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Influence of carbon availability on denitrification in the central Baltic Sea

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Abstract

Denitrification was investigated in the Baltic proper at two stations with different conditions in the deep water. The Gotland Deep was examined as an example of a basin with anoxic, H_2S-containing deep water and station T was taken as an example of low-oxygen (<0.2 ml liter $^{-1}$), sulfide-free deep water. Denitrification was measured by the acetylene blockage method; in addition, N_2O reduction was followed in samples without acetylene. To shed light on the factors limiting denitrification, we compared in situ rates to denitrification after adding nitrate or electron donors. Denitrification was restricted to the layer of the oxic–anoxic interface in the Gotland Deep and to the water layer near the sediment of station T. For both stations it could be shown that denitrification was not limited by nitrate availability. A lack of available organic C seemed to limit denitrification rates and growth of denitrifiers. As a result of C limitation in the water column, denitrification was restricted to energy-rich interfaces. In the low-oxygen water away from energy-rich interfaces, the less C-demanding nitrification-denitrification coupling ($NH_4^+ \rightarrow N_2O \rightarrow N_2$) seemed to be favored. Denitrification in the water of the central Baltic seems to be subjected to strong variability due to changing C supply during the course of the year. However, limitation by C availability can be assumed for most of the year and should be taken into account in calculating the N budget of the Baltic.

The Baltic Sea as a whole can be described as a big estuary. The Baltic proper represents the largest and southernmost part of it. The Baltic Sea is strongly influenced by human activity insofar as loads of N and P are concerned (Elmgren 1989). The Baltic proper is an ecosystem whose primary production is controlled by N availability (Wulff and Rahm 1988; Graneli et al. 1990). Denitrification, defined as the bacterially mediated process of dissimilatory reduction of ionic nitrogen oxides (NO_3^- and NO_2^-) to gaseous nitrogen compounds (NO, N_2O, and N_2), can be regarded as a major process of elimination of available N (Goering 1985). Thus, denitrification is an important process in counteracting eutrophication (Rönner 1985).

The Baltic proper is characterized by a permanent halocline (60–90 m) that inhibits seasonal vertical mixing deeper than 70 m. The renewal of water masses below the halocline relies on horizontal exchange processes that are discontinuous and, especially below 130 m, rare events. Stagnation periods between deep-water renewal may last up to 10 yr, as was the case before our investigation. During these stagnation periods, oxygen level decreases in the water masses below the halocline and, especially in basins like the Gotland Deep, H_2S from the sediment accumulates in the deep water (Stigebrandt and Wulff 1987).

This investigation was aimed at understanding the factors limiting denitrification in the central Baltic. It is generally accepted that oxygen concentration, the availability of organic C (as electron donor), and NO_3^- (as electron acceptor) are the most important factors controlling the occurrence and rate of denitrification (Hattori 1983). Although NO_3^- and the more reduced nitrogen oxides can all serve as electron acceptors for denitrification, it is assumed that NO_3^- is preferred to the more reduced intermediates of the denitrification path (Goering 1985). The importance of the availability of organic C for the rate of denitrification was pointed out by Liu and Kaplan (1984), who

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showed a strong correlation between the flux of organic particles derived from phytoplankton primary production that reach the denitrification layer and denitrification rates observed in the respective marine ecosystems.

The first investigations of denitrification in the Baltic were done by Rönner and Sörensson (1985) in the western part of the Baltic proper. According to their measurements made in late spring 1980, denitrification seemed to be limited by the availability of NO_3^- and occurred when the oxygen concentration fell below 0.2 ml liter^{-1}. Comparable or lower oxygen values for the onset of denitrification have been reported by many others (*see* Goering 1985).

Our aim was to take a closer look at the factors regulating denitrification in the central Baltic. Emphasis was put on the interaction between electron donor availability and denitrification. For this purpose, two sampling stations were selected that are considered to be representative of the Baltic proper. As shown by Wulff and Rahm (1989), the Gotland Deep (also called station BY 15) is believed to be the most representative station of the Baltic proper; station T is very close to BY 28, which Wulff and Rahm claimed to be representative for the northern part of the Baltic proper. The Gotland Deep was investigated as an example of denitrification in a water column with sulfide-containing deep water and station T as an example of a low oxygen, sulfide-free water column. The period of investigation (midsummer) was more representative of the year's average at least as far as the supply of organic C is concerned (Stigebrandt and Wulff 1987).

To answer the above questions, we located the denitrification zone and determined the in situ rates. These in situ rates were compared to denitrification after addition of NO_3^- and possible electron donors. The acetylene inhibition method was used to quantify denitrification rates. Additionally, reduction of N_2O to N_2 was followed by recording the disappearance of N_2O in samples without acetylene addition. To estimate the organic C available for denitrification, we followed degradation of particulate organic C (POC) and change of

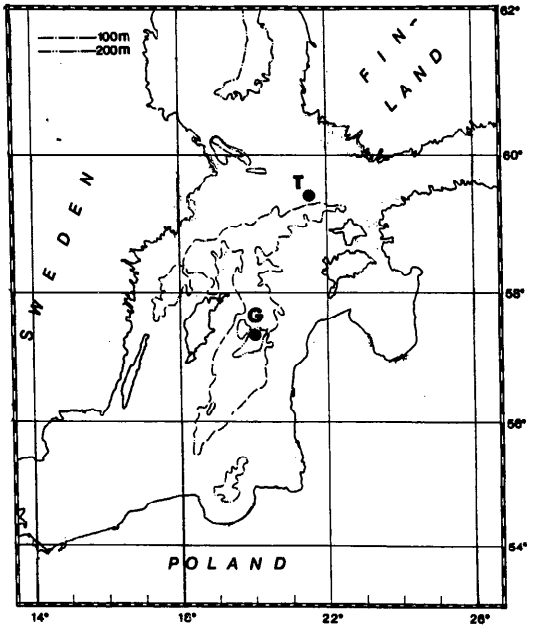

Fig. 1. Positions of the sampling stations in the Baltic proper. G—Gotland Deep; T—station T.

the nitrogenous compounds during a 100-d incubation period. We also followed microbiological background parameters, total bacterial numbers, and the number of denitrifying bacteria as detected by N_2O production and N_2 formation.

Material and methods

Sampling and field measurements—Samples were taken aboard RV *Poseidon* from 15 to 19 August 1986 and aboard RV *Aranda* from 29 to 31 July 1987 in the Gotland Deep (=BY 15) and at station T (close to BY 28). Positions of the Gotland Deep (57°20.0′N, 20°03.0′E) and of station T (59°25.0′N, 21°30.0′E) are shown in Fig. 1. Water was collected in 5-, 10-, and 30-liter Niskin PVC bottles for all purposes, except for samples used to count saprophytes and denitrifying bacteria. For the latter, sterile champagne bottles mounted on modified ZoBell samplers were used. Salinity and temperature was determined by a CTD probe. Oxygen was measured by the Winkler method as described by Grasshoff (1983). NH_4^+ was determined by the indophenol blue method as modified by Koroleff (1983).

NO_3^- was reduced by a Cu-Cd column to NO_2^- and determined as NO_2^- as outlined by Grasshoff (1983). H_2S was determined photometrically by the methylene blue method as modified by Fonselius (1983). The above methods are specified in "Methods of seawater analysis" (Grasshoff et al. 1983). The parameters were analyzed by the scientific crew of the respective ships directly after sampling.

Determination of denitrification rate—Rates of denitrification were determined by the acetylene blockage method (Balderston et al. 1976; Yoshinari and Knowles 1976). The method used was a modification of those described by Rönner and Sörensson (1985) and Andersen et al. (1984). Samples were filled in 120-ml glass serum bottles directly after sampling, with overflowing to reduce oxygen contamination. Bottles were closed immediately with a Teflon-coated butyl rubber septum (3 mm) in an aluminum cap and crimped. This procedure allowed air-bubble-free and airtight containment of the sample. To create space to introduce acetylene to the sample, we replaced 10 ml of water by the same volume of nitrogen (N_2, 5.0 ml). We added 18 ml of acetylene [C_2H_2 (2.6 ml) in cylinder, purified and controlled for $PH_3 < 5$ ppm by the manufacturer] to each sample. Acetylene was added directly to the water phase with a syringe after inverting the bottle. Solution of acetylene was accelerated by vigorous shaking during and after acetylene addition. We had a final concentration of 15% acetylene in the water sample. An increased acetylene concentration of 15% compared to 10% used by other investigators (e.g. Rönner and Sörensson 1985) was done to ensure inhibition of N_2O reduction even in the presence of H_2S, which was of special relevance at the oxic–anoxic interfaces (Brettar and Rheinheimer 1991). We did not observe any influence of this increased acetylene addition on any of the processes followed during incubation of the samples. Gases were supplied by Messer-Griesheim. Samples were incubated at 4.8 ± 0.5°C in the dark for 2, 4, 6, and 12 d. Addition of substrates (NO_3^-, Na_2S, $Na_2S_2O_3$, Na-acetate, glucose) was always done before introduction of gases to the sample. For comparison, parallels were always run without added acetylene.

N_2O measurement—For N_2O measurements, we withdrew gas samples from the headspace by a gastight lockable syringe after equilibrating the sample for 15 min at 20°C in a shaking water bath. Samples were immediately frozen after this procedure and stored for later analysis (e.g. NO_3^-, NO_2^-, NH_4^+). Gas samples were either directly injected into a gas chromatograph or stored for later analysis in evacuated vials (4-ml Vacutainer, Becton Dickinson). N_2O concentrations were quantified on a gas chromatograph (model 438A, Packard Instr. Co., Inc.) with an electron capture detector (10 mCi ^{63}Ni) operated at 320°C. Separation was done by injecting 1 ml of the gas sample on a stainless steel Poropak Q (80/100 mesh) column (3 m), at 60°C and a gas flow of 18 ml min^{-1}. The carrier gas was an Ar/CH_4 (95 : 5, vol/vol) mixture.

The standard deviation for N_2O determinations after storage of the samples in evacuated vials was better than 2% for concentrations >30 nmol $liter^{-1}$, better than 5% above 10 nmol $liter^{-1}$, and better than 9% above 5 nmol $liter^{-1}$. The detection limit was 3 nmol $liter^{-1}$. The N_2O measurements were calibrated against standard N_2O mixtures provided by Messer-Griesheim and Alltech Europe. These gases were compared to standards provided by the Frauenhofer Institut für atmosphärische Umweltchemie. The N_2O concentrations in the water were calculated according to Weiss and Price (1980). To calculate the saturation value in the water sample, we used a value of 300 ppbv N_2O as the mean air value according to the mean air concentration close to the surface of the Baltic as determined by Rönner (1983*a*).

Bacterial parameters—Total counts of bacteria were done under an epifluorescence microscope after staining with acridine orange according to Zimmermann et al. (1978). All samples were fixed in 2% formaldehyde.

Saprophytes were grown aerobically on yeast extract-peptone agar medium ZoBell 2216E (Oppenheimer and ZoBell 1952) prepared with natural seawater diluted to 8‰

salinity. Colony-forming units (CFU) were counted after incubation for 14 d in the dark at 20°C.

Denitrifying bacteria were grown in a nutrient-broth-nitrate medium modified from Sreenivasan and Venkatarman (1956); it had the following composition: meat extract (Merck), 3.0 g; Bacto-peptone (Difco), 5.0 g; KNO_3, 2.0 g; aged seawater, 250 ml; deionized water, 750 ml; pH 7.35±0.05. The medium was filled into Hungate tubes; Durham tubes were added to observe gas formation. The inoculum ranged from 0.001 to 10 ml, achieved in three parallels in 1986 and five parallels in 1987. Tubes were incubated for 5 weeks in the dark at 20°C. As a criterion for denitrifiers, gas formation in the Durham tubes was observed and N_2O in the headspace was measured gas chromatographically. Gas formation was attributed (most likely) to N_2 production, as CO_2 production was quantified concomitantly. Sampling of gases in the headspace was done after shaking and equilibration of the liquid medium with the gas phase. As gas sample (1 ml) was analyzed for N_2O and CO_2 by the same method as described above for N_2O measurement, but with a lower detector temperature of 300°C that decreased the detection limit for CO_2 to 0.8% vol/vol. There was no interference between the measurements of CO_2 and N_2O under the running conditions used. The concomitant measurement of CO_2 enabled us to recognize gas production due to CO_2 production. Data evaluation was done according to the MPN tables of de Man (1975).

Particulate organic C—The POC content of water samples was determined after filtering 1 liter of water through a 25-mm glass-fiber filter (Whatman GF/F, precombusted at 450°C). The sample was combusted at 960°C in a Perkin-Elmer CHN analyzer.

The change of the POC content was followed during a 100-d incubation of 1-liter samples (in 1-liter glass bottles, acid-rinsed, with screwcap equipped with Teflon-coated butyl rubber septum) after adding 500 μmol liter^{-1} $NaNO_3$. Filling of the bottles and incubation was done as described for determination of in situ denitrification. Because no acetylene was added to the samples, denitrification was followed by measuring NO_2^-, NO_3^-, NH_4^+, and N_2O concentrations.

This high amount of NO_3^- was added to compare our results to the findings of Rönner and Sörensson (1985), where a high percentage of the added 500 μmol liter^{-1} was reduced to N_2 or NO_2^- during a 4-week incubation period. Furthermore, we wanted to make sure that NO_3^- was not used up during the long incubation period, to enable a comparison of the amount of NO_3^- reduced and the amount of POC consumed.

Statistical analyses—All calculations were done with the Statgraphics statistical graphics system (Statistical Graphics Corp.). All regressions (least-squares) were calculated with a 95% confidence limit (=inner line shown in graphs, outer line = 95% prediction limits).

Results

In situ measurements—The depth profiles of physical, chemical, and microbiological parameters are summarized in Figs. 2 and 3. Profiles of the Gotland Deep obtained in 1986 and 1987 were quite similar and the one shown is typical for summer (Rheinheimer et al. 1989).

The water column was characterized by two density gradients: the thermocline at 20 m and the halocline at 60–90 m. The thermocline formed the lower boundary of the layer of phytoplankton primary production (Gocke 1989).

At both stations, oxygen was close to saturation level (>80%) in the water above the halocline. O_2 decreased strongly in the halocline. Oxygen deficiency was met only in water samples of the Gotland Deep that contained H_2S. Lowest O_2 levels in water samples without H_2S were >0.35 ml liter^{-1} in the Gotland Deep. In the Gotland Deep, H_2S concentrations increased from the oxic–anoxic interface toward the sediment; in 1987 this increase was almost linear. The water of station T displayed a low oxygen layer from 100 m down to the sediment with oxygen concentrations from 0.20 to 0.09 ml O_2 liter^{-1}. H_2S could not be detected.

In general, NO_3^- was low in the upper 10 m and reached only low concentrations

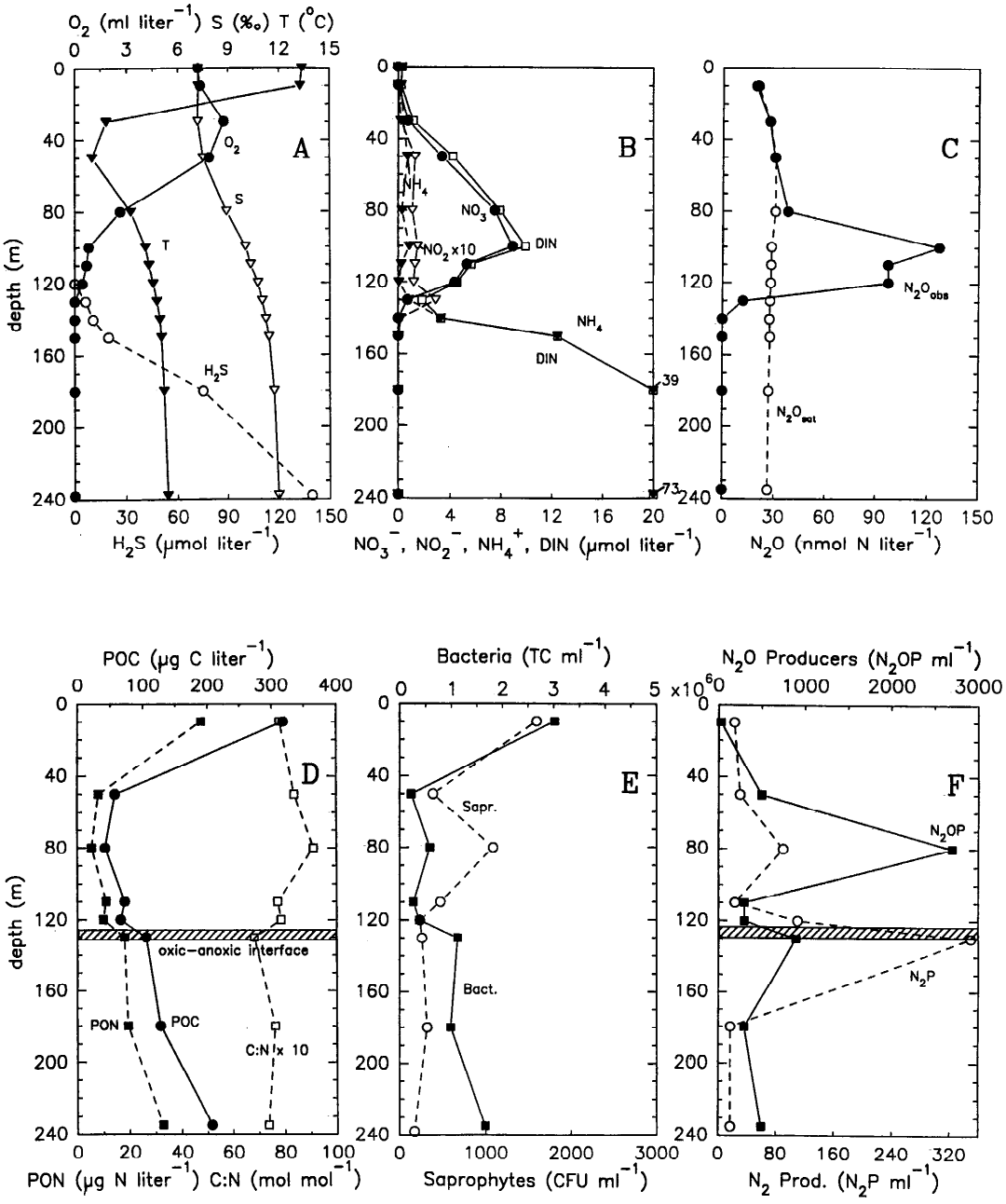

Fig. 2. Depth profiles of hydrographical, chemical, and microbiological parameters for the Gotland Deep, 29–31 July 1987. A. Oxygen, salinity, temperature, and H_2S. B. Vertical distribution of NO_3^-, NO_2^-, NH_4^+, and DIN (=sum of NO_3^- + NO_2^- + NH_4^+). C. Observed N_2O concentration (N_2O_{obs}) and calculated saturation level (N_2O_{sat}). D. POC and PON distribution. E, F. Distribution of total bacterial numbers (TC), saprophytes, and denitrifying bacteria, detected as N_2O-producing (N_2OP) and N_2-producing bacteria (N_2P).

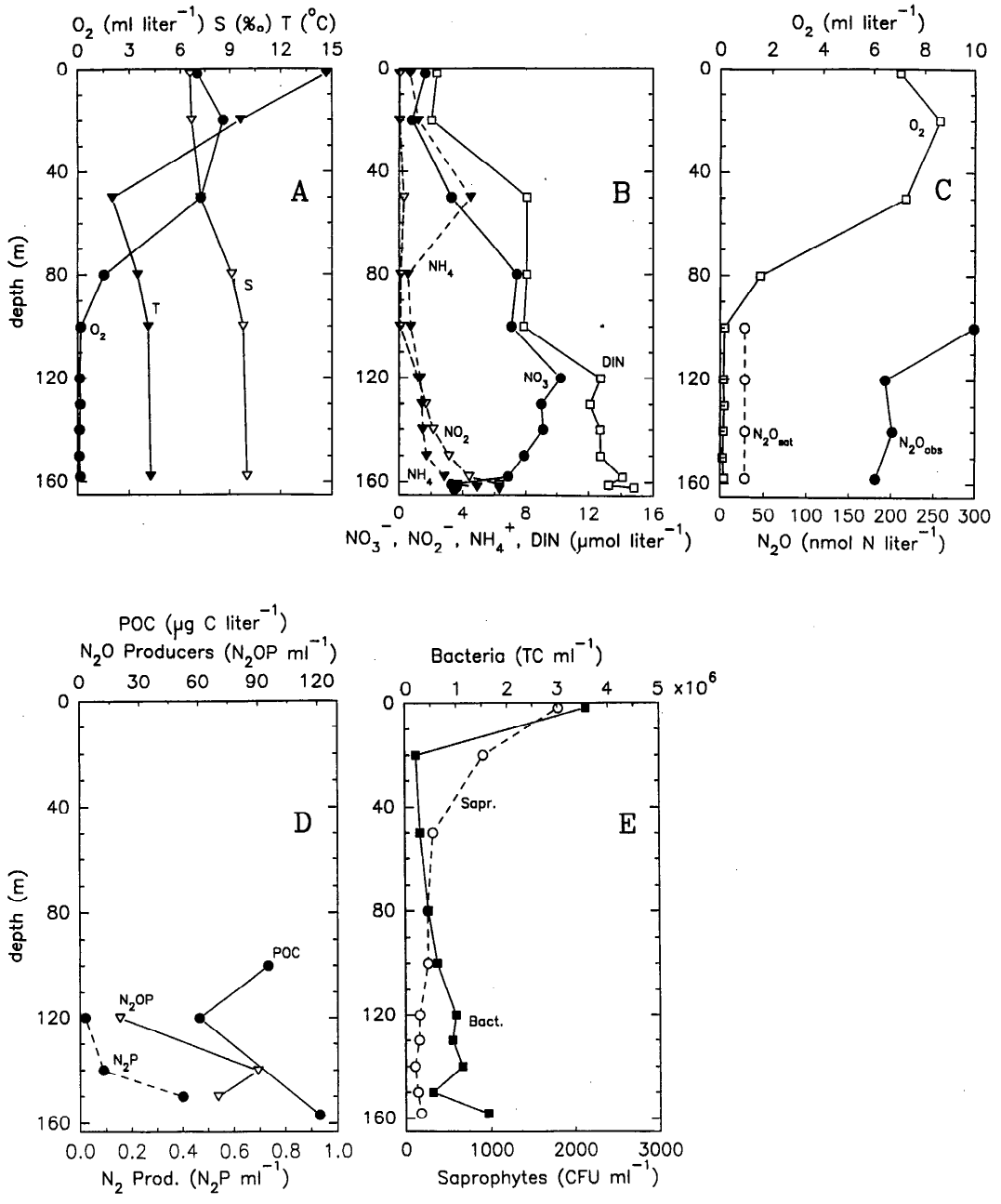

Fig. 3. As Fig. 2, but for station T, 19 August 1986.

above the halocline. Within and below the halocline NO_3^- increased up to 11 µmol liter^{-1}. In the Gotland Deep, typically a pronounced peak was observed above the oxic–anoxic interface, followed by a strong decline in the interface layer. In the water column of both stations, NO_3^- concentrations were always highest in the water that was low in oxygen. In the Gotland Deep, low oxygen water showed with a significant decrease of NO_3^- above the H_2S layer. At the top of the H_2S layer concentrations of NO_3^-

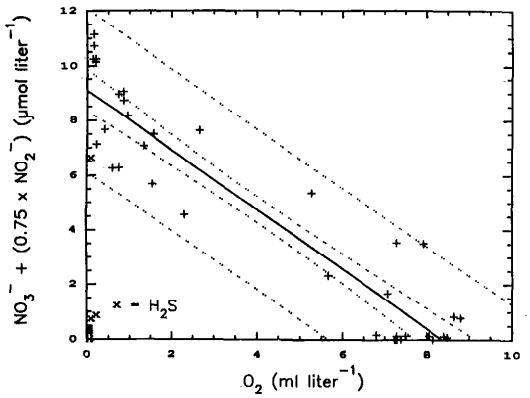

Fig. 4. Plot of oxygen vs. $NO_3^- + (0.75 \times NO_2^-)$. The linear regression for data from the Gotland Deep 1986 and 1987 and station T gave a correlation coefficient of $r = -0.937$ ($a = 9.09$, $b = 1.086$). In this calculation, values from samples with H_2S were omitted but their positions are indicated.

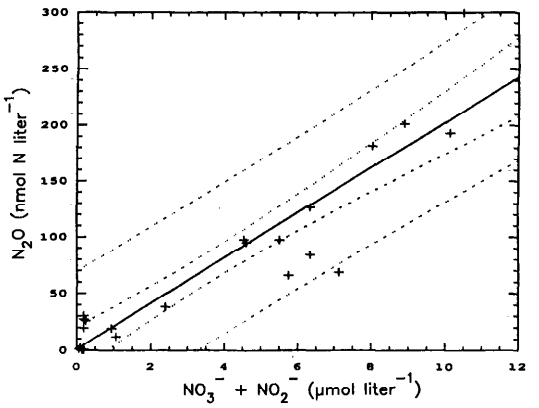

Fig. 5. Linear regression of N_2O on $NO_3^- + NO_2^-$ ($r = 0.923$; $a = 1.19$, $b = 20.159$). Data source as in Fig. 4.

were very low in the presence of low concentrations of H_2S. NO_3^- disappeared with increasing H_2S concentrations (>10 µmol liter^{-1}) in the deeper part of the anoxic layer.

In the Gotland Deep NO_2^- showed only low concentrations. At station T there was a marked increase of NO_2^- in the layer of low oxygen water toward the sediment—up to 6.3 µmol liter^{-1} at 2 m above the sediment (samples taken with a bottom-water sampler).

The correlation between oxygen and the nitrification products NO_3^- and NO_2^- can be seen from the plot of $NO_3^- + NO_2^-$ vs. O_2 when data from both stations were used (Fig. 4). NO_2^- was multiplied by the factor 0.75 because of the lower oxygen demand for oxidation of NH_4^+ to NO_2^- compared to oxidation to NO_3^-.

Nitrification occurred in the low oxygen water as long as no H_2S was present. All samples from the low oxygen water of both stations showed increased NO_3^- concentrations during incubation. Additionally, samples from station T showed a decrease of the endogenous NO_2^- concentration with a concomitant increase of NO_3^- concentration (data not shown).

The NH_4^+ concentrations were usually very low in the oxic part of the water column. Higher concentrations occasionally occurred below the euphotic zone and in the halocline. In the Gotland Deep high concentrations occurred in the anoxic part of the water column, where NH_4^+ showed an increase similar to that of H_2S toward the sediment. A minor increase toward the sediment was also seen at station T.

The sum of NO_3^-, NO_2^-, and NH_4^+ that represents the major fraction of the dissolved inorganic N (DIN) showed a stepwise increase toward the sediment. In the Gotland Deep this increase was always interrupted by a pronounced minimum in the zone of the oxic–anoxic interface. Maximum concentrations were always observed above the sediment. In 1987, the Gotland Deep displayed much higher DIN concentrations above the sediment than those observed for both stations in 1986. DIN concentrations are given here as potential indicators for layers of pronounced nitrogen utilization (e.g. due to strong nitrogen assimilation in the euphotic layer or to denitrification).

N_2O concentrations were close to the atmospheric saturation value in the water column above the halocline. Supersaturation was always found in the water of the lower part of the halocline and below the halocline. In the Gotland Deep maximum values of N_2O saturation were 450% at 100 m in 1987 and 330% at 90 m in 1986. N_2O decreased to 43% (1987) and 70% (1986) of saturation at the oxic–anoxic interface and was depleted in the anoxic deep water. At

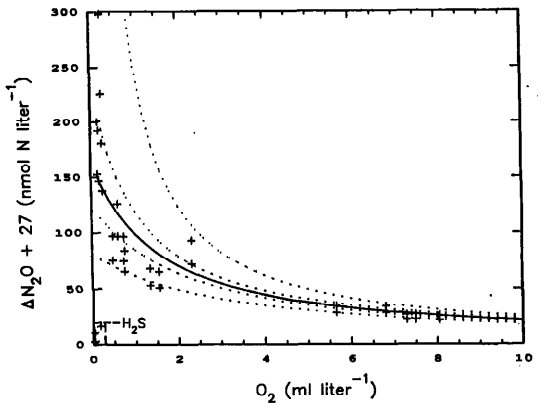

Fig. 6. Reciprocal regression of ΔN_2O (=measured value minus calculated saturation value) on O_2. For the regression shown, 27 nmol N_2O-N liter^{-1} was added to all ΔN_2O values to avoid negative values in the regression. 27 nmol N_2O-N liter^{-1} was the mean of the calculated saturation values of all water samples used in the regression. Values of H_2S-containing samples were not used in the calculation, but their positions are indicated. The correlation coefficient was $r = 0.978$ ($a = 6.10 \times 10^{-3}$, $b = 4.05 \times 10^{-3}$; for comparison without addition of the mean N_2O saturation value: $r = 0.962$; $a = 5.45 \times 10^{-3}$, $b = 4.59 \times 10^{-3}$). Data source as in Fig. 4.

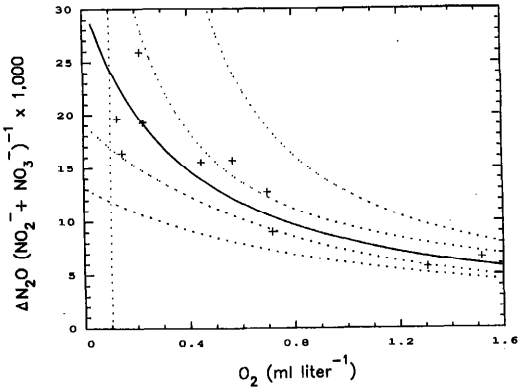

Fig. 7. Reciprocal regression of the ratio of ΔN_2O: (NO_2^- + NO_3^-) on O_2 for the low oxygen water (<2.0 ml O_2 liter^{-1}) below the halocline ($r = 0.937$; $a = 0.032$, $b = 0.089$). Data source as in Fig. 4.

station T the low oxygen water showed a pronounced supersaturation of N_2O—1,050% at 100 m and decreasing to 640% at 157 m. As an overall tendency, N_2O increased with increasing NO_3^- + NO_2^- concentration (Fig. 5) and showed a decrease with increasing actual oxygen concentration. This relationship could be described by a reciprocal regression (Fig. 6). As with decreasing oxygen concentrations, the production of N_2O as nitrification product increases (Goreau et al. 1980); the ratio of N_2O vs. the sum of NO_2^- + NO_3^- was plotted (Fig. 7). For the low oxygen water below the halocline the ratio increased from 0.6% at 1.6 ml O_2 liter^{-1} to ~2.5% at 0.1 ml O_2 liter^{-1}.

Concentrations of POC in the Gotland Deep were between 43 and 318 μg C liter^{-1} in 1987 (Fig. 2D) and between 75 and 446 in 1986. Maxima were always observed in the euphotic zone and in the water near the sediment. Values were always lowest in the oxic water layer between the euphotic zone and oxic–anoxic interface. The C:N ratio (mol C:mol N) had a maximum of 9.1 at the bottom of the halocline (80 m) and a minimum of 6.8 at the oxic–anoxic interface. At station T, POC concentrations (values available only for the low oxygen water between 100 and 157 m) ranged between 61 (120 m) and 121 μg C liter^{-1} (157 m) (Fig. 3). The C:N ratio of the 157-m sample was 9.6.

Bacterial numbers were in the range of 0.1–4.4 × 10^6 cells ml^{-1}, with the maximum in the euphotic zone. Less pronounced peaks were in the water layer near the sediment (~1.8 × 10^6) and, in the Gotland Deep, at the oxic–anoxic interface (~1.6 × 10^6 cells). Bacterial numbers were always lowest in the oxic water with oxygen higher than 0.2 ml O_2 liter^{-1} below the euphotic zone. The mean values for the water samples from this part of the oxic layer of both stations ranged between 2.1 and 3.2 × 10^5 cells ml^{-1}.

The numbers of aerobically growing saprophytes always reached highest values in the euphotic zone. Peaks in the halocline were less pronounced. Lowest values were usually recorded in the low oxygen water (<0.2 ml O_2 liter^{-1}) of station T and in the anoxic water of the Gotland Deep.

In the Gotland Deep, heterotrophic N_2O-producing bacteria that grew in nutrient-broth-nitrate medium (N_2OP in Fig. 2F) ranged from 26 to 2,400 bacteria ml^{-1}. In 1987, they showed a pronounced maximum at the bottom of the halocline and another increase was noticed at the oxic–anoxic in-

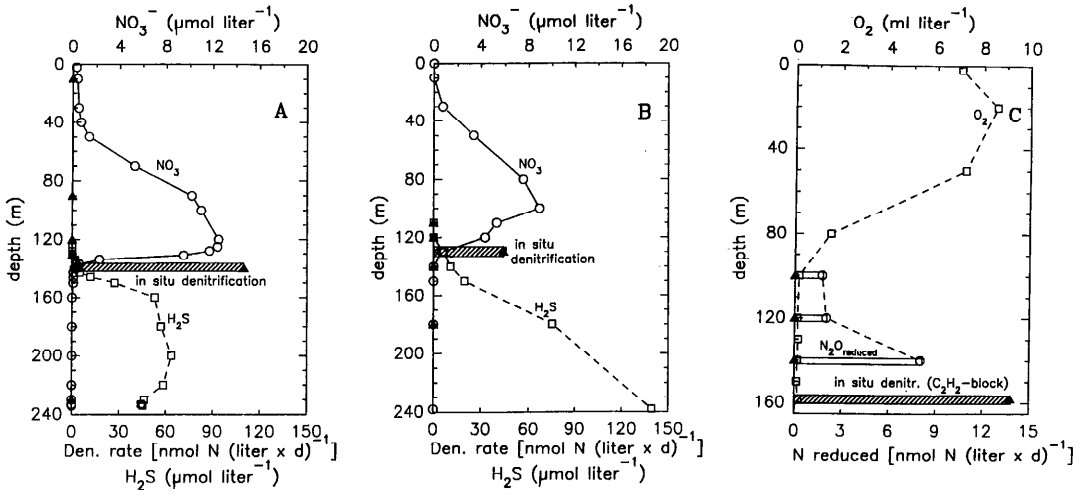

Fig. 8. In situ denitrification for the Gotland Deep in summer 1986 (A) and 1987 (B) and for station T (C) in summer 1986. For station T, rates of N_2O reduction are also shown.

terface. A strong decrease was observed in the low oxygen water between the halocline and chemocline. N_2-forming bacteria (N_2P) ranged from 17 to 350 bacteria ml^{-1}. The most pronounced maximum was at the oxic–anoxic interface. Counts from 1986 showed a similar trend for N_2O-producing and N_2-forming bacteria as that in 1987 (Brettar 1991). For station T, data are available only for samples from the low oxygen layer (*see Fig. 3D*): between 120 and 150 m numbers of N_2O-producing bacteria ranged from 20 to 90 ml^{-1}, and the number of N_2-forming bacteria increased with depth from undetectable (<0.03 ml $liter^{-1}$) to 0.4 ml^{-1}.

In situ denitrification—Denitrification was measured in the water of the Gotland Deep and station T with emphasis on low oxygen water below the halocline. By means of the acetylene blockage method, denitrification could be detected only at the oxic–anoxic interface of the Gotland Deep (1987: 130 m, 1986: 140 m) and in the water near the sediment (157 m) at station T (Fig. 8). The denitrifying samples from the Gotland Deep contained concomitantly low concentrations of H_2S and NO_3^- and came out of a layer with high rates of CO_2 dark fixation (Gocke 1989). The rates of denitrification were higher in interface samples from the Gotland Deep (1986: 110 nmol $liter^{-1}$ d^{-1}; 1987: 44 nmol $liter^{-1}$ d^{-1}) than in samples from water near the sediment at station T (1986: 13.5 nmol $liter^{-1}$ d^{-1}). Rates were calculated from incubation periods of 4 d. For interface samples it was shown that linearity of the denitrification rates was given for incubation periods up to 4 d (Brettar and Rheinheimer 1991). The endogenous NO_3^- was in no case used up in a 4-d period.

The water layer low in oxygen and far from the sediment (100–140 m) at station T did not show any denitrification detectable by the acetylene blockage method. In this layer, reduction of N_2O in samples without added acetylene was observed, whereas samples with acetylene added did not show a significant change of N_2O concentration. N_2O reduction was quantified after an incubation period of 12 d. In the upper part of this low oxygen water layer, reduction of the N_2O present was very low (~2 nmol N $liter^{-1}$ d^{-1}). Samples from 140 m showed a higher reduction rate of 8 nmol N $liter^{-1}$ d^{-1} (Fig. 8C).

Denitrification after nitrate addition—Denitrification after NO_3^- addition was measured in all samples where in situ denitrification was measured (Fig. 9). This comparison should indicate if the availability of NO_3^- had an influence on the rate of denitrification in samples where in situ denitrification occurred or on extension of the denitrification layer.

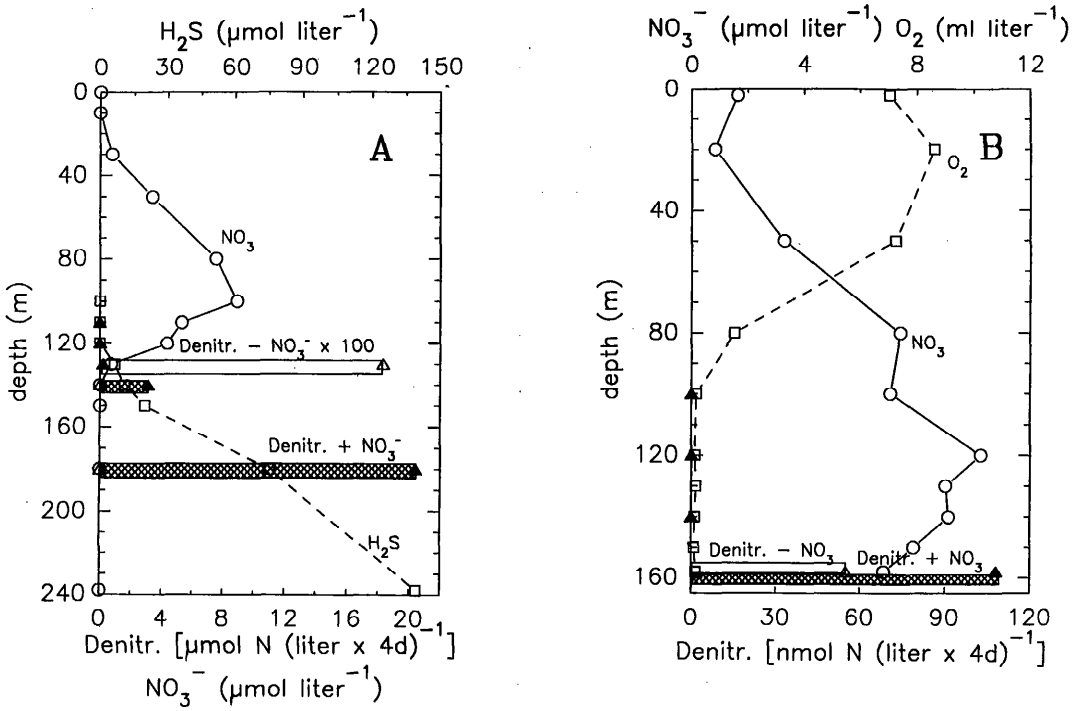

Fig. 9. Denitrification with and without added NO_3^- after a 4-d incubation. Data shown are from the Gotland Deep in 1987 (A) and station T in 1986 (B).

Denitrification was enhanced only in samples from water near the sediment from station T (Fig. 9B). The in situ rate was doubled by adding 50 µmol NO_3^- liter^{-1}, although in situ denitrification consumed <1% of the endogenous NO_3^- within 12 d. In the Gotland Deep, no enhancement of denitrification was observed.

Samples from the sulfide-free water of the Gotland Deep or station T that did not show in situ denitrification (=without any addition except acetylene) never showed denitrification after NO_3^- was added, which was also the case for incubation periods up to 12 d for samples with acetylene added or incubations up to 100 d without added acetylene.

In contrast, sulfide-containing and NO_3^--free samples from the anoxic water of the Gotland Deep showed denitrification after NO_3^- was added. The amount of NO_3^- finally reduced increased with increasing H_2S concentration (*see Fig. 9A*).

Denitrification after addition of electron donors—Denitrification in water samples from the oxic–anoxic interface of the Gotland Deep (1987) was strongly enhanced by adding H_2S, $S_2O_3^-$, and acetate as electron donors plus NO_3^-. The interface samples displayed a very strong increase in denitrification rate within 2 d at 4.5°C after adding the above substrates. This increase was not observed when NO_3^- was added without electron donors. The utility of reduced sulfur compounds as electron donors for denitrification was shown in more detail by Brettar and Rheinheimer (1991).

Water samples from the nondenitrifying water far from the sediment (120 m) at station T were tested for denitrification after adding glucose + NO_3^-, acetate + NO_3^-, and NO_3^- only. Figure 10 shows the reaction of the samples during an 8-d incubation period with and without added acetylene. No change of the nitrogenous compounds NO_2^-, NO_3^-, NH_4^+, and N_2O was observed during the first 4 d of incubation for all assays. After 8 d, samples with acetate and

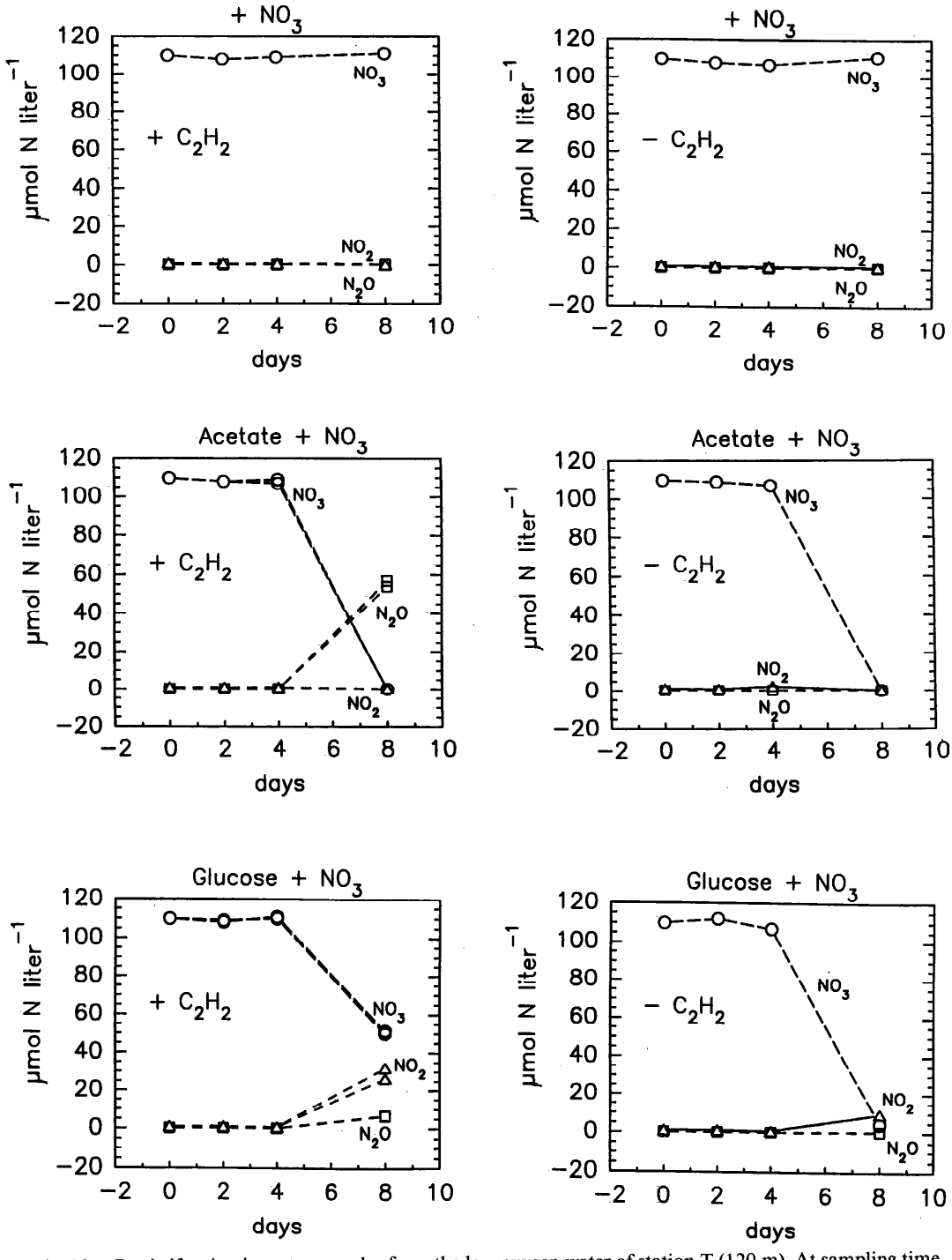

Fig. 10. Denitrification in water samples from the low oxygen water of station T (120 m). At sampling time, concentrations were 0.14 ml O_2 liter^{-1}, 1.4 µmol NO_2^- liter^{-1}, 8.7 µmol NO_3^- liter^{-1}, and 0.6 µmol NH_4^+ liter^{-1}. After a 2-d preincubation phase, 150 µmol acetate + 100 µmol NO_3^-, or 50 µmol glucose liter^{-1} + 100 µmol NO_3^- liter^{-1}, or 100 µmol NO_3^- liter^{-1} only were added. During the preincubation phase NO_3^- had increased to 9.8 µmol liter^{-1}, NO_2^- decreased to 1.0 µmol liter^{-1}, and denitrification was not detectable. Assays with C + acetylene added were done as duplicates; results of both parallels are shown (same line and point symbols used for duplicates).

Table 1. Initial POC concentration (POC^0), POC change (ΔPOC), NO_2^- increase (ΔNO_2^-), initial O_2 concentration (O_2^0), and O_2 consumption (ΔO_2^{POC}, calculated for POC degradation) for samples from the low oxygen water incubated for 100 d. Values given are means of duplicates.

Sta.	Depth (m)	POC^0 (μg C liter^{-1})	ΔPOC (μg C liter^{-1})	ΔNO_2^- (μmol liter^{-1})	O_2^0 (ml liter^{-1})	ΔO_2^{POC} (ml liter^{-1})
Gotland	120	74.7	−39.0	0	1.30	−0.15
Gotland	130	82.7	−33.4	0	0.71	−0.12
Gotland	140	84.9	+42.6	+3.2	0.13	—
T	120	60.6	−4.1	0	0.14	−0.02

glucose added showed denitrification. No denitrification could be detected in samples where only NO_3^- was added. The fact that denitrification was much faster after adding acetate than with glucose was obviously due to the added acetylene, as the comparison of the samples with and without acetylene reveals. Acetylene obviously had a retarding effect on samples with glucose added.

POC degradation in low oxygen water samples—The observation that there was no denitrification without added electron donors in samples away from interfaces was also confirmed by the results from incubation of 1 liter of water from the low oxygen water of the Gotland Deep (1986: 120 m, 130 m, 140 m) and of station T (120 m) for 100 d. These samples were identical to those where in situ denitrification (with acetylene added) was measured. No acetylene, but 500 μmol NO_3^- liter^{-1}, was added. Except for the interface sample (Gotland, 140 m) where formation of 3.2 μmol NO_2^- liter^{-1} was observed, monitoring of the nitrogen oxides did not give any hint on reduction of the added NO_3^-.

Changes of POC during the 100-d incubation period are listed in Table 1. As the bacterial biomass in the filtrate of the GF/F filters never exceeded 1 μg C liter^{-1}, different retainments of the bacterial cells on the filter due to changes of the mean cell volume during incubation should not have introduced a major error to the POC measurements. The low oxygen samples from the Gotland Deep showed changes in POC content of between 33 and 39 μg C liter^{-1}. The interface sample showed an increase, rather than a decrease, of the initial POC content of 85 μg C liter^{-1}. The sample from station T (120 m) showed only a minor decrease from 60.6 to 56.5 μg liter^{-1} within 100 d. With the simplifying assumption that the decrease of POC means degradation to CO_2, the amount of oxygen consumed or NO_3^- reduced was calculated from the difference in POC content according to Liu and Kaplan (1984) (1 μmol POC consumed = 1 μmol O_2 consumed or 0.8 mol NO_3^- reduced to N_2 or 2 mol NO_3^- reduced to NO_2^-).

According to these assumptions and conversion factors, the decrease of POC observed (degradation assumed) in the low oxygen water, samples from above the interface (Gotland Deep) may have consumed 2.75 and 3.25 μmol O_2 liter^{-1}, respectively, within 100 d. The oxygen concentration would have been lowered to 1.15 ml O_2 liter^{-1} in the 120-m sample and to 0.59 in the 130-m sample. If the POC degradation was used for denitrification, 2.6 (120 m) and 2.2 (130 m) μmol NO_3^- liter^{-1} could have been reduced to N_2. For samples from the low oxygen water of station T (120 m), the amount of POC that disappeared could have been used for a maximum oxygen consumption of 0.34 μmol liter^{-1}, thereby reducing the oxygen concentration to 0.12 ml liter^{-1} or for a maximum reduction of 0.27 μmol NO_3^- liter^{-1} to N_2.

Discussion

Restriction of denitrification to electron donor-rich interfaces—Measurements in the Gotland Deep and station T showed that denitrification was restricted to interfaces like the oxic–anoxic and the sediment–water. These interfaces seemed to be the only places where conditions were appropriate for denitrification.

As shown by NO_3^- addition experiments at both stations, NO_3^- was not the factor that restricted extension of the denitrification zone. An increase of the denitrification

rate was observed only with NO_3^- addition to water near the sediment at station T. Consumption of <1% of the endogenous NO_3^- in the samples without NO_3^- added indicates that the NO_3^- concentration obviously had a kinetic control (higher NO_3^- concentration needed for saturation of the enzymes involved than the actual NO_3^- concentration), but was not the limiting factor in denitrification.

Limitation of denitrification by available organic C in water far from the sediment—The availability of organic C seemed to be the factor that restricted denitrification to electron donor-rich interfaces. This influence was most obvious for the low oxygen water at station T. Although NO_3^- and oxygen concentrations were adequate for denitrification from 100 m down to the sediment (163 m), denitrification was detectable by the acetylene blockage method only in water near the sediment (157 m). Water far from the sediment did not seem to provide enough organic C for denitrification.

C limitation is also indicated by the experiments with water samples far from the sediment (120 m) of station T where adding acetate or glucose, but not NO_3^- alone resulted in denitrification. The long lag phase of >4 d until denitrification started can be interpreted as the absence of an actively denitrifying microflora. Probably only a few of the denitrifiers responsible for the final denitrification were present at the beginning of the incubation, and the long lag phase was due to the time needed for growth of the denitrifiers. This assumption is supported by the low number of N_2O- and extremely low number of N_2-producing bacteria detected in the relevant sample as well as in the whole of the low oxygen water of this station. A reason for the low numbers of denitrifiers in the water far from the sediment at station T could be a longer lasting supply low in organic material. Low uptake rates for glucose, acetate, and lactate were reported by Rheinheimer et al. (1989) and Gocke (1989) for the whole oxic water column between the euphotic layer and chemocline of the central Baltic. These findings may hint at low availability of organic C. A low C supply is also indicated by the low POC degradation during a 100-d incubation period.

The dependence of the number of denitrifiers on the availability of organic C was already shown by Tiedje (1988). It could be assumed that a longer lasting low C supply in the low oxygen water of station T resulted in the synergistic effect of reducing the denitrifying microflora and limiting the denitrification rate by supply of C that was too low.

A similar situation in terms of low C supply could also occur in the low oxygen water between the halocline and the chemocline of the Gotland Deep: similar low POC content, a pronounced decrease of the numbers of denitrifying bacteria, and the finding that denitrification did not occur within 100 d of incubation may demonstrate the comparability of the situation. Occasional input of organic C provided by the high chemosynthetic activity of the interface layer (Gocke 1989) may be responsible for the higher availability of POC in this layer compared to the low oxygen water of station T.

Nitrification-denitrification coupling as an energetically more favorable alternative in C-poor environments—There was no reduction of NO_3^-, NO_2^-, or NO that resulted in production of N_2O in the presence of acetylene in samples of water far from the sediment at station T. We conclude therefore that no denitrification in the classical sense starting from NO_3^- or NO_2^- occurred. In contrast, reduction of N_2O with rates increasing toward the sediment could be seen (*see Fig. 8C*). In the following we will differentiate between N_2O reduction and denitrification. The term denitrification is used to define the reduction of NO_3^-, NO_2^-, and NO, resulting in detection of N_2O with the acetylene inhibition method. N_2O reduction describes the process of N_2O reduction in the absence of acetylene.

Coincidence of the inability to denitrify NO_3^- and NO_2^- and the ability of N_2O reduction under conditions of low C supply substantiated the hypothesis that reduction of N_2O could be energetically more favorable per mole of electrons consumed than the reduction of the less-reduced nitrogen oxides. According to our hypothesis that no

Table 2. Changes of free energy ($\Delta G^{0\prime}$) for the reduction of different nitrogen oxides. All calculations refer to pH 7.0.

Substrates	Products	$\Delta G^{0\prime}$ (kcal) (per mol H_2)	$\Delta G^{0\prime}$ (kcal) (per mol N)
Modified from Thauer et al. 1977:			
$NO_3^- + H_2$	$NO_2^- + H_2O$	−39.0	−39.0
$NO_2^- + 0.5\ H_2 + H^+$	$NO + H_2O$	−35.0	−17.5
$2NO + H_2$	$N_2O + H_2O$	−73.2	−36.6
$N_2O + H_2$	$N_2 + H_2O$	−81.6	−40.8
$2NO_3^- + 2H^+ + 5H_2$	$N_2 + 6H_2O$	−53.6	−133.9
For comparison:			
$O_2 + 2H_2$	$2H_2O$	−56.7	
According to Delwiche 1978:			
$2NO_3^- + 2H^+ + 5H_2$	$N_2 + 6H_2O$	−53.6	−134.1
$2NO_3^- + 2H^+ + 4H_2$	$N_2O + 5H_2O$	−46.7	−93.4
Nitrate ammonification:			
$NO_3^- + 4H_2 + 2H^+$	$NH_4^+ + 3H_2O$	−37.3	−149.0

microflora was present in water far from the sediment that was able to denitrify under the given conditions, we further assume that the bacteria that reduced N_2O were not denitrifying bacteria. The ability to reduce N_2O must not necessarily have been coupled to the capability to reduce NO_3^- or NO_2^-.

Under conditions of C limitation, reduction of N_2O should be thermodynamically more favorable than reduction of the less-reduced nitrogen oxides, as can be seen from the free energy changes (according to Thauer et al. 1977) (Table 2). Reduction of N_2O provides double the amount of energy per electron consumed than reduction of NO_3^- or NO_2^-. In this case energy is calculated for pH 7.0 with H_2 as electron donor. To our knowledge, there are no known bacteria that can convert this higher free energy change to a higher ATP yield than that obtained from reduction of the less-reduced nitrogen oxides (i.e. NO_3^-, NO_2^-, and NO). The preferred reduction of N_2O observed in the water column suggests that there were bacteria present able to utilize it. Perhaps they are "normal" aerobic bacteria, with an additional ability to use high concentrations of N_2O, especially when oxygen is low. Such an organism could use the higher energy yield usually obtained by aerobic degradation of organic compounds compared to denitrification (Boogerd et al. 1984; Koike and Hattori 1975) and additionally being more versatile in the case of low oxygen concentrations. N_2O reduction is probably relevant only in environments with low oxygen and high N_2O concentrations. It may provide an energetically favorable path to use high N_2O concentrations at a given low C supply.

The high N_2O concentrations are likely to be the product of nitrification. A hint on that could be the linear increase of the N_2O concentration with increasing $NO_3^- + NO_2^-$ concentrations (*see Fig. 5*). Also, the increasing ratio of N_2O vs. the sum of the nitrification products NO_3^- and NO_2^- with decreasing oxygen concentrations is in the same range as that reported for *Nitrosomonas* sp. by Goreau et al. (1980). For *Nitrosomonas* sp. the ratio increased from ~0.6% at 1.6 ml liter^{-1} to 10% at 0.13 ml liter^{-1}. For the same range of oxygen concentrations the ratio increased from 0.6 to 2.5% in the low oxygen water below the halocline (*see Fig. 7*). The lower ratio for the lower oxygen concentrations may have several causes, starting with the physiology of the present nitrifiers. However, as consumption processes by N_2O-reducing organisms have been shown to occur, it is most likely that these reduction processes will counteract N_2O accumulation.

Nitrification as the most important source for nitrous oxides has been pointed out by Hahn (1981) for the oceans, and a dominant role for the Baltic was shown by Rönner

Table 3. Rates, carbon demand, and extension of different denitrification mechanisms in the central Baltic. Key: 1—Gotl.—Gotland Deep (BY 15); T—station T. 2—Time period of investigation. 3—*Denitrification mechanism:* H_2S-den.—denitrification at the oxic-anoxic interface with H_2S as electron donor; den. NO_3^--denitrification with organic C, starting from NO_3^- or NO_2^-; N_2O-red.—N_2O reduction observed as N_2O decrease without acetylene addition. 4—Assumed most important electron donor: H_2S—H_2S and(or) other reduced sulfur compounds; *Org C—organic C.* 5—*Limiting factor of* denitrification: don.—electron donor; acc.—electron acceptor. 6—Depth (1) and estimated extension (2) of denitrification layer. 7—Denitrification rates: a—nmol liter^{-1} d^{-1}; b—mmol m^{-2} d^{-1}. 8—Method for quantifying denitrification rate: C_2H_2—acetylene blockage method; N_2O-decr.—observation of N_2O reduction in samples without acetylene addition; NO_3^--cons.—NO_3^- conservation method according to Shaffer and Rönner 1984. 9—C demand for denitrification of the measured or calculated rate in the part of the water column under consideration: 1—C demand for denitrification of NO_3^- to N_2; 2—C demand for denitrification of NO_3^- to N_2O; 3—C demand for reduction of N_2O to N_2. Value 2 was only calculated when denitrification was measured by acetylene blockage method, where N_2O is the final product of denitrification. For rates here it is more likely that value 2 is the more correct value, because of electron donor limitation of denitrification. For the data of Rönner and Sörensson value 1 should be more accurate, because of electron acceptor limitation.

1	2	3	4	5	6	7		8	9
						Den. rate			Org C
Sta.	Date	Mech.	e-don.	Limit. factor	Depth (1), ext. (2) (m)	a	b	Meth.	(mg C m^{-2} d^{-1})
Data from this work:									
Gotl.	16 Aug 86	H_2S-den.	H_2S	don.	(1) 135–145 (2) 10	110	1.1	C_2H_2	(1) 16.5 (2) 13.2
Gotl.	30 Jul 87	H_2S-den.	H_2S	don.	(1) 125–135 (2) 10	44	0.44	C_2H_2	(1) 6.6 (2) 5.3
T	19 Aug 86	den. NO_3^-	Org C	don.	(1) 150–163 (2) 13	14	0.18	C_2H_2	(1) 3.1 (2) 2.5
T	19 Aug 86	N_2O-red.	Org C	don.	(1) 95–135 (2) 40	2	0.08	N_2O-decr.	(3) 0.24
T	19 Aug 86	N_2O-red.	Org C	don.	(1) 135–150 (2) 15	8	0.12	N_2O-decr.	(3) 0.36
Calculated from data of Rönner and Sörensson 1985:									
BY 31	24 May–14 Jun 80	den. NO_3^-	Org C	acc.	(1) 100–440 (2) 340	264 (250–300)	90.0	C_2H_2	(1) 1,350 (2) 1,080
BY 38	24 May–14 Jun 80	den. NO_3^-	Org C	acc.	(1) 70–108 (2) 38	80 (10–200)	3.0	C_2H_2	(1) 45.6 (2) 36.5
According to model calculations of Shaffer and Rönner 1984:									
Baltic proper	For yearly averages				Sediment		1.79	NO_3^--cons.	(1) 26.8
					Water column	36		NO_3^--cons.	

Table 3. Continued.

For comparison, oxygen consumption at different depths and C consumption below different isohalines (modified from Rahm 1987)

	S (‰)	Depth (m)	O_2 consumption (mmol liter^{-1} d^{-1})	Org C (mg C m^{-2} d^{-1})
	9	85	138	62
	10	106	69	31
Baltic proper	11	140	17	14
Data from 1957–1982				

(1983*a*). That even strong nitrification by marine bacteria is possible under low oxygen (0.1 ml liter^{-1}) concentrations has been reported by Carlucci and McNally (1969). For the low oxygen water of the Baltic proper, high rates of nitrification have been reported by Enoksson (1986) and have also been observed as NO_3^- increase during incubation of the water samples during this investigation.

The relevance of the reduction of N_2O produced by nitrification was first demonstrated by Codispoti and Christensen (1985). They showed that this mechanism can play a major role for nitrogen transformations at the boundary of oxygen-deficient waters (0.1 ml liter^{-1}) in the eastern tropical South Pacific Ocean.

Changing C supply in the course of the year as followed by corresponding denitrification rates—In this paper we drew a picture of C-limited denitrification in the central Baltic. Our findings are in contrast with those of Rönner and Sörensson (1985). Their investigations, made in late spring 1980, indicated NO_3^--limited denitrification that concerned much more extended parts of the water column, usually from below the halocline down to the sediment. Their rates were 2–10 times as high as those reported here.

Calculations of the organic C needed as electron donor for the denitrification rates measured in the Baltic proper are given in Table 3. Calculations are based on the assumption that denitrification of 1 mol NO_3^- consumes 1.25 mol C_{org} for reduction to N_2 or 1 mol C_{org} for reduction to N_2O, as suggested by Liu and Kaplan (1984). Table 3 demonstrates that a high C supply in the water is necessary to sustain the high rates and the large denitrification layers reported by Rönner and Sörensson (1985). In contrast, rates and extension of the denitrification layers reported here demand a C supply that is less by 1–3 orders of magnitude. Rahm (1987) calculated the C consumption in a depth profile of the Baltic. His calculations suggest that the maximum amount of C available in the relevant depths for denitrification is about an order of magnitude lower than that needed for the rates measured by Rönner and Sörensson.

As demonstrated by these calculations, the most likely explanation for the different findings by Rönner and Sörensson (1985) and this study should be the different C supply in the central Baltic during the course of the year. Late spring—the investigation period of Rönner and Sörensson—is the time period that displays the highest C supply in the water column due to sedimentation of the spring bloom (Elmgren 1989). Our investigation period in the end of July to mid-August usually shows a much lower C supply in the water. This situation is more representative for most times of year (Stigebrandt and Wulff 1987). A high C supply in late spring may explain the higher rates, the more extended denitrification layer, and the observed NO_3^- limitation of denitrification. Because the low C supply is the typical situation for most of the year, a C-limited denitrification, restricted to the interfaces, is likely to be more representative for the year's average. Splitting the year into short periods of high denitrification and long-lasting periods of low denitrification can result in a C demand for denitrification that is in the range of Rahm's (1987) calculations. Additionally, it explains the high N loss observed in the deep water of the Baltic proper (Rönner 1983*b*; Shaffer and Rönner 1984).

The phenomenon of C-limited denitrification during a large portion of the year should be taken into account in calculating future denitrification capacities in the Baltic. On the basis of the observed C limitation, it cannot be assumed that a continual increased anthropogenic N load will be compensated by denitrification.

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