Denitrification in coastal bay sediment: regional and seasonal variation in Aarhus Bight, Denmark

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ABSTRACT: The seasonal pattern of denitrification activity was studied in sediment from 3 stations in the Aarhus Bight, Denmark. At all 3 stations, a dramatic increase in denitrification activity was found in early spring coincident with sedimentation of the spring phytoplankton bloom. The maximum of activity was followed by declining rates in the early summer and low and relatively constant rates throughout the rest of the year. For the 3 sediments, the total range of denitrification activity was <0.05 to 0.5, 0.05 to 0.7 and 0.2 to 1.0 mmol N m^{-2} d^{-1}, respectively, and the total annual activity 35, 70 and 180 mmol N m^{-2} yr^{-1} Regional differences in activity were comparable to differences in organic content at the 3 stations; throughout the year, highest activities were observed in the most organic-rich sediment and lowest were observed in the relatively organic-poor sediment. Results indicate that annual denitrification may increase in response to higher nutrient load and primary production in the coastal sea.

INTRODUCTION

The increasing nitrogen loads in coastal environments (Maybeck 1982) have led to considerable interest in the marine nitrogen cycle. Denitrification in sediments has received much attention due to its role as a natural sink for combined nitrogen. During denitrification, NO_3^- is reduced primarily to N_2 und to a lesser extent to N_2O, both of which can escape to the atmosphere. Denitrification thus mitigates coastal nitrogen loading from terrestrial sources (e.g. Seitzinger & Nixon 1985), and may in some cases limit coastal primary production (Boynton et al. 1980, Nixon 1981). In order to estimate the role of denitrification in coastal eutrophication, it is of prime importance to determine the temporal and regional variations of the process and to identify the regulating environmental factors.

Until recently, measurements of in situ rates of denitrification were sparse for coastal environments. A few investigations have dealt with the natural activity and local variation of denitrification in estuaries (Andersen et al. 1984, Sørensen 1984, Jørgensen & Sørensen 1985, Smith et al. 1985, Seitzinger 1987, Jørgensen & Sørensen 1988 [preceding article]) and other near-coastal environments (Seitzinger et al. 1984, Kaspar et al. 1985). Not surprisingly, a variety of denitrification patterns have been observed.

The purpose of the present investigation, which took place over 16 mo in 1985–86, was to determine the seasonal and regional variations of denitrification in a coastal bay area characterized by a significant phytoplankton bloom and rapid detritus sedimentation in early spring.

MATERIALS AND METHODS

Study area and sampling. The investigation was carried out in Aarhus Bight on the east coast of Jutland, Denmark (Fig. 1). In terms of hydrography and biological oceanography, the bight is representative of many near-coastal areas in the Kattegat, which separates the Baltic from the North Sea. In spite of the shallow water, there is often a marked stratification in the water column when low-salinity surface water from the Baltic meets saline bottom water from the North Sea. Primary production and sedimentation of organic matter are high in spring and significant O_2 deficits may develop in the bottom water and sediments from spring to fall. For the present investigation, we collected water and sediment samples frequently at 3 localities in the bay: Stns 10, 16 and 22, all at a water depth of 15 m (Fig. 1).

Temperature, salinity, and concentrations of O_2, NO_3^- and NO_2^- were determined in samples from both the
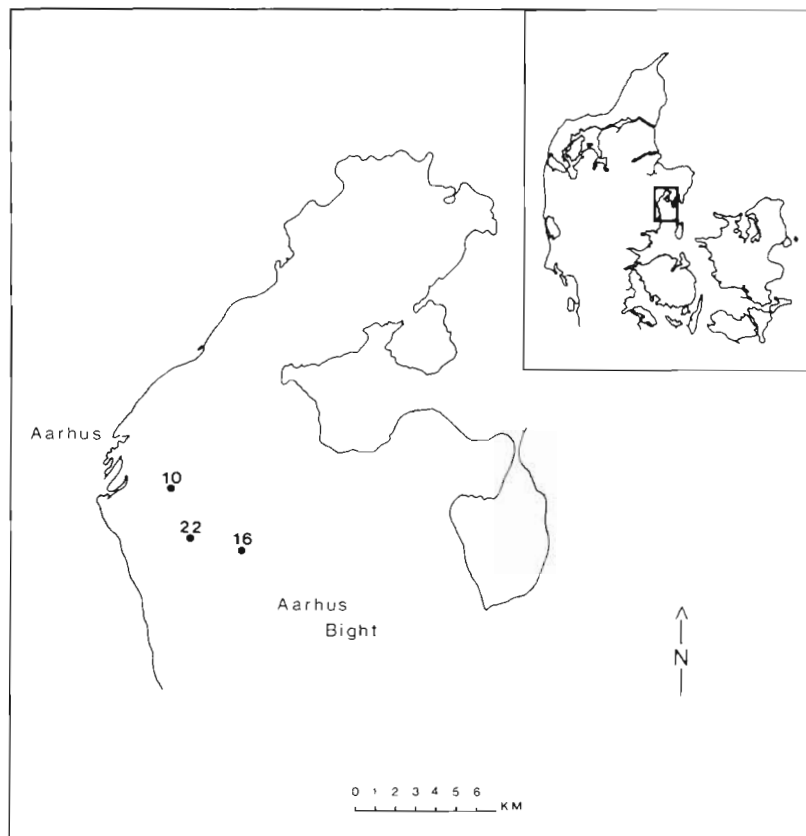
Fig. 1. Study sites in Aarhus Bight, Denmark

surface water (2 m below the surface) and the bottom water (0.5 m above the sediment). On the day of sampling, a water sample taken at one of the stations was assumed to be representative for the whole sampling area.

Sediment was collected with a 'Haps' sampler which took 12.5 cm (i.d.) cores of the sediment (Kanneworff & Nicolaisen 1973). From the main core, a number of smaller subcores were taken in 3.6 cm (i.d.) wide and 12 cm long acrylic tubes for determinations of density, water content, organic content, and denitrification activity in the sediment. All cores were carefully inspected and only those with an undisturbed sediment-water interface were used.

The sediment was collected at approximately monthly intervals (fortnightly in the spring period) at each of the 3 stations. The determinations of denitrification activity in the cores were initiated within few hours after sampling. Determinations of the other sediment characteristics were done the next day after storage of the cores at 3 °C.

Water and sediment chemistry. Salinity was measured potentiometrically (Conductivity Meter CDM 3, Radiometer, Copenhagen, Denmark) and a Winkler titration was performed to determine dissolved O_2 concentrations (Strickland & Parsons 1972). Water samples

for NO_3^- and NO_2^- assays were stored frozen (-20 °C) in 25 ml polyethylene vials which contained 50 µl of chloroform. After thawing, the NO_3^- and NO_2^- concentrations were determined colorimetrically by an auto-analyser (Chemlab Instr. Ltd, Essex, England) using the method of Armstrong et al. (1967).

Duplicate determinations of density (specific gravity), water content (weight loss after 24 h at 105 °C), and organic content (ignition loss of dry samples after 3 h at 540 °C) were performed on 1 cm segments of the sediment to a depth of 5 cm.

Assay of denitrification activity. The measurements of denitrification were based upon the acetylene inhibition technique (Sørensen 1978). Acetylene, which inhibits the reduction of N_2O to N_2, is distributed into closed sediment cores and the rate of N_2O accumulation is used as a measure of denitrification. We essentially used the core design and incubation procedure of Andersen et al. (1984). A modification, by which the total N_2O accumulation was determined in whole cores of C_2H_2-treated sediment, was used.

Eight sediment cores were taken for each determination of denitrification activity. The water phase above the sediment was ca 7 cm high (70 ml volume). The tubes were filled completely with bottom water from the locality and gastight acrylic caps were mounted on

top. Small magnetic stirring bars were placed underneath the caps to mix the water phase. C_2H_2-saturated, distilled water (200 µl, 0°C) was injected through a vertical series of silicone-filled holes (i.d. 3 mm) placed at 5 mm intervals along the side of the tubes. The water phase also received C_2H_2-saturated water and the final inhibitor concentration in the porewater and overlying water phase was about 10% of saturation (vol./vol.). The cores were incubated in the dark at in situ temperature using a thermostatted water bath. Incubation time was from 0 to 1.5 h and the cores were sacrificed in duplicate at 20 to 25 min intervals.

After incubation, the exact heights (volumes) of the water and sediment phases in the tubes were recorded. Twenty ml of the water phase was then transferred to a closed serum bottle (40 ml volume) which was pre-evacuated to obtain atmospheric pressure after the water sample was injected. The bottle was shaken vigorously for 2 min to equilibrate the dissolved N_2O with the gas phase. From the latter, duplicate 3 ml gas samples were then taken to evacuated 'Venoject' tubes (Terumo Europe N.V., Leuven, Belgium). After the water samples were removed, the tubes were capped and shaken vigorously for 2 min to equilibrate the remaining N_2O with the 20 ml gas phase. Again, duplicate 3 ml gas samples were transferred to evacuated 'Venoject' tubes. The gas samples were analysed for N_2O on a Packard model 427 gas chromatograph equipped with a ^{63}Ni Electron Capture Detector held at 320°C. A backflush system prevented C_2H_2 contamination of the detector and allowed for a short analysis time (3 min). Gases were separated on a 80/100 mesh Porapak Q column (2 m long and 3.2 mm wide) operated at 80°C. Pure N_2 was used as the carrier gas at a flow rate of 15 ml min^{-1}.

In the headspace analysis, the volumetric solubility coefficients for N_2O at various salinities and temperatures were used to correct for dissolved N_2O in the equilibrations (Weiss & Price 1980). Total N_2O accumulation in the cores was the sum of N_2O in the water sample and the suspension of sediment and water. The cores sacrificed at zero time were to correct for the in situ N_2O content in the sediment. Denitrification rates (mmol N m^{-2} d^{-1}) were calculated as the mean of the N_2O accumulation rates measured in 6 different cores. The detection limit for the assay was estimated to be 0.01 mmol N m^{-2} d^{-1}.

RESULTS

In Figs. 2 to 4, which are presented in the following, the spring bloom of primary production (and sedimentation of algae) is identified from the rapid NO_3^- deple-

tion in the surface water (Fig. 3). The shaded area, which has been inserted in the figures, marks this period (about 1 mo) in the early spring.

Hydrography

During the sampling period surface water temperatures varied from about 0 to 18°C (Fig. 2, upper panel), which is typical of these shallow coastal waters; the bottom water temperature varied only from about 3 to 13°C. Maximum temperature in the bottom water (and at the sediment surface) was reached in September. Thermal stratification was observed in both summer and winter; in summer, the temperature difference was up to 10 C° between surface and bottom waters. In winter, the difference between the warmer bottom water and the cooler surface water was about 3 C°.

The stratification periods were interspersed by periods of mixing during the spring and fall as indicated by both temperature and salinity profiles. During mixing periods, the salinity was typically about 23‰, compared to an overall range of 13 to 27‰ for the surface water and 20 to 33‰ for the bottom water. During stratification, salinity of the bottom water was substantially higher than in the surface water (Fig. 2, lower panel). The mixing periods in both spring (early May in 1985 and mid-April in 1986) and fall (September to December) are also evident from the data.

O_2 and NO_3^- concentrations in the water

Oxygen in the water column was never seriously depleted during the investigation period; in the bottom water, the lowest O_2 concentrations were about 130 µM corresponding to about 50% air saturation (data not shown).

Maximum NO_3^- concentrations (about 10 µM NO_3^-) were found in the winter and minimum concentrations (below 1 µM) in the summer (Fig. 3). The rapid NO_3^- consumption (marked by the shaded area in Fig. 3) in early spring (March–April) and the subsequent low concentrations (below 1 µM) from mid-spring (April–May) to fall (October–November) were explained by assimilation in phytoplankton (primary production) in the area. During a year, NO_2^- concentrations were never higher than 0.5 µM in the water column. Depletion of NO_3^- in the surface water, which marked the period of the spring phytoplankton bloom, thus seemed to occur several weeks earlier in 1986 than in 1985. In both years, the water column was strongly stratified while NO_3^- was being depleted (Fig. 2).
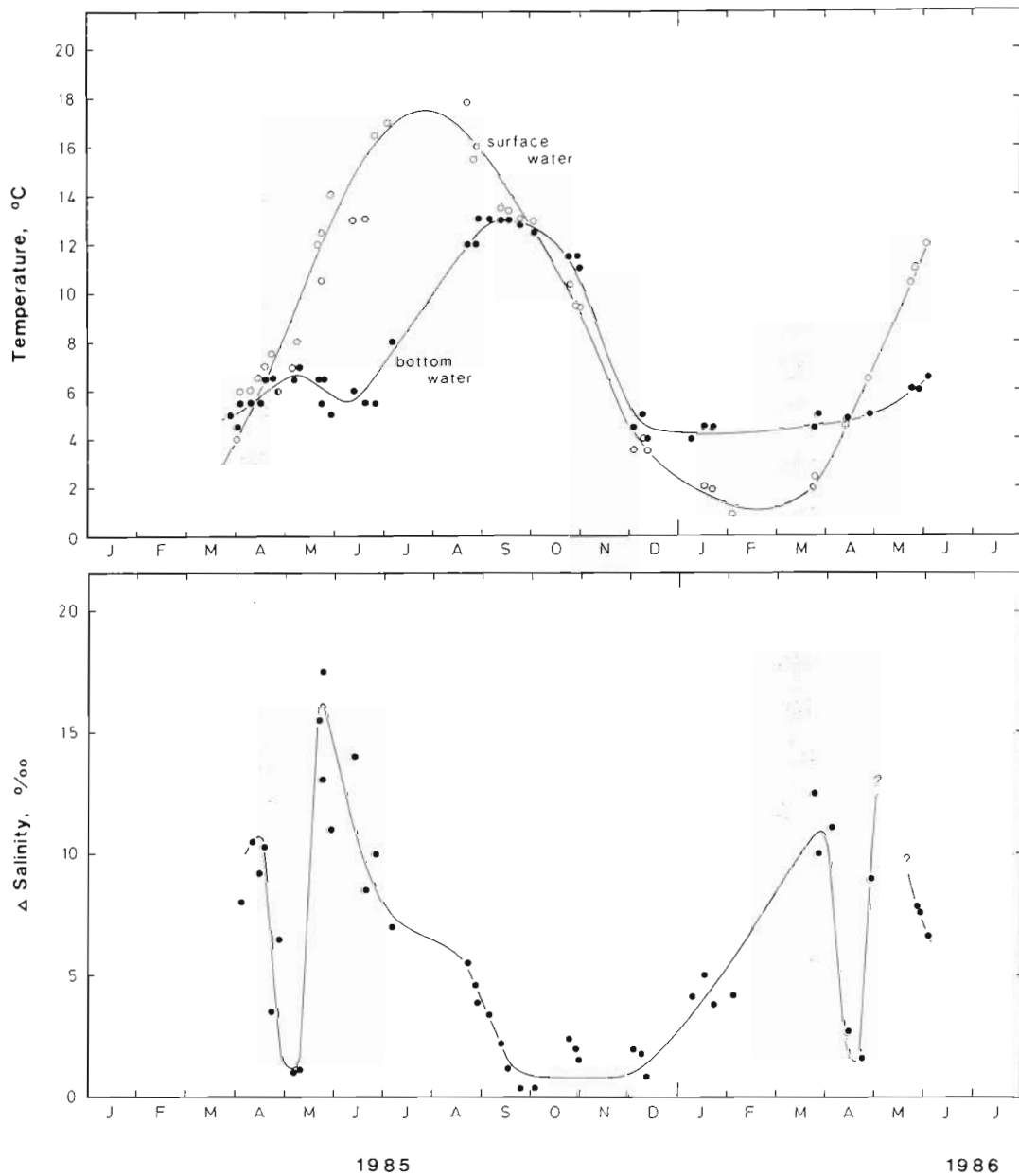
Fig. 2. *Upper panel*: Seasonal variation of temperature in surface water (○) and bottom water (●). *Lower panel*: Seasonal variation of the difference in salinity between bottom water and surface water from Aarhus Bight. Shaded areas indicate sedimentation period for spring phytoplankton bloom

Denitrification in the sediment

Fig. 4 shows the seasonal variation of denitrification activity recorded at the 3 stations. A general pattern was discerned for the process: maximum activities in the spring were followed by decreasing denitrification in early summer and minima were reached in late summer and fall. Peak activities for Stn 10, 16 and 22 were about 0.5, 0.7 and 1.0 mmol N m^{-2} d^{-1} and minimum activities about <0.05, 0.05 and 0.2 mmol N m^{-2} d^{-1}. Standard

errors of the mean ($n = 6$) are indicated; even if the core variability could be high, the seasonal and regional patterns of denitrification activity were still apparent.

Sediment characteristics

Table 1 characterizes the sediments at Stns 10, 16 and 22 by density, water content, and organic content. Stns 16 and 22 had higher water content and contained

Fig. 3. Seasonal variation of NO_3^- concentration in the surface water from Aarhus Bight. Shaded areas indicate sedimentation period for spring phytoplankton bloom
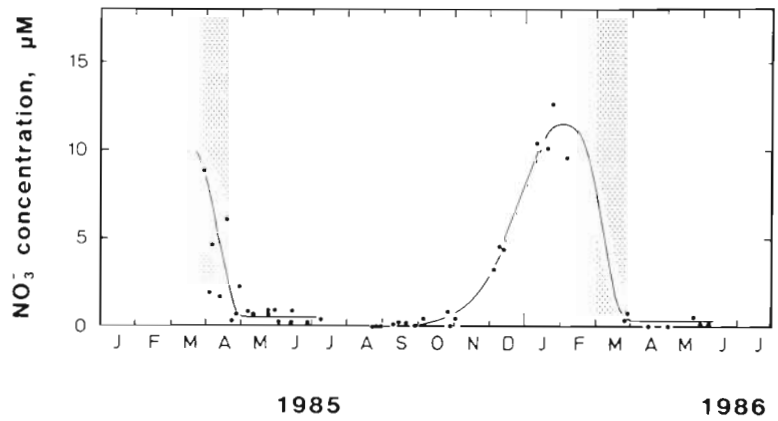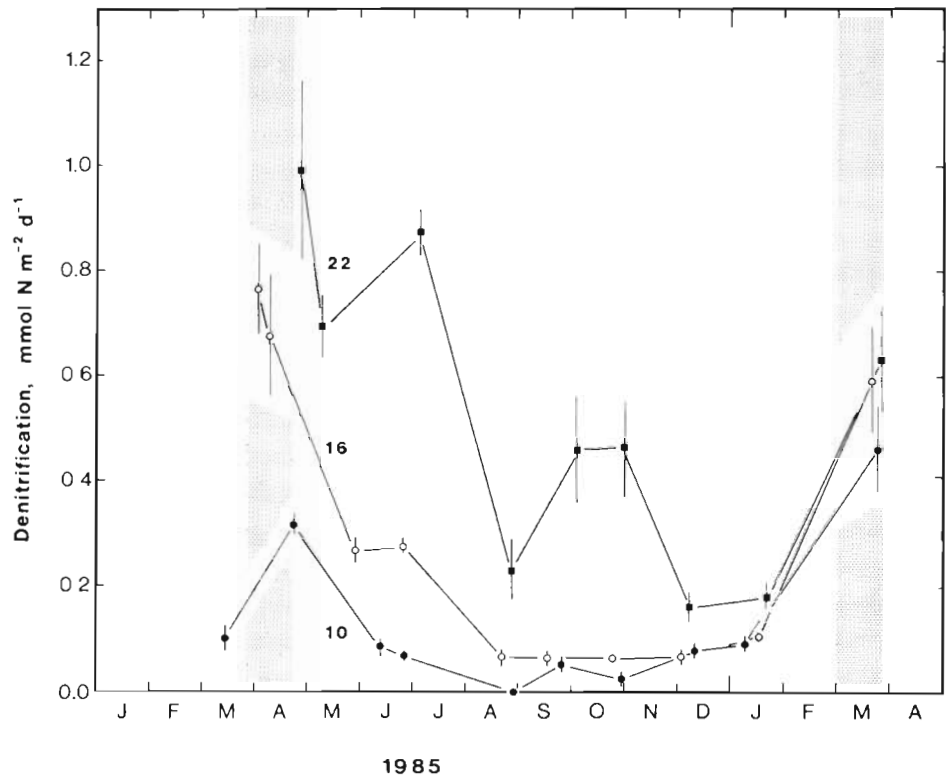
Fig. 4. Seasonal variation of denitrification activity in the sediment from Stns 10, 16 and 22 in Aarhus Bight. Standard errors of the mean ($n = 6$) are indicated. Shaded areas indicate sedimentation period for spring phytoplankton bloom

more fine-grained, organic material than Stn 10 sediment which had more clay and fine sand and less organic matter. Stn 22 had a particularly high organic content in the surface layer. During spring, all stations had a 1 to 2 mm thick, flocculent layer of organic material on the sediment surface, which was evidence for the rapid sedimentation of algal detritus in this period. The flocculent organic material was least pronounced at Stn 10, possibly reflecting lower net sedimentation at this location. The detritus layer sometimes appeared in patches within the 'Haps' cores; similar observations were made in a comparable type of coastal sediment by Christensen & Kanneworff

(1986). This heterogeneity should explain some of the variability between cores from the same sampling event.

DISCUSSION

Spring bloom and detritus sedimentation

The spring bloom of phytoplankton resulted in the rapid NO_3^- consumption in the surface waters during March–April. In these shallow waters, a large fraction of the spring production sinks quickly from the water

Stn	Depth (cm)	Density (g cm^{-3})	Water content (% of wet wt.)	Organic content (% of dry wt.)
	0–1	1.51	41	2.6
	1–2	1.54	37	2.8
10	2–3	1.53	36	2.9
	3–4	1.50	37	3.2
	4–5	1.50	40	3.9
	0–1	1.18	70	9.4
	1–2	1.23	65	8.7
16	2–3	1.24	63	8.5
	3–4	1.24	61	8.2
	4–5	1.25	57	7.6
	0–1	1.13	75	11.4
	1–2	1.18	67	9.5
22	2–3	1.26	63	8.3
	3–4	1.30	57	7.0
	4–5	1.34	51	5.8

and reaches the bottom as easily-degradable detritus. Rapid transport of phytoplankton may also be facilitated by the fact that the water column is stratified (Fig. 2) and that grazing zooplankton are almost absent at this time of year (Smetacek 1985). Others have reported considerable increases in chlorophyll a, phaeopigments and total organic matter in the upper 4 to 6 mm of sediment following the sedimentation of a spring bloom (Christensen & Kanneworff 1986). In spite of the low temperature at this time, benthic metabolism has further been reported to increase immediately after sedimentation of a spring bloom (Graf et al. 1983, Meyer-Reil 1983, Kanneworff & Christensen 1986). The sudden input of fresh organic material to the sediment surface may therefore trigger the onset of rapid mineralisation and release of NH_4^+ which in turn could be converted to NO_3^- by nitrifying bacteria (Kemp et al. 1982, Henriksen & Kemp 1988). Alternatively, if the increased heterotrophic activity (O_2 consumption) results in decreased O_2 penetration into the sediment, nitrification may actually be suppressed; bottom water NO_3^- may in this case be important for denitrification following a spring bloom sedimentation. (Jensen et al. unpubl.)

Denitrification in the sediment

Both in spring and fall, sectioning of C_2H_2-treated cores showed that about 90 % of the N_2O accumulated in either the upper 1 cm segment or in the water phase of the sediments (data not shown). The data demonstrated that denitrification was dependent on either supply of NO_3^- from the bottom water or NO_3^- produc-

tion (nitrification) in the O_2-containing surface layer (Nishio et al. 1983, Jenkins & Kemp 1984).

Various difficulties of the acetylene inhibition technique should be considered in measurements of such surface-located denitrification activity. Koike & Sørensen (1988) have listed a number of potential drawbacks of the assay: (1) Physical disturbance of the surface sediment seems inevitable during the injection of inhibitor solution; this could alter the O_2 and NO_3^- gradients or, in the worst case, wash the compounds completely out of the sediment. (2) Complete inhibition of N_2O reduction may be difficult to obtain immediately after the inhibitor is applied, especially if the C_2H_2 is not rapidly and homogenously distributed during injection. (3) Complete C_2H_2 blockage of N_2O reduction may be difficult to obtain at low NO_3^- concentrations (Kaspar 1982, Oremland et al. 1984) or in the presence of sulfide (Tam & Knowles 1979, Sørensen et al. 1987). (4) Blockage of bacterial NH_4^+ oxidation (nitrification) by C_2H_2 (Hynes & Knowles 1982) could lower denitrification during the incubation due to a depletion of the NO_3^- and NO_2^- pools (Andersen et al. 1984).

The denitrification assay was always based on short-term incubations of cores, and there was no significant difference between the rates determined from the first cores (sacrificed after 20 to 25 min) and those recorded in the last cores (sacrificed after 1.5 h). Therefore, we argue that a constant level of inhibition of N_2O reduction was obtained from the beginning of the incubation period. The constant rate of N_2O accumulation also indicated that the change of the NO_3^- concentrations with time did not significantly affect the denitrification rate, even if nitrification was inhibited and the NO_3^- pool decreased during the incubation. We conclude that the variation of denitrification, which was 5- to 10-fold on a seasonal scale and 2- to 5-fold between the stations, was correct, in spite of the potential drawbacks of the acetylene inhibition technique and the apparent heterogeneity of the sediment.

Seasonal variation of denitrification

The steep increase of denitrification in the sediments in early spring seemed to be directly related to sedimentation of the preceding bloom of phytoplankton. The minor increase in temperature could hardly explain the rapid increase of denitrification between February and April. Most likely, the process was stimulated by the supply of fresh phytodetritus, which served as a suitable organic substrate. It was uncertain, however, if the sedimentation also stimulated nitrification due to increased NH_4^+ release or if nitrification was actually suppressed during this period. In the latter case, the NO_3^- demand for the denitrification maximum

must partly be met by the pool of NO_3^- in the bottom water. The low denitrification in the summer and fall must be a result of lesser sedimentation and limitation by NO_3^-. We did not measure nitrification in the present investigation, but earlier measurements using sediment from the same area have suggested that this activity is indeed relatively low during summer (Hansen et al. 1981, Henriksen et al. 1981). Further, NO_3^- was almost absent in the bottom water during summer and early fall (data not shown).

Regional variation of denitrification

The data reported here comprise one of the first detailed seasonal records of the annual pattern of denitrification in coastal marine sediments. In Norsminde Fjord, which is a NO_3^--polluted estuary adjacent to Aarhus Bight, annual denitrification rates were 1800 mmol N m^{-2} yr^{-1} for the inner estuary (Jørgensen & Sørensen 1988) and 450 mmol N m^{-2} yr^{-1} for the outer estuary (Jørgensen & Sørensen 1985). Based on the rate measurements shown in Fig. 4, the annual denitrification activities in Aarhus Bight were about 35, 70 and 180 mmol N m^{-2} yr^{-1} at Stns 10, 16 and 22, respectively. An earlier indirect estimate of denitrification in the same area gave 130 mmol N m^{-2} yr^{-1}, based on 2 cruises in July and November (Blackburn & Henriksen 1983). Seitzinger et al. (1984) measured N_2 emission (denitrification) in 3 sediments from Narragansett Bay (2 to 5 occasions during 1 yr of investigation); denitrification rates ranged from 0.2 to 2.8 mmol N m^{-2} d^{-1}, a range which is somewhat higher than reported here for the Aarhus Bight (<0.05 to 1.0 mmol N m^{-2} d^{-1}).

As for the seasonal variation, the regional differences in denitrification could relate directly to the sedimentation of the phytoplankton bloom in early spring. The increasing level of activity found at Stns 10, 16 and 22, (in this order) was likely to be a result of a similar increase in the sedimentation rate for organic detritus. It was evident that Stns 10, 16 and 22 (in this order) had an increasing organic content in the surface sediment (0 to 5 cm depth) , and that Stn 22 (and to a minor extent Stn 16) had a particularly high organic content in the uppermost cm.

It was recently shown from large experimental enclosures that a higher nutrient (NH_4^+) loading in the water may lead to higher phytoplankton production and increased denitrification in the sediment (Seitzinger & Nixon 1985). The in situ activities measured in this study also suggest that denitrification increases with higher sedimentation and organic loading of the sediment. It is evident, however, that a continued and heavy organic sedimentation may eventually stimulate

heterotrophic metabolism to such an extent that availability of O_2 for nitrification will be reduced or disappear completely. In case the water column is an insignificant NO_3^- source, it is therefore of prime importance for the maintenance of denitrification in the sediment that nitrification occurs throughout the season.

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