 ml. Half of the cores were treated with nitrapyrine, the specific inhibitor of ammonium oxidation (Fig. 2, Step 1), while the remaining cores served as blanks. The required amount of nitrapyrine solution to obtain a final concentration of 20 ppm in the sediment was estimated from the thickness of the oxic sediment surface layer. Amounts of 30 to 60  $\mu\text{l}$  nitrapyrine solution (3.3 mg ml<sup>-1</sup>) were injected with a 10  $\mu\text{l}$  syringe through silicon-filled side-ports in different directions. The water column received 0.1 ml of the nitrapyrine solution to achieve a final concentration of 5 ppm. The overlying water was aerated and stirred by injecting air through a thin needle. Samples from the water column (2.5 ml) were taken at 7 to 10 h intervals and replaced by overlying water from the sampling location.

Average incubation times varied from 30 to 35 h in the dark at *in situ* temperature. After incubation, pore water was extruded from both series of cores (see pore-water profiles). The nitrification rate was calculated by subtracting the total amount of ammonium in the control cores from the amount in the inhibited cores.

**Inhibition of nitrate production by acetylene (Method 2):** A second estimate of nitrification was obtained by subtracting the flux of nitrate in acetylene amended (denitrification) cores from the mean nitrate flux in uninhibited cores. This approach is based on the fact that ammonium oxidation is inhibited by acetylene (Walter et al. 1979; Fig. 2, Step 1). It is assumed that the application has no influence on nitrate reduction (Binnerup et al. 1992).

**Inhibition of nitrification by depletion of oxygen (Method 3):** The third approach is based on the fact that ammonium oxidation by nitrifiers (Fig. 2, Step 1) occurs only under oxic conditions. Bell jars which were used to determine the nutrient fluxes were made anoxic after 2 h by flushing the water column with nitrogen gas. Removal of oxygen was checked using Clark electrodes mounted inside the bell jar. Without oxygen, nitrate production is stopped effectively, so that the difference in nitrate flux before and after nitrogen flushing is an estimate for nitrification (e.g. in Fig. 3). It is assumed that the depletion of oxygen does not seriously affect denitrification and other nitrate reducing processes. Estimates based on differences in ammonium fluxes failed due to the high variability of these fluxes which may be caused by the impact of anoxia on microbial ammonium production.

**Disappearance of nitrate after nitrapyrine addition (Method 4):** The fourth approach is based on the disappearance of nitrate in pore water and overlying water in nitrapyrine-amended cores after ammonium oxidation is effectively stopped (Fig. 2, Step 1). The nitrification rate was calculated from the difference in nitrate content at the start and at the end of the experiment.

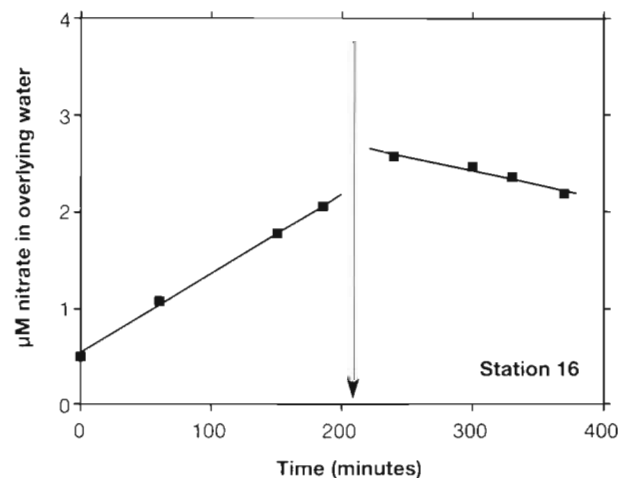


Fig. 3. Impact of flushing overlying water with  $\text{N}_2$  on nitrate concentration at the Frisian Front. Arrow indicates time of flushing

**Potential nitrification rates.** Potential rates of nitrification in the upper 0.5 cm were measured at Stns 5, 7, 13 & 16 by incubating 15 cm<sup>3</sup> of homogenized sediment with 15 ml of North Atlantic surface water. Ammonium was added to obtain a final concentration of 400 µM. Incubation took place at *in situ* temperature in plastic tubes in a gently rotating shaker. Oxic conditions in the slurry were checked with oxygen micro-electrodes at the beginning and at the end of the incubations. Rates were calculated by the accumulation of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> after 24 h in the tubes. All values were corrected for temperature by assuming a Q<sub>10</sub> value of 3 (Helder & de Vries 1983).

## RESULTS

### Nitrate and ammonium concentrations in the water column

In August nitrate concentrations in bottom waters did not exceed 1 µM at all stations except at the Helgoland Bight (Stn 13; 2.8 µM). In February concentrations were 4 µM at Stns 5, 6 & 7, and even higher values were found at Stns 12, 13 & 14 (10 to 40 µM). Contrary to nitrate, ammonium concentrations at the deeper Stns 5, 6 & 7 were highest in August (1.5 to 4.8 µM), whereas only 1.1 to 2.2 µM was measured in February. A similar pattern was found at the coastal Stns 12 & 14 which showed elevated ammonium concentrations in August (3.5 µM) compared to February (2.1 µM). However, ammonium concentrations at the coastal Stn 13 were higher in February (5.7 µM) than in August (2.9 µM).

### Oxygen penetration

Oxygen penetration depths differed considerably in both periods (Table 3). In August, oxygen penetration was not deeper than 4.5 mm into the sediment. There was a clear spatial trend from Stns 5, 6, 7 & 16 in the central North Sea and the Dutch coast to Stns 12, 13 & 14 in the German Bight. While the former stations had oxygen penetration depths of 3 to 4.5 mm the latter stations were apparently anoxic in this period. The oxygen penetration increased by a factor of 2 to 3 from August to February and even stations that were anoxic in August had an oxygenated layer of ca 5 mm in February.

### Pore-water profiles of inorganic nitrogen

Representative pore-water profiles of inorganic nitrogen compounds are shown in Fig. 4. Ammonium was always the prevailing component in the interstitial water. There was a trend from lower concentrations at offshore stations to higher values along the German and Danish coast. In August values ranged from 10 to 60 µM at Stns 5, 6 & 7, while concentrations up to 170 µM were detected at Stns 12 & 14. Extremely high values were found at Stn 13. Here the ammonium concentration was 2800 µM at 9 cm depth and seemed to increase further with depth. Lower concentrations throughout the whole profile were found in February. Gradients were less steep and the strongest increase of ammonium occurred at a depth of 1 to 2 cm.

In August nitrate concentrations were less than 10 µM. Most profiles showed small peaks in the upper interval (0 to 4 mm). Below that interval nitrate de-

Table 3. Oxygen penetration (mm), benthic ammonium and nitrate fluxes, nitrification and denitrification rates (all in µmol N m<sup>-2</sup> h<sup>-1</sup>) in August and February (mean ± SD, positive values indicate release from the sediment, negative values indicate uptake). nm: not measured

Station	Month	O <sub>2</sub> penetration (mm)	NH <sub>4</sub> <sup>+</sup> flux	NO <sub>3</sub> <sup>-</sup> flux (µmol N m <sup>-2</sup> h <sup>-1</sup> )	Nitrification	Denitrification
5	Aug	4.3	14 ± 10	13 ± 9	12 ± 6	0.5
	Feb	nm	-2 ± 2	9 ± 3	8 ± 4	0.8
6	Aug	3.0	14 ± 9	11 ± 4	9	0.9
	Feb	5.8	3 ± 2	2 ± 3	5 ± 5	0.7
7	Aug	3.1	3 ± 4	9 ± 4	18 ± 7	0.1
	Feb	7.5	2 ± 5	3 ± 2	11 ± 5	0.0
12	Aug	<0.2	22 ± 24	12 ± 6	17 ± 9	0.4
	Feb	26.0	3 ± 13	5 ± 3	7 ± 7	3.8
13	Aug	<0.2	497 ± 151	-3 ± 2	0	1.9
	Feb	5.18	13 ± 20	-2 ± 4	9 ± 51	8.2
14	Aug	<0.2	84 ± 94	12 ± 4	11 ± 3	0.2
	Feb	5.1	5 ± 9	11 ± 3	12 ± 4	1.3
16	Aug	4.5	29 ± 13	23 ± 6	26 ± 12	0.7
	Feb	18.5	4 ± 8	5 ± 6	8 ± 3	1

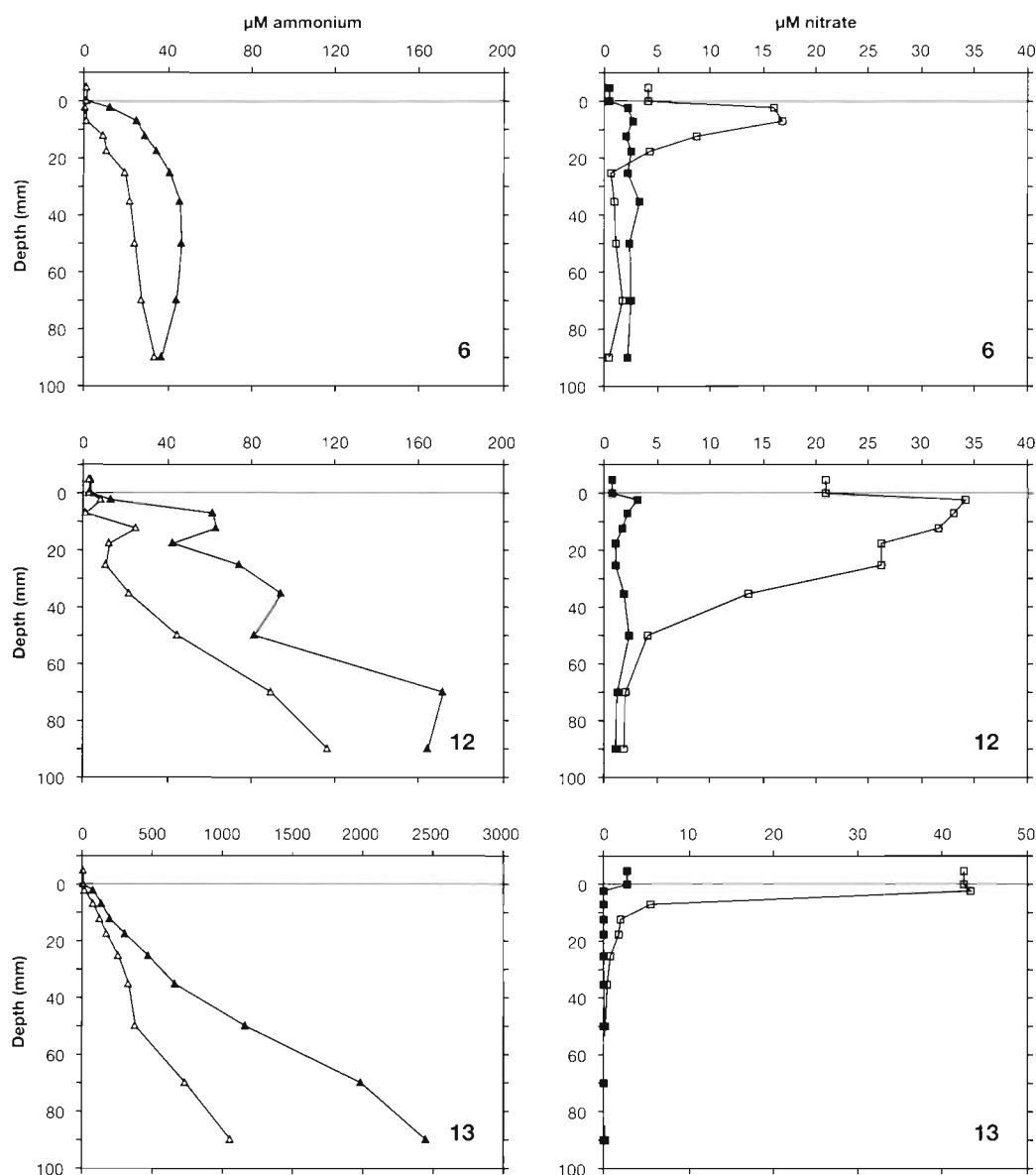


Fig. 4. Pore-water profiles of ammonium ( $\blacktriangle$ ,  $\triangle$ ) and nitrate ( $\blacksquare$ ,  $\square$ ) in August (closed symbols) and February (open symbols)

creased to values between 3 and 4  $\mu\text{M}$ . Nitrate was apparently absent in the sediment of Stn 13 (Fig. 4). In February 2 types of nitrate profiles could be distinguished. The first type represents stations where the nitrate concentration in the upper few mm of the sediment approximately equals the concentration in the overlying water (Stns 13 & 14). The second type consists of profiles where the pore water concentrations exceeded the concentration in the overlying water considerably, indicating active nitrification. This pattern was found at all other stations. Below the surface peak, nitrate decreased due to denitrification to values between 2 and 4  $\mu\text{M}$  in deeper layers. Nitrite concentrations in pore water were always less than 1  $\mu\text{M}$ .

### Sediment-water fluxes

Ammonium and nitrate fluxes are shown in Table 3. Average values include all observations, since statistical analysis by the nonparametric Wilcoxon test for matched pairs did not indicate any significant differences between the methods applied. However, differences between August and February were highly significant for both ammonium and nitrate fluxes (test statistics are  $t = 1$ ,  $p < 0.01$ ,  $n = 7$  for nitrate;  $t = 0$ ,  $p < 0.01$ ,  $n = 7$ , for ammonium).

The seasonal variation in ammonium fluxes was larger than was found for nitrate. In August on average  $28 \mu\text{mol m}^{-2} \text{ h}^{-1}$  was released by the sediment. This

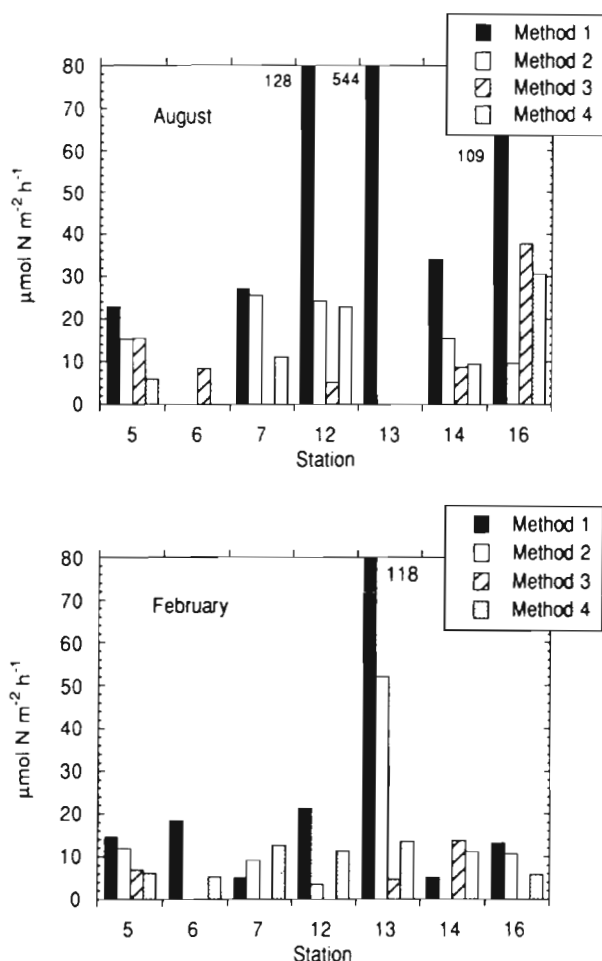


Fig. 5. Nitrification as obtained by Methods 1 to 4 in August (upper panel) and February (lower panel). All values in  $\mu\text{mol N m}^{-2} \text{h}^{-1}$ . For explanation see text

value dropped to  $3.8 \mu\text{mol m}^{-2} \text{h}^{-1}$  in February. In this period even a slight influx of ammonium was detected at Stn 5. These averages do not include the Helgoland Bight Stn 13, where ammonium was released in extremely high rates by the sediment in August ( $496 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). Much lower, but still considerably high rates, were found in February ( $13 \mu\text{mol m}^{-2} \text{h}^{-1}$ ).

Nitrate was generally released at rates between  $8.5$  and  $23 \mu\text{mol m}^{-2} \text{h}^{-1}$  in August. Again, the Helgoland Bight Stn 13 deviates from the other stations and showed a nitrate uptake by the sediment ( $\sim 2.5 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). On average, fluxes were 3 times lower in February as compared to August.

#### Denitrification rates

The observed denitrification rates varied between  $0$  and  $1.9 \mu\text{mol m}^{-2} \text{h}^{-1}$  in August (Table 3). Highest

values in this period were found at the Helgoland Bight Stn 13, lowest values were found at Stn 7. Denitrification increased by a factor of 1.5 to 5 in February. In this period highest activities were detected at Stns 12 & 13 ( $3.8$  and  $8.2 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). Denitrification was below the detection limit at the most offshore Stn 7 in February.

#### Nitrification rates

Nitrification rates were higher in August than in February. The average rate of all stations including all methods applied was  $47 \mu\text{mol m}^{-2} \text{h}^{-1}$  (Fig. 5). The nitrapyrine approach, however, gave significantly higher values in both periods compared to the other methods ( $p < 0.01$ ,  $n = 11$ ). The differences were most pronounced in August for Stns 12, 13 & 16 ( $128$ ,  $544$  and  $109 \mu\text{mol m}^{-2} \text{h}^{-1}$  respectively) where the sediment had a thin oxic layer and/or high rates of ammonium release. Here, the average nitrapyrine based rate was  $144 \mu\text{mol m}^{-2} \text{h}^{-1}$ . The other approaches, which were statistically not different from each other ( $p < 0.05$ ,  $n = 6$  to  $11$ ) resulted in lower values of  $18$  (Method 2),  $15$  (Method 3) and  $16$  (Method 4)  $\mu\text{mol m}^{-2} \text{h}^{-1}$  at these stations. In February, nitrification rates were on average  $15 \mu\text{mol m}^{-2} \text{h}^{-1}$  but still highest values were obtained with the nitrapyrine application ( $28 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). Average values for Methods 2, 3 and 4 were  $17$ ,  $6$  and  $9 \mu\text{mol m}^{-2} \text{h}^{-1}$  respectively.

Potential activities of nitrification were always 1.3 to 2.2 times higher in August than in February (results not shown). The range of the activities was from  $10$  to  $26 \text{ nmol cm}^{-3} \text{h}^{-1}$  in August and from  $5$  to  $20 \text{ nmol cm}^{-3} \text{h}^{-1}$  in February.

## DISCUSSION

#### Pore water and fluxes of nitrate and ammonium

Maximum porewater ammonium concentrations at offshore Stns 5, 6 & 7 did not exceed  $60 \mu\text{M}$  (Fig. 4), whereas at coastal Stns 12, 13, 14 & 16 considerably higher values up to  $2500 \mu\text{M}$  (Stn 13) were found, indicating more intense ammonium production from organic matter. Besides these spatial differences seasonal differences can be deduced from the shape of the profiles. In August, all stations are characterized by high ammonium concentrations and corresponding steep gradients in the upper mm's of the sediment. Apart from Stn 13, maximum values were generally reached between  $3$  and  $7 \text{ cm}$ , indicating no further increase in mineralisation beyond that depth. In February, decreased ammonification rates can be inferred



from the 2 to 3 times lower ammonium concentrations compared to August. The spatial and seasonal pattern of sediment-water exchange of ammonium corresponds to the pore-water concentrations. The mean fluxes at the coastal stations exceeded that of the offshore stations by a factor of 15 in August. However, these average values include the flux at Stn 13 ( $496 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) which was exceptionally high compared to ammonium fluxes measured in other coastal areas (Jensen et al. 1990, Kemp et al. 1990). B. B. Jørgensen (1989) reported extremely high sulfate reduction rates at this station, which may be partly responsible for these high releases. In February the ammonium flux at the coastal stations was still 5 times higher than at the offshore stations but compared well to earlier investigations (Billen 1978, van Raaphorst et al. 1992).

The distribution of nitrate is directly linked to the seasonal variation in oxygen penetration. In August, when the oxygen penetration was restricted to the upper mm's, pronounced nitrate peaks were absent (Fig. 4). This may, however be due to an insufficient resolution of the pore-water profile (0 to 4 mm) of nitrate. In February, when oxygen and nitrate gradients were expanded over the first few cm, pronounced nitrate peaks were detected. This peak coincides with very low ammonium concentrations in this layer. Similar seasonal pore-water distributions, suggesting relatively high nitrification rates, have been described by several authors in sediments of the North Sea area (Rutgers van der Loeff 1980, van Raaphorst et al. 1990, 1992), as well as for other areas (Kattegat: Henriksen et al. 1981; Chesapeake Bay: Kemp et al. 1990).

The sediment-water fluxes of nitrate were approximately 2 times higher in August compared to February. There was, however, no difference between average rates at offshore and coastal stations, although the latter showed more site-to-site variability. The nitrate release at Stns 12 & 14 seems to contradict the apparent absence of an oxic layer at these stations in August. This might be due to the fact that the cores remained unstirred during the oxygen electrode measurements thus causing an underestimation of the  $\text{O}_2$  penetration in these relatively coarse-grained sediments (Booij 1989). In any case, the released nitrate seems to be produced in a very thin layer at the sediment-water interface. The most striking feature of the nitrate pore-water profiles and fluxes is their contradictory pattern. Although no substantial peaks of nitrate were visible in the oxic layer, effluxes were high in August compared to February. In the latter period, pronounced nitrate peaks were found, although the fluxes were half as high as in August. The effluxes confirm that nitrate was produced during summer although nitrate peaks were not resolved by our slicing technique. Similar observations were reported for other areas as well

(Rutgers van der Loeff 1980, Henriksen et al. 1981, van Raaphorst et al. 1992).

### Denitrification rates

Our measured denitrification rates represent a lower limit due to the fact that the blockage of nitrous oxide reduction may be incomplete at low nitrate concentrations (Christensen et al. 1989, Slater & Capone 1989). However, the range of benthic denitrification rates ( $0$  to  $8 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) is more typical for continental shelf sediments than for coastal sediments (overview in Koike & Sørensen 1988). Only a limited data set on denitrification in the North Sea is available. In a recent study, Law & Owens (1990) found rates between  $0.3$  and  $6 \mu\text{mol m}^{-2} \text{h}^{-1}$  in midsummer. Van Raaphorst et al. (1992) performed measurements at 2 offshore stations in the southern North Sea (including our Stn 16) during an annual cycle in 1989/90. They estimated denitrification rates between  $1$  and  $23 \mu\text{mol m}^{-2} \text{h}^{-1}$  with highest activities in spring and early summer. Denitrification decreased during summer and was low in fall and winter. Jensen et al. (1990) indicated that highest denitrification rates occur after deposition of the spring bloom. Because our investigation was restricted to February and August we missed the period with maximum activity.

In August as well as in February coastal denitrification was 2 to 3 times higher than at the offshore stations. Since nitrate was always released by the sediments (except Stn 13), it can be assumed that benthic nitrification was the exclusive nitrate source for denitrification. Nitrification, however, is inhibited by acetylene (Walter et al. 1979) and denitrification may thus be suppressed during the denitrification measurements. We calculated that in the applied experimental set-up the flux of nitrate out of the sediment combined with the measured denitrification rate would exhaust the pool of nitrate in the sediment within 1 to 2 h in August and within 10 to 30 h in February. Therefore, we used the initial slope of nitrous oxide accumulation (0 to 1 h) to calculate the denitrification rates. Additionally, these short term incubations avoid substantial changes of *in situ* conditions.

### Nitrification rates

Basically, 2 types of seasonal cycles of benthic nitrification can be distinguished in the literature. The first type occurs in areas which are highly productive and receive high loads of nutrients, e.g. Chesapeake Bay (Kemp et al. 1990), Kysing Fjord, Denmark (Hansen et al. 1981). These areas are characterized by

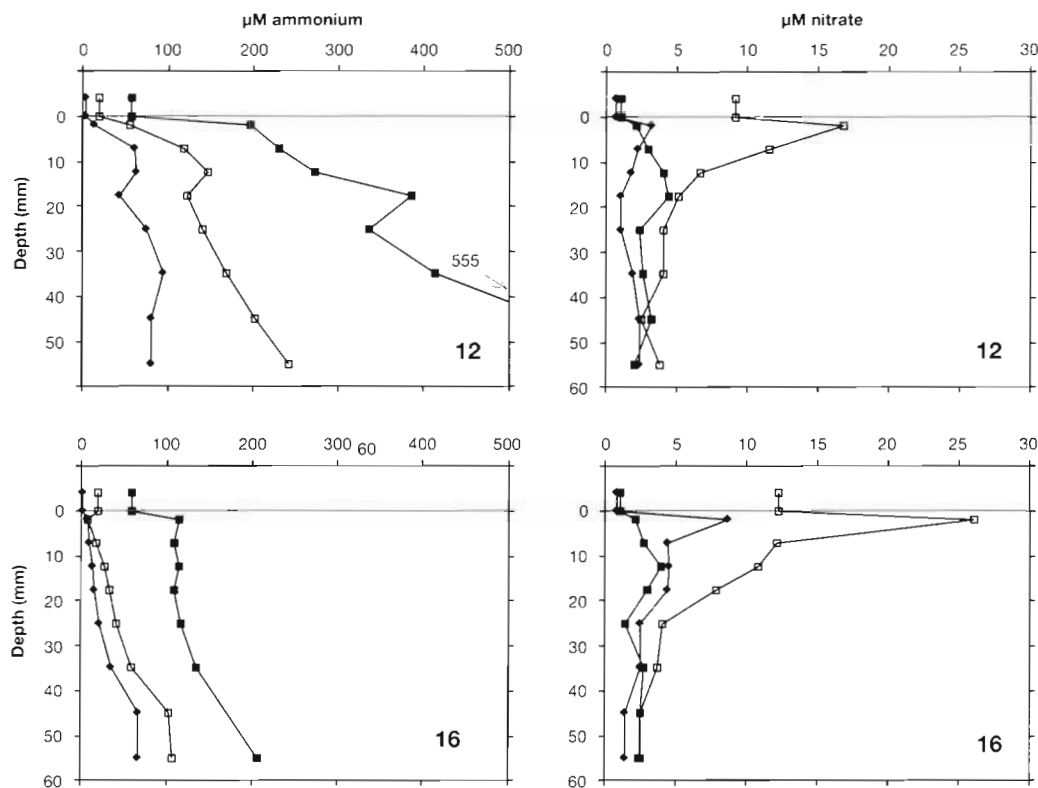


Fig. 6. Ammonium (right panels) and nitrate pore-water profiles (left panels) in blank (initial profiles,  $\blacklozenge$ ), control (after 35 h without N-Serve additions,  $\square$ ) and inhibited cores (after 35 h with nitrapyrine,  $\blacksquare$ ) at Stns 12 & 16 (all data from August)

a summer depression of nitrification due to the occurrence of anoxia and free sulphide in the sediment. The second type occurs in offshore sediments where the input of nutrients and organic matter is rather low, as in the Kattegat (Hansen et al. 1981), at an offshore station of Narragansett Bay, USA (Seitzinger 1987) and at Kingogoodie Bay, Scotland (McFarlane & Herbert 1984). At these stations increased temperatures and pore-water ammonium concentrations have a positive influence on nitrification during summer. Although our study did not comprise an annual cycle it can be concluded that the Helgoland Bight Stn 13 belongs to the first type whereas all other stations belong to the second type.

The strikingly high nitrification rates at Stns 12, 13 & 16 as obtained by the nitrapyrine technique (Method 1) are not in line with average rates published previously (overview in Henriksen & Kemp 1988) and with our observed denitrification rates. Therefore they seem suspect. That *in situ* conditions were not maintained during the relatively long incubation periods can be derived from the nitrate profiles at the start and at the end (with and without inhibitor) of the incubations (Fig. 6). The control profile exhibits a clear nitrate peak in the upper sediment layer after 35 h, although this

peak was much less pronounced at the start of the experiment. Nitrification may have been stimulated by an increased oxygen supply caused by bubbling air through the water column. Consequently, more ammonium was probably oxidized to nitrate, resulting in an overestimation of nitrification. A second reason for the overestimation by the nitrapyrine technique originates from changes in the ammonium concentrations during the incubation. Ammonium profiles at the start of the experiments were always lower than concentrations in the control cores after the incubation period (Fig. 6). The differences were most pronounced in the 4 to 6 cm layer where no nitrification took place due to the absence of oxygen. We conclude that a substantial part of the ammonium increase cannot be coupled to the blockage of nitrapyrine. Consequently all rates obtained with this approach are rejected. Nitrification data are based on the averages of the remaining methods (Methods 2, 3 & 4). We are aware that these methods also have drawbacks, e.g. the necessary assumption that the nitrate reduction rates remained unchanged during manipulations. The resulting rates are given in Table 3. In both periods, nitrification tends to be higher at coastal stations compared to offshore stations, although activity was suppressed at Stn 13 in

August. At this station, the cores had a free sulphide smell during processing in that period. This, together with the absence of an oxic layer, probably inhibited nitrification.

The primary factors controlling nitrification are temperature, abundance of nitrifying bacteria, ammonium and oxygen. In August, increased temperatures exhibit a positive influence on the abundance of nitrifying bacteria as well as on the *in situ* activity (Focht & Verstraete 1977, Helder & de Vries 1983). The role of oxygen and ammonium is more difficult to assess. Pure culture experiments have shown that nitrification activity continues even at very low oxygen concentrations (Carlucci & McNally 1969, Focht & Verstraete 1977). However, Kemp et al. (1990) calculated that nitrification was absent when oxygen concentrations in the overlying water declined below 125  $\mu\text{M}$  and sediments were anoxic. In our study, nitrification was increased when the oxic layer was thinner in August, suggesting that nitrification was not suppressed at low oxygen concentrations. Ward (1987) demonstrated that at lowered oxygen concentrations the affinity of the ammonium oxidizing enzyme to ammonium increased. However, this situation occurred only when the ammonium concentration was  $<20 \mu\text{M}$ . This value was not exceeded in most of the oxic layers of the present sediments. On the other hand, addition of ammonium to the overlying water (50  $\mu\text{M}$ , data not shown) was followed by an immediate 2- to 3-fold increase in nitrate release. In February, the response was much stronger (up to 9-fold). The bacterial production ( $^3\text{H}$  leucine incorporation), as measured in sediment slurries from the same sampling site, remained unchanged during the additions (F. van Duyl pers. comm.). This clearly points out that nitrification can be stimulated by ammonium additions in both seasons. We conclude that the interaction between ammonium and oxygen

rather than oxygen alone probably controls benthic nitrification activity in North Sea sediments.

### Nitrogen cycling

It is obvious that nitrification and denitrification in the North Sea show opposite patterns in the periods investigated here. Generally, nitrification is thought to have a stimulatory effect on denitrification (e.g. Seitzinger 1987, Kemp et al. 1990). The present study, however, suggests that high nitrification rates were accompanied by low denitrification rates in August. In February, when nitrification activity was low, denitrification had increased. At offshore stations, only 4 and 20% of the nitrate produced by nitrification is denitrified in August and February, respectively. The values for coastal stations were 2 and 29% (excluding Stn 13 in February: 12%) in August and February respectively. Obviously, bulk of the nitrate produced by nitrification is released to the overlying water, particularly in August (Table 3). In this month nitrification was confined to a very thin, oxygenated surface layer of the sediment. At the same time, nitrate in the overlying water was almost exhausted (0.2 to 1.2  $\mu\text{M}$ ). By comparing the nitrate gradients across the sediment-water interface with the maximum downward directed gradients, it becomes obvious that the upper gradient is ca 2 to 10 times steeper than the latter (Table 4). This situation favours a nitrate release to the overlying water, rather than a downward diffusion to anoxic sediments where denitrification takes place. In February, however, the oxic zone is extended, thus nitrification occurs in deeper sediment layers. Then, the nitrate concentration in the overlying water is relatively high (around 4  $\mu\text{M}$  offshore, 10 to 40  $\mu\text{M}$  coastal) so that the nitrate gradient between overlying water and the nitrifi-

Table 4. Calculated upwards- (across the sediment-water interface,  $(d[\text{NO}_3^-]/dz)_{z=0}$ ) and downwards- (maximum decrease of nitrate,  $(d[\text{NO}_3^-]/dz)_{z=\text{max}}$ ) directed nitrate gradients

Station	August		February		August	February
	$(d[\text{NO}_3^-]/dz)_{z=0}$	$(d[\text{NO}_3^-]/dz)_{z=\text{max}}$	$(d[\text{NO}_3^-]/dz)_{z=0}$	$(d[\text{NO}_3^-]/dz)_{z=\text{max}}$	ratio	$\frac{(d[\text{NO}_3^-]/dz)_{z=0}}{(d[\text{NO}_3^-]/dz)_{z=\text{max}}}$
	$(\mu\text{mol cm}^{-4}) \times 10^{-2}$		$(\mu\text{mol cm}^{-4}) \times 10^{-2}$			
5	0.35	-0.18	5.89	-1.11	-1.9	-5.3
6	0.86	-0.09	6.05	-1.48	-9.6	-4.0
7	1.55	-0.28	9.65	-1.14	-5.5	-8.5
12	1.23	-0.18	6.55	-1.24	-6.8	-5.3
13	-1.40	-1.40	0.45	-7.56	1	<-0.1
14	0.90	-0.25	-0.30	-0.47	-3.6	0.6
16	3.98	-0.86	4.20	-0.81	-4.6	-5.2



Table 5. Response of the denitrification rate after nitrate enrichment of the overlying water (100  $\mu\text{M}$ ) in August. Incubation time: 2 h

Station	<i>In situ</i> denitrification	Denitrification after enrichment	Factor of increase
$(\mu\text{mol N m}^{-2} \text{ h}^{-1})$			
5	0.50	11.40	23
6	0.90	14.79	16
7	0.10	14.31	143
12	0.42	30.46	73
13	1.90	104.02	55
14	0.24	30.35	126
16	0.72	17.13	24

fication zone is diminished. Although nitrification rates were lower than in August, less nitrate is released to the water column but is available for denitrification. Additionally, low porosity (Table 1) and relatively high bottom currents in the investigation area (Tryggstad et al. 1983) favour a nitrate release to the water column instead of downward diffusion. To demonstrate a potential nitrate limitation of denitrification the water phase of duplicate cores was enriched with 100  $\mu\text{M}$  nitrate in August. The sediments responded with a 16- to 143-fold increase in the denitrification rate (Table 5). Therefore, we conclude that denitrification was limited by nitrate availability in spite of relatively high nitrification rates. Apparently the coupling between the 2 processes is indirectly influenced by the nitrate concentration in the overlying water. Evidence for this assumption may be derived from the positive correlation ( $r^2 = 0.989$ ,  $p < 0.01$ ,  $n = 7$ ) between the nitrate concentration in the overlying water and denitrification

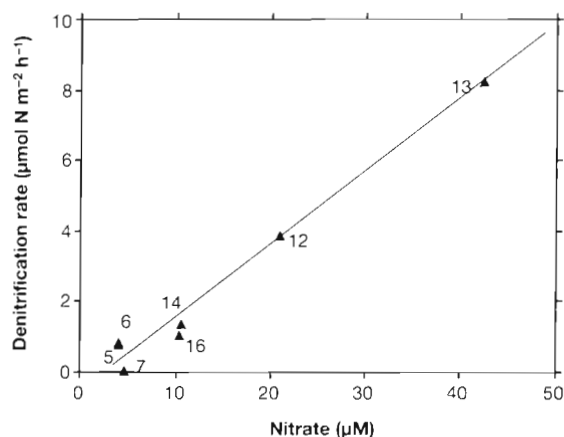


Fig. 7 Concentration of nitrate ( $\mu\text{M}$ ) in the overlying water plotted against denitrification rate ( $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ ) in February 1992

rates in February (Fig. 7). If the nitrate concentration is relatively high, the concentration gradient between the nitrification zone and the overlying water is minimized, resulting in a more efficient nitrification/denitrification coupling.

The situation at Stn 13 may be typical for areas in which the coupling of denitrification and nitrification is relatively strong as long as the nitrate concentration in the overlying water is high enough (Kemp et al. 1990, Kieskamp et al. 1991). During August, however, nitrification was inhibited due to the absence of an oxic layer. Consequently, the ambient denitrification rate ( $1.9 \mu\text{mol m}^{-2} \text{ h}^{-1}$ ) is dependent on the nitrate influx at this station ( $3 \mu\text{mol m}^{-2} \text{ h}^{-1}$ ). Although nitrification had recovered in February the overall nitrate flux was still directed towards the sediment, the total nitrate consumption being  $11 \mu\text{mol m}^{-2} \text{ h}^{-1}$  ( $9 \mu\text{mol}$  nitrification plus  $2 \mu\text{mol}$  nitrate influx). The observed denitrification rate was  $8.2 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ , indicating a very efficient coupling between nitrification and denitrification. The difference (25 to 37 %) in both periods may be due to an underestimation by the acetylene technique (Christensen et al. 1989, Rudolph et al. 1991) or to nitrate ammonification (Steps 3 & 10, Fig. 2), which probably occurs in this reduced sediment (K. S. Jørgensen 1989).

Total nitrogen mineralisation can be estimated from the assumption that ammonification is the sum of the ammonium flux and nitrification rates (Kemp et al. 1990). In agreement with the estimates of van Raaphorst et al. (1992), approximately 50 % of the produced ammonium is nitrified at the offshore stations in August and more than 85 % in February (Fig. 8). At the coastal stations, where the ammonification was about 8 times higher than at the offshore stations in August, most of the ammonium was released to the water col-

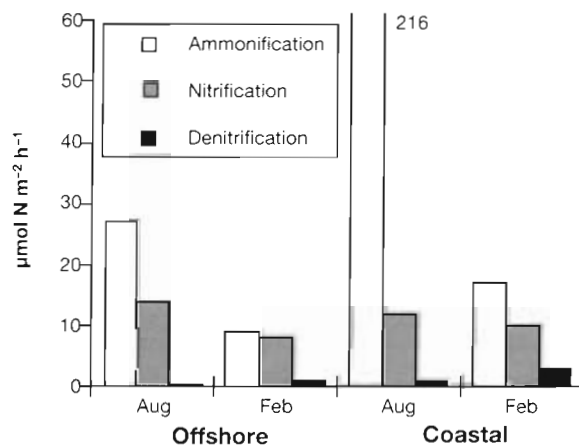


Fig. 8. Average rates of ammonification, nitrification and denitrification at coastal and offshore stations in August and February ( $\mu\text{mol N m}^{-2} \text{ h}^{-1}$ )

umn. Only 16 and 60% was nitrified in August and February, respectively. Because the bulk of nitrate is released to the water column as well, the total DIN fluxes in August are almost identical to ammonification. In February, a substantial part of the nitrate is denitrified, particularly in the German Bight.

### Ecological implications

The contribution of benthic ammonium and nitrate release to the requirements of phytoplankton can be calculated assuming an annual primary production of  $250 \text{ g C m}^{-2} \text{ yr}^{-1}$  and a C/N ratio in phytoplankton of 6.6 (Stefansson & Richards 1963). On an average annual basis 4 to 8% of the nitrogen requirements of phytoplankton can be delivered by benthic nitrogen releases. Only at the Helgoland Bight Stn 13 where high N fluxes were accompanied by an elevated primary production did the contribution amount to more than 50%, pointing out the important role of the sediments in nutrient cycling in the German Bight.

The role of denitrification as nitrogen sink can be estimated when the anthropogenic nitrogen input to the continental coastal waters (area:  $65\,000 \text{ km}^2$ ) is assumed to be  $971\,000 \text{ t N}$  (Nelissen & Stefels 1988). Our average denitrification rate of  $0.2 \text{ g N m}^{-2} \text{ yr}^{-1}$  results in a removal of  $12\,500 \text{ t N yr}^{-1}$ , which corresponds to 1.5 to 3% of the estimated input of nitrogen. This value is lower than previous estimations of 20% (Brockmann et al. 1990), 8 to 12% (Law & Owens 1990) and 10 to 15% (van Raaphorst et al. 1992). The difference is largely caused by the fact that our calculation is based on a restricted area, directly affected by anthropogenic inputs, whereas other authors compared the denitrification rate of the total North Sea sediment surface area with anthropogenic nitrogen inputs to the coastal zones. By comparing the total anthropogenic nitrogen input into the entire North Sea ( $2.3 \times 10^6 \text{ t N}$ ; Nelissen & Stefels 1988) with our average denitrification rate ( $0.2 \text{ g N m}^{-2} \text{ yr}^{-1}$  over  $575\,000 \text{ km}^2$  is  $0.12 \times 10^6 \text{ t N}$ ) it becomes clear that denitrification can remove only a minor part of the nitrogen loading of the North Sea. If these low denitrification rates are common for other continental shelf seas with low nitrate concentrations in the overlying water, the role of continental shelves in nitrogen removal should be re-evaluated.

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