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Rates of Nitrification, Distribution of Nitrifying Bacteria, and Nitrate Fluxes in Different Types of Sediment from Danish Waters

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Abstract

Nitrification rates, measured in different sediment types from Danish waters, are in the range of 0.3 to 1.4 mmol NO_3^- m^{-2} d^{-1}. There is no significant difference between sandy and muddy sediments, nor between shallow and deeper stations. The extent of nitrification is probably limited to the zone of oxygen penetration, 1.5 to 5.5 mm. There are, however, nitrifying bacteria located in the anoxic sediment layers. There relative numbers were found by measuring the "nitrification potential" of the sediment. These potential rates (22 °C) can also be used to calculate actual rates of nitrification, by adjusting to *in situ* temperature and oxygen penetration. These calculated rates agree with the actual measured rates of nitrification for a wide range of sediment types and may be used for the estimation of actual nitrification rates. Nitrate flux out from the sediment/water interface is in the range of 0 to 0.7 mmol NO_3^- m^{-2} d^{-1}. There is no correlation between concentration gradients of nitrate across the sediment/water interface and the measured flux of nitrate. Approximately 50% of nitrate production is released to the water column. The remainder (0 to 0.35 mmol N m^{-2} d^{-1}) may have been denitrified.

Introduction

The measurement of rates of nitrification in sediments is technically difficult. Two methods used to measure nitrification are a mixed sediment procedure (Billen, 1976) and an intact core method (Henriksen, 1980). The mixed system has the disadvantage that oxygen is made available to bacteria in the lower sediment strata, where oxygen does not normally occur. The intact core procedure maintains natural oxygen concentrations in the sediment, but it is time-consuming. We now compare this intact core procedure with a method, which measures potential nitrification rates, using a mixed sediment with optimum aeration (Hansen, 1980). These potential rates can be converted to actual *in situ* rates, when the depth of oxygen penetration into the sediment is known. The two methods give similar results, suggesting that the potential nitrification procedure can be used to give actual nitrification rates. Potential nitrification measurements have the advantage that in addition to being technically simple, they give the depth distribution of nitrifying bacteria in the sediment.

Materials and Methods

This study was carried out during 2 cruises with "R/V Martin Knudsen" in Danish waters. The stations were situated in Langelandsbaelt (III), Storebaelt (II), Kattegat (V, VI, VIII, IX and XIII) and Skagerak (XI). The positions are shown on Fig. 1 and some sediment characteristics are given in Table 1. Stations VI and IX were situated in the eastern part of Kattegat, where special current and salinity conditions prevailed with bottom water of high salinity moving from Skagerak towards the Baltic Sea (Steemann-Nielsen, 1940).

All sediment cores were sampled by a "Haps" bottom corer (Kanneworf and Nicolaisen, 1973), and subsamples from this were taken with Plexiglas tubes. Only cores with undisturbed stratification were used. Samples were incubated at ambient temperature aboard ship immediately after sampling. Five sediment cores from each station were sectioned in 1 and 2 cm segments. Segments from similar depth were pooled and duplicate samples were taken for measurement of NH_4^+ and $NO_2^- + NO_3^-$ concentrations in pore water, KCl-extractable NH_4^+ and potential nitrification rates. Pore water and KCl-extracts (1 : 1 sediment, 1 N KCl) were obtained by centrifugation at 2000 x g for 10 min. The samples were analysed immediately aboard ship.

Actual nitrification rates were measured in the dark at *in situ* temperature in undisturbed sediment cores by

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Table 1.

Station	Depth	Geographical position		Sediment type	Ignition loss
	m	N	E		(450 °C)
II Storebaelt	25	55°25.0′	10°57.3′	mud	7.5%
III Langelands Baelt	15	55°06.3′	10°49.4′	mud	13.9%
V Aalborg Bugt	14	56°46.8′	10°44.5′	sand	0.8%
VI Anholt SE	43	56°39.4′	11°45.5′	mud	7.0%
VIII Aarhus Bugt	17	56°07.8′	10°21.2′	mud	7.5%
IX Laessø E	65	57°19.6′	11°35.8′	mud	12.3%
XI Hirtshals N	65	57°52.2′	09°58.2′	mud	7.0%
XIII Aalbaek Bugt	25	57°36.8′	10°47.6′	sandy mud	3.7%

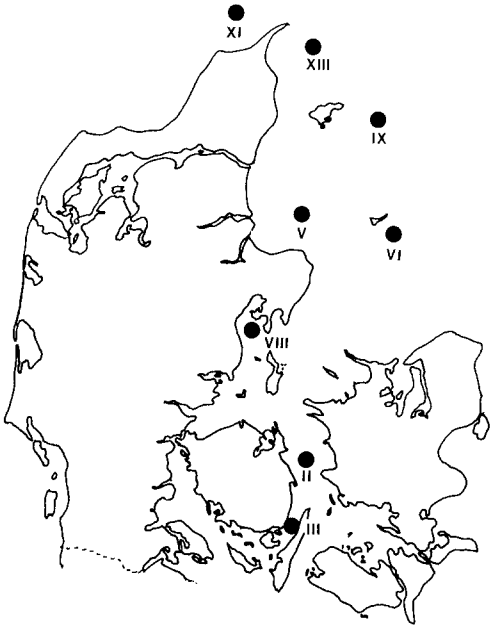

Fig. 1. Location of the sampling stations

use of N-serve, a specific inhibitor of the nitrification process (Henriksen, 1980). Potential nitrification rates were mesured in the dark at 22 °C (Hansen, 1980). Duplicate samples from different depths were incubated in 100 ml of filtered sea water in a shaker at 130 rpm. The sea water was enriched with NH_4Cl (0.5 mmol l^{-1}) and KH_2PO_4 (0.1 mmol l^{-1}). Denitrification was inhibited by the aerobic incubation conditions used (Henriksen, 1980). Samples were taken after 0, 4, 12 and 24 h and analysed for NO_2^- + NO_3^-. From the accumulation of NO_2^- + NO_3^-, potential nitrification rates of sediment at different depths (0 to 8 cm) were calculated. The NO_2^- + NO_3^- accumulation was linear with time during the first 24 h of incubation ($R \geqslant 0.95$), indicating that ammonium oxidation started almost immediately and that no significant growth of nitrifying bacteria occurred during this time interval. The measured value, therefore, gives an estimate of the potential activity of viable nitrifying bacteria in the sediment, when oxygen and ammonium are not limiting factors. The potential nitrification activity is presumably proportional to numbers of nitrifying bacteria.

Oxygen penetration of sediment was measured with membrane covered oxygen microelectrodes in undisturbed sediment cores with gentle stirring of the water column (Revsbech *et al.*, 1980).

The nitrate flux between sediment and water column was measured in short term incubations (4 to 6 h) in undisturbed sediment cores (3.6 cm diameter) with gentle stirring of the water column to maintain *in situ* gradients of oxygen in the sediment surface layer (Jørgensen, 1977). The water column of the cores was replaced with bottom water from the same station before incubation in the dark at *in situ* temperature. Increase in NO_3^- + NO_3^- and NH_4^+ concentrations during the incubation period (4 to 6 h) were measured. A series of 10 cores were used for each station.

NH_4^+, NO_2^- + NO_3^- were measured by an autoanalyser (Chemlab Instruments, Ltd., Essex) aboard ship using the methods of Solórzano (1969) and Armstrong *et al.* (1967). Density and water content of sediment were determined for conversion of measured concentrations and activities into appropriate units.

Results

Potential nitrification rates were measured to a depth of 8 cm (Fig. 2). The 3 selected stations represent the range of profiles found in different sediment types. The sandy Station V and the deep muddy Station VI had little decrease in numbers of nitrifying bacteria to a depth of 8 cm, whereas at the muddy Station VIII, there was a decrease in nitrification potential below 4 cm depth. The depth of oxygen penetration was in the range of 1.5 to 6 mm (Revsbech *et al.*, 1980). At all the stations there were significant numbers of nitrifying bacteria in the anoxic sediment layers.

Nitrifying bacteria are strictly aerobic, and depth of oxygen penetration is, therefore, a good estimate of the depth of nitrification zone in the sediment under *in situ* conditions. The Q_{10} was 2.5 for the potential nitrification rate in these sediment types between 2° and 22 °C (Hansen, 1980). This and the depth of oxygen

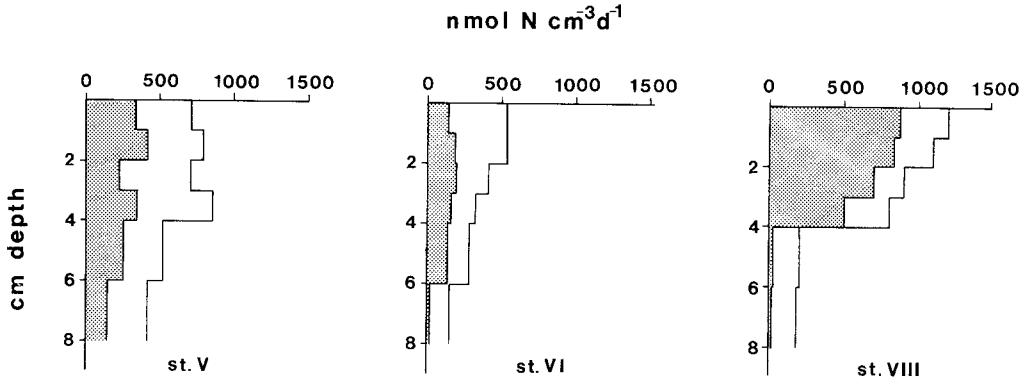

Fig. 2. Potential nitrification rates (22 °C) in 0 to 8 cm depth at Stations V, VI and VIII in Nov. 1978 (shaded) and July 1979 (unshaded)

Table 2.

	Temperature	O_2 penetration	Actual nitrification[a]		Potential nitrification[b]	NO_3^- flux
	°C	mm	Measured	Calculated	(22 °C)	nmol cm^{-2} d^{-1}
NOV						
Station II	10	2.5	40 (31)[c]	90	1080	30 (9)[d]
Station III	10	2.0	70 (29)	105	1590	60(31)
Station V	6.4	2.0	30 (46)	15	330	10 (4)
Station VI	9.9	5.5	30 (24)	25	130	20 (6)
Station VIII	7.6	4.5	80 (35)	105	870	50 (14)
JULY						
Station II	5.0	2.5	60 (29)	75	1410	40 (16)
Station III	8.5	2.5	130 (72)	120	1610	70 (13)
Station V	7.5	1.8	35 (86)	35	710	0 (0)
Station VI	3.5[e]	4.5	110 (41)	60	530	40 (8)
Station VIII	10	1.5	80 (43)	60	1200	30 (19)
Station IX	4.8	5.5	90 (33)	45	385	40 (11)
Station XI	6.7	5.5	90 (23)	110	790	55 (15)
Station XIII	11.8	4.0	100 (47)	140	880	55 (17)

[a] nmol NH_4^+ oxidised cm^{-2} d^{-1}
[b] Potential nitrification rates in 0 to 1 cm depth in nmol NH_4^+ oxidised cm^{-3} d^{-1}
[c] Standard deviation of 5 cores
[d] Standard deviation of 10 cores
[e] Incubated at 5 °C aboard ship

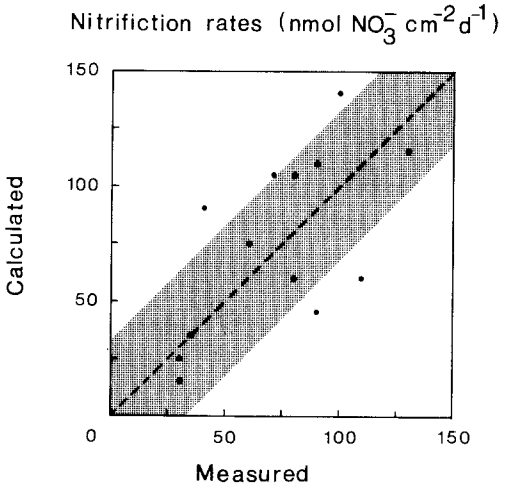

Fig. 3. Measured nitrification rates plotted against calculated nitrification rates. The shaded area represents the standard error

penetration were used to transform potential nitrification rates (22 °C), measured in the surface layer of the sediments, to actual nitrification rates (calculated). No correction was made for the effect of ammonium concentration (Table 2). The measured and calculated actual nitrification rates were plotted against each other (Fig. 3). There was some scatter, but the points mostly lay close to the slope of 0.5.

The actual nitrification rates were higher in July than November for Stations II, III and VI; there was no change in Stations V and VIII. The remaining stations were not sampled on both cruises.

The factors, which influence nitrification rates are temperature, oxygen penetration, ammonium concentration and number of nitrifying bacteria. These factors changed seasonally as follows: Temperatures at the sediment surface of Stations V and VIII, where total mixing of the water column occurs, had only increased by 1 to 2 °C in July compared to November. At Stations

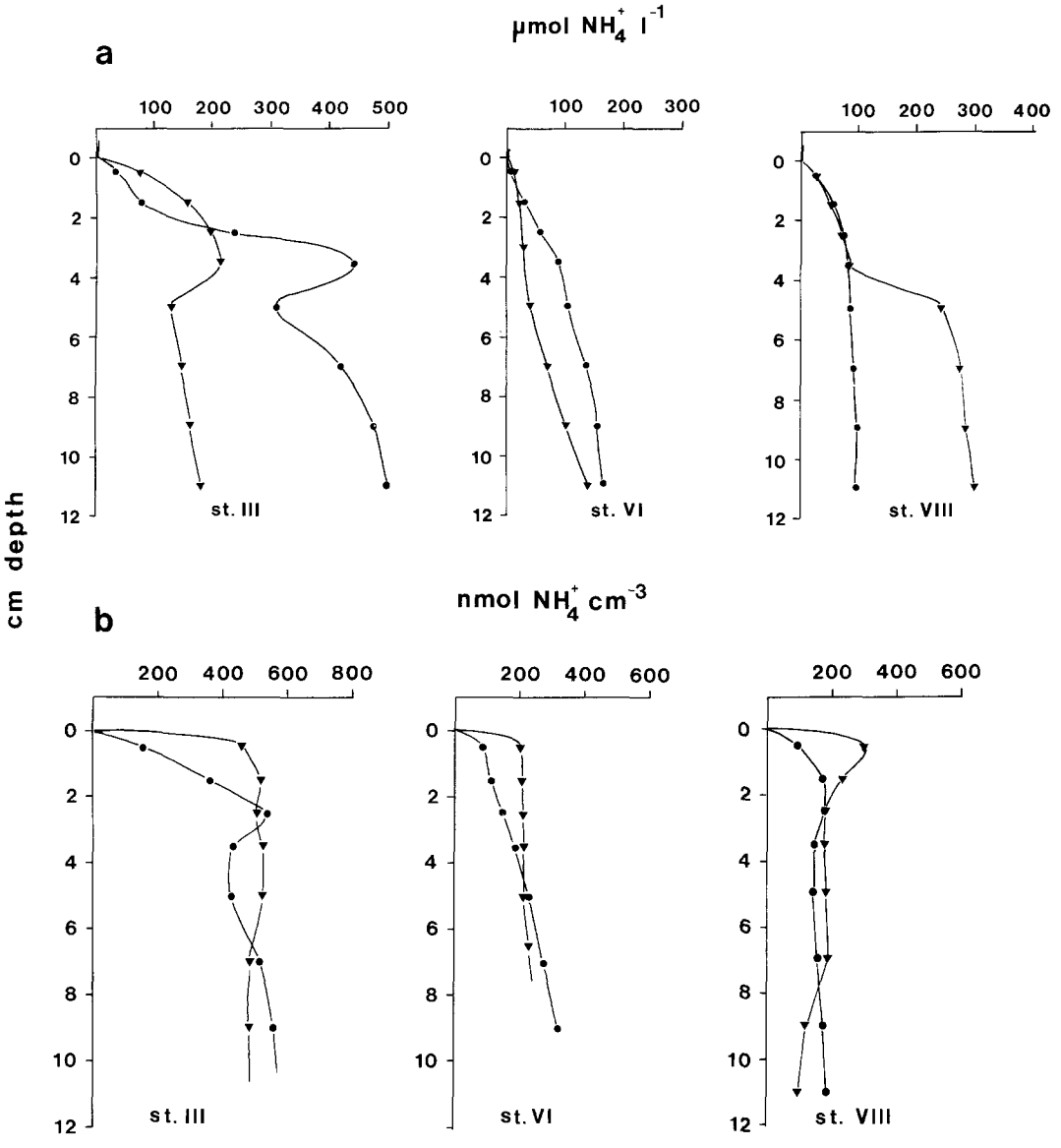

Fig. 4. (a) Pore water concentrations of ammonium and (b) KCl-extractable ammonium at Stations III, VI and VIII in Nov. 1978 (●—●) and in July 1979 (▼—▼)

II, III and VI temperatures in July were lower than for November due to cold winter water of higher salinity moving at the bottom from Skagerak towards the Baltic Sea. Oxygen penetration at the sediment surface in July was almost identical (Stations II, III, V and VI) or lower (Station VIII) than for November (Table 2). Ammonium concentration in the pore water of the 0 to 1 cm layer of sediment was in the range of 10 to 100 µmol NH_4^+ l^{-1}. There was neither a consistent seasonal variation in ammonium concentration in the 0 to 1 cm layer nor in lower sediment layers (Fig. 4a). The concentration of soluble plus extractable ammonium in the surface layer was in the range of 100 to 400 nmol NH_4^+ cm^{-3} and there was a consistent increase in the surface layer in July compared to November (Fig. 4b).

The numbers of nitrifying bacteria in the surface layer and in the deeper layers increased significantly from November to July, except for Station III (Table 2, Fig. 2). This increase was not reflected in actual nitrification rates at all stations due to different changes in temperature, oxygen penetration and possibly also ammonium concentrations.

The measured flux of nitrate between sediment and water column (Table 2) was not correlated with concentration gradients of nitrate across the sediment/water interface (Fig. 5). These profiles represent the range of nitrate concentrations which were measured at all stations in July. At Station III the lowest nitrate concentrations were measured in the surface layer, whereas the highest flux of nitrate from sediment to the water

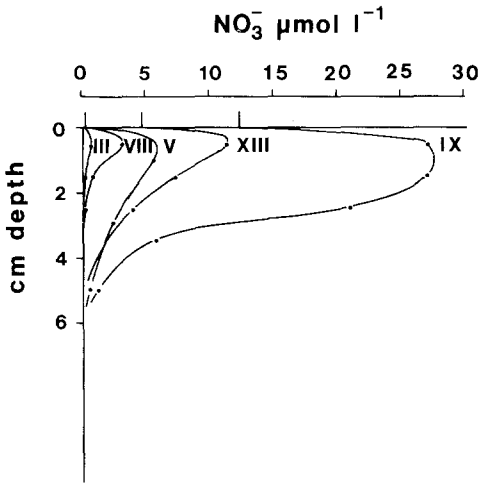

Fig. 5. Concentration profiles of NO_3^- in the pore water at Stations III, V, VIII, IX and XIII, in July

column (70 nmol NO_3^- cm^{-2} d^{-1}) was measured at this station. At Station V the nitrate flux was zero, probably due to the layer of benthic diatoms present at the sediment surface of this station (14 m depth). Station IX had the steepest nitrate gradient, but an intermediate flux of 40 nmol NO_3^- cm^{-2} d^{-1} was recorded at this station. The flux of nitrate measured at different stations in July ranged from 35 to 65% of the actual measured nitrification rate, except for Station V (0%). The mean nitrate flux from the sediment for November and July was 53% of the actual nitrification rate.

Discussion

The integrated potential nitrification rates per sediment area, corrected to *in situ* temperature, were 20 to 30 times higher than the measured actual nitrification rates. Oxidation of ammonium and growth of nitrifying bacteria are restricted to the narrow zone of oxygen penetration at the sediment surface, but nitrifying bacteria are able to survive for a prolonged period under anoxic conditions (Hansen, 1980). The large population of nitrifying bacteria present in the anoxic sediment layers were activated immediately by exposure to oxygen in the potential nitrification assay. This caused the high potential nitrification rate, when uncorrected for oxygen availability in sediment. There is a fairly good correlation between the actual nitrification rates and the potential nitrification rates (22 °C) in the surface layer (0 to 1 cm), when the latter are corrected for both temperature and oxygen penetration. The procedure for measuring potential nitrification rates is simple and quick compared to the procedure for measurement of actual nitrification rates in intact sediment cores. When the depth of oxygen penetration is known, a good estimate of actual nitrification rates can be obtained by this method. This correlation is true for a broad spectrum of different sediment types. Furthermore the method gives information about the distribution and relative population sizes of nitrifying bacteria in different sediments.

Actual nitrification rates, which were measured in different sediment types in November and July, are in the range of 30 to 130 nmol NH_4^+ oxidized cm^{-2} d^{-1}.

There is no significant difference between the sandy Station V (0.8% ignition loss) and the remaining stations of fine textured sediment (7–14% ignition loss), nor is there a definite seasonal trend in actual nitrification rates. This lack of correlation is reasonable since the main parameters which control acutal nitrification (temperature, oxygen penetration, NH_4^+ concentration and number of nitrifying bacteria) show only small seasonal variations or their effects cancel each other out. There are fewer nitrifying bacteria at deeper stations (VI, IX and XI,), but here oxygen penetrates to a greater depth. There is no evidence of limitation by ammonium concentrations in the surface layer, although pore water concentrations of ammonium were low (10 µmol l^{-1}) at most of the stations. This is, however, close to the K_m for Nitrosomonas at the measured temperature range (Knowles *et al.*, 1965).

The increase in the number of nitrifying bacteria at most stations from November to July had little effect on actual nitrification rates, due to counteracting changes in temperature and oxygen penetration. In more detailed studies of seasonal changes in potential nitrification rates at Station VIII, a maximum in the number of nitrifying bacteria was found in spring at low temperatures, whereas minima occurred in the autumn at high temperatures (J. I. Hansen *et al.*, unpublished data).

There is no correlation between the measured flux of nitrate from sediment to the water column and the concentration gradient across the sediment/water interface, at the different stations. Nitrification is limited to the upper 2 to 5 mm of the sediment, where transport due to turbulence in the watercolumn may be of greater importance than molecular diffusion. In the eastern part of Kattegat, bottom currents are 4 cm s^{-1} (Steemann-Nielsen, 1940).

The coarse measurements of the nitrate concentration profiles (1 cm intervals) compared to the narrow zone of nitrification, probably give an underestimate of concentration gradients across the sediment/water interface, but cannot explain the large difference between flux and concentration gradients measured (Fig. 4). There was, however, a dense layer of benthic diatoms at Station V, which probably contributed to the zero flux of nitrate and ammonium from this sediment. During daytime, benthic diatoms create an effective filter for nutrients diffusing towards the sediment surface from below, and the filter effect also operates during the first hours of darkness (Henriksen *et al.*, 1980).

We conclude, that quantitative estimates of the nitrate flux between sediment and water column in coastal areas cannot be based on models where concentration gradients and diffusion alone are used as the main parameters. On the average, 50% of the nitrate

production was released into the water column. Bacteria prefer ammonium to nitrate as nitrogen source (Payne, 1973), so that it is likely that only a small part of the sediment nitrate is assimilated by bacteria. The difference between nitrate production in the sediment and the nitrate flux to the water column (50% of actual nitrification) might, therefore, be an indirect estimate of the denitrification rate in these sediments.

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