Shift from denitrification to anammox after inflow events in the central Baltic Sea

M. Hamnig
Department of Biological Oceanography, Baltic Sea Research Institute Warnemünde, Seestraße 15, 18119 Rostock, Germany

G. Lavik and M. M. Kuypers
Nutrient Group, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany

D. Woebken
Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany

W. Martens-Habbena
Paleomicrobiology Group, Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Carl-von-Ossietzky Strasse 9-11, 26111 Oldenburg, Germany

K. Jürgens
Department of Biological Oceanography, Baltic Sea Research Institute Warnemünde, Seestraße 15, 18119 Rostock, Germany

Abstract

Incubation experiments with 15N-labeled compounds (NO3− and NH4+) were performed during three cruises (2002, 2004, and 2005) to study the loss of inorganic N as dinitrogen gas (N2) via denitrification and anammox in the water column of the Gotland Deep (central Baltic Sea). 15N incubations did not provide evidence for direct conversion of NO3− to N2 (heterotrophic denitrification) in the suboxic (O2 < 10 μmol L−1 sulfide-free waters. Substantial denitrification rates (up to 2.7 μmol L−1 d−1 N2) were measured in water samples collected from the NO3− -H2S interface (redoxcline) in 2002 and in water from the sulfidic zone in 2004, which indicates chemolithotrophic denitrification as the dominant N-loss process in both years. Massive inflows of oxygenated North Sea water from 2002 to 2003 caused a complete ventilation of the Baltic Sea with high oxygen concentrations in the Gotland Deep bottom water. After the reestablishment of the redoxcline in 2004, a newly formed suboxic zone above sulfidic waters—with NO3−, NO2−, and NH4+ at the detection limit—was observed in spring 2005. The development of this zone was associated with a severalfold increase in reduced and oxidized manganese and with a shift from denitrification to anammox as the main N-loss process. Fluorescence in situ hybridization analysis confirmed the presence of anammox bacteria and the number of anammox cells was consistent with the observed N2 production rates in 2005.

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The Baltic Sea is the world’s largest brackish water system and, as a semiclosed sea, it is surrounded by highly industrialized countries with over 80 million inhabitants in its drainage area. Hence, the Baltic Sea is strongly influenced by anthropogenic input of nutrients such as phosphorus and nitrogen. The main sources of biologically available nitrogen in the Baltic Sea are (1) nitrogen fixation, converting molecular dinitrogen (N2) into organic nitrogen; (2) terrestrial input; and (3) the water exchange with the North Sea (Voss et al. 2005). The episodic inflow of North Sea water with higher salinity into the Baltic Sea causes ventilation of the generally anoxic basins, such as the Arkona, Bornholm, and Gotland. Stagnation periods between those renewal events may last up to 10 yr and because of the limited water exchange, H2S mainly diffusing from the sediments accumulates in the deeper basins (Nausch et al. 2003). The last deepwater renewal that reached the Gotland Deep (250 m, central Baltic Sea) took place during 2002 and 2003 (Feistel et al. 2004) and pushed the suboxic–sulfidic interface (redoxcline) back into the sediments.
The water column of the Gotland Deep is characterized by biogeochemical gradients formed from a strong and stable halocline and influenced by internal currents. The halocline is localized at about 60–70 m depth and prevents vertical mixing of deep and surface waters. During stagnant periods, oxygen decreases below the halocline and suboxic \((O_2 < 10 \, \mu\text{mol} \, \text{L}^{-1})\) conditions are prevalent toward the redoxcline. Substantial loss of inorganic nitrogen (ammonium \([\text{NH}_4^+]\), nitrate \([\text{NO}_3^-]\), and nitrite \([\text{NO}_2^-]\)) (Brettar and Rheinheimer 1991), high microbial activity (Höfle and Brettar 1995), and high \(\text{CO}_2\) dark fixation rates (Labrenz et al. 2005) are characteristic for the suboxic–sulfidic interface of the Gotland Deep. Manganese (Mn) and iron (Fe) are important both as electron donor and electron acceptor in redox processes at the redoxcline (Neretin et al. 2003). In the suboxic zone, Mn(II) is oxidized mainly by microorganisms to particulate Mn oxide \((\text{MnO}_2)\), which sinks through the redoxcline and can then be responsible for various oxidation processes (Neretin et al. 2003). Furthermore, occasional inflows of dense seawater into the basins result in the recycling of manganese from the sediments into the water column (Heiser et al. 2001).

Respiratory reduction of \(\text{NO}_3^-\) to \(\text{N}_2\) by heterotrophic denitrifiers was regarded to play a major role in the nitrogen budget of the Baltic Sea because it counteracts eutrophication (Römer 1985). A recent study, which investigated the distribution and diversity of denitrifying bacteria in the water column of the Gotland Deep by functional gene-based analysis (\(\text{ nirS}\)), revealed a low diversity in suboxic waters and a change in the community across the suboxic–sulfidic interface (Hannig et al. 2006). Activity of denitrifiers in the Gotland Deep waters, measured by the acetylene method, was restricted to a narrow zone at the redoxcline (Brettar and Rheinheimer 1991). This N loss was attributed to the activity of chemolithotrophic denitrifiers, which are assumed to use \(\text{NO}_3^-\) for the oxidation of reduced sulfur compounds. The importance of these organisms was supported by recent investigations showing that chemolithoautotrophic denitrification in the Gotland Deep’s redoxcline is catalyzed mainly by epsilonproteobacteria (Brettar et al. 2006), which are closely related to \(\text{Sulfurimonas denitrificans}\) (formerly \(\text{Thiomicrospira denitrificans}\)) (Takai et al. 2006). These denitrifiers could also be enriched from the suboxic–sulfidic interface by \(\text{NO}_3^-\) and thiosulfate additions (Labrenz et al. 2005).

Recently, anaerobic ammonium oxidation (anammox), which combines \(\text{NO}_3^-\) and \(\text{NH}_4^+\) to produce \(\text{N}_2\), was discovered as an alternative pathway for the loss of inorganic nitrogen (Van De Graaf et al. 1995). Anammox, a process found originally in wastewater treatments, was also identified in natural environments such as marine sediments (Dalsgaard and Thamdrup 2002), oxygen minimum zones (Kuypers et al. 2005), and anoxic fjords/basins (Dalsgaard et al. 2003; Kuypers et al. 2003), as well as in Artic sea ice (Rysgaard and Glud 2004). It is carried out by a few anaerobic, autotrophic bacteria belonging to the order \(\text{Planctomycetales}\) (Strous et al. 1999). So far, all identified but yet uncultivated anammox bacteria in marine systems belong to the genus \(\text{Scalindua}\) (Kuypers et al. 2006). Molecular methods and stable isotope approaches give us the possibility to verify both the presence and the activity of anammox bacteria in marine environments.

Our main objective in this study was to assess the role of denitrification and anammox in the N loss from suboxic and anoxic waters of the Gotland Basin. Therefore, \(^{15}\text{N}\)-labeling experiments were applied during three cruises in the period from 2002 to 2005, which covered the major inflow event of North Sea water into the Baltic. Associated with this ventilation event, we recorded a shift in N-loss processes for the water column. Our results imply that the vertical location and temporal dynamics of denitrification and anammox in the water column are strongly affected by changes in physicochemical conditions resulting from deepwater renewal events.

Materials and methods

Study site and nutrient analysis—Water samples were obtained from the 250-m-deep water column at the Baltic Sea monitoring station 271 (57°19′2″N, 20°03′E) in the Gotland Deep (Fig. 1) during cruises with the RV \textit{Professor Albrecht Penck} (July 2002 and August 2004), the RV \textit{Alexander von Humboldt} (August 2003), and RV \textit{Alkor} (May 2005). Profiles of temperature, conductivity, oxygen \((\text{O}_2)\), fluorescence, and turbidity were obtained by CTD (Sea-Bird Electronics). Water samples were collected with FreeFlow bottles attached to the CTD-rosette system (Hydrobios). In 2002, nitrate \((\text{NO}_3^-)\), nitrite \((\text{NO}_2^-)\), ammonium \((\text{NH}_4^+)\), and phosphate \((\text{PO}_4^{3-})\) concentrations (detection limits 0.1, 0.03, 0.3, and 0.1 \(\mu\text{mol} \, \text{L}^{-1}\), respectively) were determined on board with an autoanalyzer (TRAACS 800; Bran & Luebbe) immediately after sampling. In 2004 and 2005, nutrients were measured manually. Ammonium was determined by the indophenol blue method; \(\text{NO}_3^-\) was reduced by a copper cadmium column to \(\text{NO}_2^-\) and determined as \(\text{NO}_2^-\) photometrically (Grass-
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15N incubations and analysis—The denitrification process—both hetero- and autotrophic—combines two NO₃⁻ ions to form one molecule of N₂. Because all suboxic water samples contained 15NO₃⁻ in addition to the added 15NO₂⁻, denitrification produced 14N15N, 14N15N, and 15N15N through random isotope pairing. In contrast, anammox combines one NO₂⁻ and one NH₄⁺ ion, which results in the production of 14N14N and 14N15N in incubation experiments with 15NO₂⁻ or 15NH₄⁺ without the production of 15N15N (Thamdrup and Dalsgaard 2002).

For 15N incubations we slightly modified the method previously published by Dalsgaard et al. (2003). On the basis of the chemical profiles, specific depths throughout the redoxcline were selected for incubations and the water for the incubations was sampled by a second CTD-rosette cast. During the 2002 cruise, the 15N incubations were performed in 250-mL serum bottles without degassing, only by adding 10 μmol L⁻¹ Na15NO₃ or 15NH₄Cl (>99% purity; Campro Scientific). In 2004 and 2005, samples were flushed with argon and ~0.5% of carbon dioxide for 20 min to reduce the background of N₂ gas to increase the sensitivity of the measurement and to maintain the in situ pH as well as the alkalinity of the water samples. All incubations were started immediately after sampling by adding 5 μmol L⁻¹ 15N-labeled compounds to the 250-mL bottles. Generally, water was transferred from the 250-mL bottles into 12-mL Exetainers (Labco) and incubated for up to 72 h at 10°C in the dark. At different time intervals (e.g., 6, 12, 24, and 48 h) biological activity was stopped by adding 0.1 mL of a 50% ZnCl₂ solution and samples were subsequently stored at room temperature. The isotope ratio (14N14N, 14N15N, and 15N15N) of the headspace was determined by gas chromatography–isotope ratio mass spectrometry (VG Optima, Micromass) by direct injections from the headspace. The concentrations of the produced 14N15N and 15N15N were assessed as excess relative to air and the N₂ production rates were calculated from the slope of increase (Thamdrup and Dalsgaard 2002). The production of 15NH₄⁺ from 15NO₃⁻ was also analyzed as N₂ after the chemical conversion of NH₄⁺ with NaOBr into N₂ (Warembourg 1993).

Fluorescence in situ hybridization (FISH) analysis—Water samples (40 mL) for analysis of anammox bacteria by FISH were collected from the same FreeFlow bottles as the samples for the 15N incubations in 2005. The samples were fixed with formaldehyde (1.85% final concentration) for 2 h at 4°C, filtered on polycarbonate filters (0.2-μm pore size), dried, and stored at −80°C until processing. For enumeration of anammox bacteria, hybridization was done with a mixture of Alexa 488-labeled oligonucleotide probes BS-820 (5’T-GGATCTCTTCTATATTGGCCC-3’; Kuyper et al. 2003; Biomers.net) and BS-820-C (5’T-CATCCCTCTTACTAGTGCCC-3’; Hamersley et al. 2007) specific for the Candidatus Scalindua sorokinii and Candidatus S. wagneri clade. Total cell counts on the basis of staining with 4,6-diamidino-2-phenylindole (DAPI) and relative proportions of hybridized bacteria were determined by epifluorescence microscopy (Glockner et al. 1996). From each hybridized filter, 20 fields of view were examined and counted. To check for unspecified staining and autofluorescence the probe Non338 (Wallner et al. 1993) was used.

For photographic documentation we used the catalyzed reporter deposition FISH (CARD-FISH) protocol (Pernthaler et al. 2002). The same probe mixture as described above as well as the general anammox probe Amx368 (Schmid et al. 2003) were used. Generally, the CARD-FISH protocol is a two-step procedure: The hybridization of horseradish peroxidase (HRP)-labeled probes with the 16S ribosomal ribonucleic acid (RNA) and the subsequent catalyzed reporter deposition of fluorescently labeled tyramides. The hybridization step is nearly identical to the standard FISH procedure. In the second step, the HRP catalyzes the deposition of a large number of fluorescently labeled tyramides inside the hybridized cells. Because of this signal amplification CARD-FISH results in a greatly enhanced sensitivity compared with the standard FISH protocol.

Results

Biogeochemical profiles—During our first cruise in July 2002, the Baltic basins were characterized by a pronounced stratification of the water column after an 8-yr period without deepwater renewal events (Nausch et al. 2003). We observed steep gradients of NO₃⁻ and H₂S in the redoxcline at the Gotland Deep (Fig. 1). Profiles of nutrients across the redoxcline of the Gotland Deep are shown in Fig. 2A. High H₂S concentrations up to 110 μmol L⁻¹ were found at 235 m depth in 2002 (Fig. 2A shows data only down to 145 m). From about 107 m toward the NO₃⁻–H₂S interface, oxygen was below the detection limit. The interface was localized at around 128 m and included a narrow zone (1–3 m) where NO₃⁻ and H₂S coexisted.

Oxygen was measured by the Winkler method (detection limit ~2 μmol L⁻¹). H₂S, comprising the sum of all sulfide species, was photometrically detected by the methylene blue method (Grasshoff 1983). Dissolved (Mn(II)) and particulate (MnO₂) manganese analysis was performed according to Hinrichs et al. (2002), where 1 liter of water sample was filtered through polycarbonate membranes (0.45-μm pore size; Millipore) directly after sampling. Each filter was subsequently rinsed with 18 mΩ water and stored in sterile plastic petri dishes in the dark until analysis. Complete acid digestion of filters was done in closed polytetrafluoroethylene (PTFE) autoclaves at 180°C in a mixture of HNO₃, HClO₄, and HF and digests were resuspended in 2% HNO₃. For the determination of dissolved manganese 50-mL subsamples of the filtrates were collected, acidified by addition of 1 mL of redistilled HNO₃, and stored in 2% HNO₃-equilibrated polyethylene bottles. Analysis of manganese was performed by inductively coupled plasma–optical emission spectroscopy (Perkin Elmer Optima 3000XL). Manganese was calibrated between 0.36 and 36 μmol L⁻¹. The detection limits for dissolved and particulate manganese were 0.36 μmol L⁻¹ and 0.01 μmol L⁻¹, respectively.
Fig. 2. Vertical profiles of chemical parameters ($O_2$, $H_2S$, $NO_3^-$, $NO_2^-$, $NH_4^+$, $Mn(II)$, and $MnO_x$ [$\mu$mol L$^{-1}$]) and rates of $^{14}N^{15}N$ and $^{15}N^{15}N$ production ($\mu$mol L$^{-1}$ d$^{-1}$) after 48 h in the water column of Gotland Deep. Panels illustrate all parameters for the cruises of (A) 2002, (B) 2003, (C) 2004, and (D) 2005. The production rates of $^{14}N^{15}N$ and $^{15}N^{15}N$ in $^{15}N$ incubation experiments are shown in (A), (C), and (D) for the years 2002, 2004, and 2005, respectively and were performed with water from a separate CTD-rosette cast at the same station. n.d. = $N_2$ production was not detected. (B) illustrates the whole water column of Gotland Deep; all other plots display a 30-m extended zone across the suboxic–sulfidic interface.
In suboxic and anoxic layers, dissolved manganese Mn(II) was in the range of 0.1 to 8 μmol L⁻¹ and manganese oxide (MnOₓ) in the range of 0 to 0.6 μmol L⁻¹.

Immense inflows of saline North Sea water into the Baltic Sea from summer 2002 to summer 2003 caused extensive deepwater renewal, which resulted in a ventilation of the anoxic Gotland Basin that persisted until the end of the year 2003. In August 2003, the redoxcline had disappeared, no H₂S was detected in the water column, and O₂ occurred in varying concentrations throughout the entire water body (Fig. 2B). No NO₃⁻ was detected in the upper 70 m of the water column, whereas from 70 to 230 m, NO₃⁻ concentrations ranged between 2 and 10 μmol L⁻¹. In a parallel study (C. Pohl unpubl. data), it was shown that Mn(II) became almost completely oxidized in the former sulfidic zone after the inflow events.

During the cruise in August 2004, we found the redoxcline reestablished and H₂S was again detected in the bottom water (Fig. 2C). The suboxic–sulfidic interface was situated at 220 m water depth, about 20 m above the sediment. Nitrate and H₂S disappeared at about 223–224 m without an obvious overlap. Maximum concentrations of MnOₓ (~2 μmol L⁻¹) in suboxic waters were up to fivefold higher than in 2002. MnOₓ concentrations decreased toward the suboxic–sulfidic interface, but were still high in the suboxic zone. Mn(II) continuously increased from the suboxic zone toward the sediment (Fig. 2C; 47 μmol L⁻¹ at 240 m). In May 2005, the redoxcline moved to 200 m water depth, ~20 m shallower than in 2004. A distinct feature observed only in 2005 was the development of a 6-m-thick suboxic sulfide-free zone (Fig. 2D). Within this suboxic zone, the concentrations of NO₂⁻, NO₃⁻, NH₄⁺, O₂, and H₂S were very low or nondetectable. MnOₓ was in the range of 0.03–0.1 μmol L⁻¹ and Mn(II) was present up to 22 μmol L⁻¹ (Fig. 2D).

15N incubation experiments—To elucidate the microbial processes responsible for N loss in suboxic and anoxic layers we incubated water samples from various depths across the NO₃⁻-H₂S interface after addition of 15N-labeled NO₃⁻ and NH₄⁺. In 2002, we did all 15N incubation experiments at in situ conditions without degassing the samples. No N₂ production was found in the suboxic waters above the redoxcline after adding 10 μmol L⁻¹ 15NO₃⁻ (Fig. 2A). However, high amounts of N₂ production were measured after the addition of 15NO₃⁻ to water collected from the top of the sulfidic zone with a NO₃⁻ concentration of 0.25 μmol L⁻¹ (128 m depth; Fig. 2B).
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Fig. 4. Rates of isotopically labeled N₂ and NH₄⁺ production (48-h incubations) at and above the suboxic–sulfidic interface at Sta. 271 (August 2004). ¹⁵NO⁻₃ (5 μmol L⁻¹) was added to all samples. Nitrate and H₂S depth profiles are shown for reference. NH₄⁺ production was only determined for water collected between 220 and 227 m depth.

Fig. 3A). We almost exclusively (98%) detected production of ¹⁵N₁⁵N (Fig. 3A). To our knowledge, these rates (2.7 μmol L⁻¹ d⁻¹ N₂) are the highest ¹⁵N₁⁵N production rates ever measured from in situ-like incubations in marine waters (Fig. 2A).

¹⁵N incubations in 2004 were performed with degassed water samples from the zone around the suboxic–sulfidic interface by adding 5 μmol L⁻¹ ¹⁵NO⁻₃. Comparable with 2002, no N₂ production was detected in suboxic waters (217 and 219 m), not even after 48 h of incubation (Figs. 2C, 4). On the other hand, the addition of ¹⁵NO⁻₃ to sulfidic waters caused the production of ¹⁵N₁⁵N (Figs. 3C, 4). However, there was a 24-h time lag between the start of the incubation and the first measurable N₂ production (Fig. 3C), and the denitrification rates (0.23 μmol L⁻¹ d⁻¹ N₂) were lower than in 2002 (Fig. 2). The addition of 5 μmol L⁻¹ ¹⁵NO⁻₃ to sulfide-containing water samples resulted in a production of ¹⁵N₁⁵N and ¹⁵NH₄⁺. The production rates (determined in 48-h incubation experiments) of ¹⁵N₁⁵N and ¹⁵NH₄⁺ increased with water depth, with up to 68% of the labeled ¹⁵NO⁻₃ being converted to ¹⁵N₁⁵N and up to 21% to ¹⁵NH₄⁺ (Fig. 4). At the redoxcline, the concentrations of NO⁻₃, O₂, NH₄⁺, and H₂S were at or below the detection limit. After adding ¹⁵NO⁻₃ to water from this depth, we measured the production of ¹⁴N₁⁵N for the first time (Figs. 2C, 3B), indicating anammox activity. Linear ¹⁴N₁⁵N production rates of 0.01 μmol L⁻¹ d⁻¹ N₂ were measured in the first 48 h of incubation, while no ¹⁵N₁⁵N production was detected even after 72 h of incubation (Fig. 3B).

Similarly, no detectable ¹⁵N₁⁵N production in suboxic waters above the sulfidic zone was found in May 2005 in incubations with ¹⁵NO⁻₃ (Fig. 2D). In contrast to 2004, the addition of ¹⁵NO⁻₃ to sulfidic waters did not result in ¹⁵N₁⁵N production (Fig. 2D). We observed production of only ¹⁴N₁⁵N in the suboxic sulfide-free zone after ¹⁵NH₄⁺ + ¹⁴NO⁻₃ addition (each 5 μmol L⁻¹) (Fig. 2D). Anaerobic ammonium oxidation rates of ~0.05 μmol L⁻¹ d⁻¹ N₂ (Figs. 2D, 3D) were measured at 202 m depth and at 206 m (0.005 μmol L⁻¹ d⁻¹ N₂; Fig. 2D). We observed ¹⁴N₁⁵N formation at a rate of 0.04 μmol L⁻¹ d⁻¹ N₂ even in sulfide-containing water samples (Fig. 3E).

Discussion

N loss in the suboxic zone of the Gotland Basin—To study the microbial processes responsible for N loss in the Gotland Basin, water samples from various depths across the suboxic–sulfidic interface were incubated after the addition of either ¹⁵NH₄⁺ or ¹⁵NO⁻₃. We defined the 10 μmol L⁻¹ as upper limit of the suboxic zone as previously suggested upper limit for the activity of denitrifiers in general (Tiedje 1994) as well as for the Black Sea (Kirkpatrick et al. 2006). Surprisingly, none of our ¹⁵N incubations with suboxic water samples showed a production of ¹⁵N₁⁵N, which would be characteristic for denitrification (Figs. 2, 4). Consequently, our ¹⁵N incubation experiments did not provide any evidence for the direct conversion of NO⁻₃ to dinitrogen gas by heterotrophic denitrifiers. The only study in which a potential of heterotrophic denitrification could be measured in the water column of the Baltic Sea was carried out by Rönnér and Sörensson (1985). Using ¹⁵N incubations and the acetylene inhibition method, they detected N₂ and N₂O production in suboxic as well as in oxic waters of the Baltic proper, respectively. However, in their experiments, the incubations lasted 1–4 weeks and were supplemented with
500 μmol L\(^{-1}\) \(^{15}\)NO\(_3^-\) as well as 500 μmol L\(^{-1}\) glucose (for \(^{15}\)N incubations), which does not represent in situ conditions.

In agreement with our results, Brettar and Rheinheimer (1992) obtained in situ denitrification rates at the suboxic–sulfidic interface of the Gotland Deep (4-d incubation), while no significant denitrification was detected for suboxic and sulfide-free waters, not even after 12 d of incubation and addition of 50 μmol L\(^{-1}\) NO\(_3^-\). The authors suggested that this was probably due to organic carbon limitation of heterotrophic denitrification in the central Baltic basins. However, not even after the spring bloom in May 2005, during which enhanced sedimentation probably occurred, were we able to measure any heterotrophic denitrification, which might indicate that there are, besides organic carbon limitation, also other, yet unknown regulating factors. These findings are in line with the results from other suboxic water masses underlying highly productive surface waters like the Namibian and Peruvian/Chilean oxygen-minimum zones, where only anammox but no heterotrophic denitrification were found in the water column (Kuypers et al. 2005; Thamdrup et al. 2006; Hamersley et al. 2007).

**Chemolithotrophic denitrification and nitrate ammonification**—Water samples from the NO\(_3^-\) -H\(_2\)S interface and the sulfidic zone, which were incubated with \(^{15}\)NO\(_3^-\), showed production of \(^{15}\)N\(_2\) during the 2002 and 2004 cruises (Figs. 2A,C, 3A,C). Laboratory experiments with sulfidic water samples indicated that purging the samples may reduce or delay the chemolithotrophic denitrification rates, probably because of degassing of reduced sulfur compounds, such as H\(_2\)S (data not shown). This might explain the low and delayed \(^{15}\)N\(_2\) production in 2004 compared with 2002 when samples were not degassed. Alternatively, the capacity may be reduced because of the less favorable conditions for NO\(_3^-\) reduction with H\(_2\)S as well as due to the reduced or sporadic mixing of NO\(_3^-\) and H\(_2\)S in 2004 compared with 2002. The \(^{14}\)C incorporation by CO\(_2\) dark fixation was determined during the cruise in August 2004 with high rates (0.7 μmol L\(^{-1}\) d\(^{-1}\)) at the redoxcline (Labrenz et al. 2005). Several microbial processes contribute to the total CO\(_2\) dark fixation and the contribution of chemolithoautotrophic denitrifiers (reducing NO\(_3^-\) by oxidizing sulfur compounds) to the fixation rates is still unknown. For the redoxcline of the Gotland Deep, a previous study indicated chemolithotrophic denitrification as the main process for N loss (Brettar and Rheinheimer 1991). Chemolithotrophic denitrification generally requires the availability of both NO\(_3^-\) and H\(_2\)S, and a clear overlap of both was observed solely during the cruise in July 2002 (Fig. 2A). However, the vertical resolution (~1 m) of sampling with FreeFlow bottles mounted on a CTD-rosette might be a limiting factor for the detection of the NO\(_3^-\) -H\(_2\)S interface. Irrespective, small-scale turbulences due to internal waves and mesoscale eddies can potentially produce local mixing events (Lass et al. 2003), which would result in the temporal mixing of H\(_2\)S- and NO\(_3^-\)-containing water. Furthermore, lateral intrusions of oxygenated and NO\(_3^-\)-rich water into sulfidic water layers may promote denitrification activity (Nehring 1987). The frequency and magnitude of such mixing events, however, is presently unknown.

The potential for chemolithotrophic denitrification was detected even 15 m beneath the NO\(_3^-\) -H\(_2\)S interface (Figs. 2C, 4). This suggests that either lateral or vertical fluxes of nitrate-containing water occur down to 15 m below the interface or that the involved organisms exhibit a large physiological flexibility. Recent investigations identified an uncultured epsilonproteobacterium, related to *Sulfurimonas denitrificans*, as a key player for chemolithoautotrophic NO\(_3^-\) reduction at the suboxic–sulfidic interface (Labrenz et al. 2005; Brettar et al. 2006). The quantification of RNA and deoxyribonucleic acid by quantitative polymerase chain reaction showed that this strain has its maximum activity at the suboxic–sulfidic interface but active organisms were found even deeper into the sulfidic zone (Brettar et al. 2006). Intriguingly, we did not detect any potential for chemolithotrophic denitrification in the sulfidic Gotland Basin waters during the May 2005 cruise. It remains to be investigated whether this was due to shifts in the community structure, which were caused by massive changes in the biogeochemical gradients around the suboxic–sulfidic interface.

**Dissimilatory nitrate reduction to ammonium (DNRA),** also known as nitrate ammonification, was detected in our \(^{15}\)N experiments with sulfidic waters (Fig. 4). DNRA is a widespread process among anaerobic heterotrophic and chemoaautotrophic prokaryotes (Canfield et al. 2005). It is normally found in environments with high input of organic carbon (Tiedje 1994), but can also be coupled to H\(_2\)S oxidation with NO\(_3^-\), for example by the giant sulfur bacteria *Thioploca* (Ötte et al. 1999). In our incubations, the contribution of DNRA to the total NO\(_3^-\) consumption is up to 21% (Fig. 4). As DNRA was solely found in sulfidic waters without measurable amounts of NO\(_3^-\) and is increasing with enhanced H\(_2\)S concentrations, it seems to depend on reduced S compounds. Consequently, in the Gotland Basin, this process appears to be restricted to the sulfidic waters, in agreement with previous findings (Samuelsson and Rönnner 1982).

**Anammox rates and biological characterization**—\(^{15}\)N incubation experiments that were performed above and directly at the redoxcline of the Gotland Deep during the 2004 and 2005 cruises exhibited characteristic features of anammox: (1) the addition of \(^{15}\)NO\(_3^-\) resulted in the sole production of \(^{14}\)N\(_{\text{NO}}\) (no \(^{15}\)N\(_{\text{N}}\)); (2) the sole accumulation of \(^{14}\)N\(_{\text{N}}\) with a small lag phase in one incubation with \(^{15}\)NO\(_3^-\) in 2004 (Fig. 3B) and without a lag phase in incubations with \(^{15}\)NH\(_4\)+ in 2005 (Fig. 3D); and (3) the recovery of \(^{13}\)NH\(_4\)+ as \(^{14}\)N\(_{\text{NO}}\) and not as \(^{15}\)N\(_{\text{N}}\) indicating that NH\(_4\)+ is oxidized by NO\(_3^-\) to N\(_2\) (Van De Graaf et al. 1995) and not by Mn(III/IV) as proposed by Luther et al. (1997). Furthermore, the low NH\(_4\)+ concentration is indicative of anammox activity in this zone. This was also suggested for the sites with high anammox activity in the Benguela upwelling system (Kuypers et al. 2005).

The anammox rates in 2004 and 2005 varied between 0.005 and 0.05 μmol L\(^{-1}\) d\(^{-1}\) N\(_2\), which is in the range of
previously published pelagic anammox rates (0.002–
0.38 µmol L$^{-1}$ d$^{-1}$) (i.e., Kuypers et al. 2003, 2005;
Hamersley et al. 2007). In laboratory bioreactors, ana-
mmox bacteria exhibited rates of 2–20 fmol cell$^{-1}$ d$^{-1}$
NH$_4^+$ (Strous et al. 1999), whereas rates of ~3–4 fmol
cell$^{-1}$ d$^{-1}$ NH$_4^+$ were estimated for the Black Sea as well as
the Benguela upwelling system (Kuypers et al. 2003, 2005). Assuming a similar rate for the Baltic Sea, $9 \times 10^2$–$9 \times 10^3$
(2004) anammox cells mL$^{-1}$ and $2.5 \times 10^3$–$2.5 \times 10^4$
anammox cells mL$^{-1}$ (2005) would be required to explain
the observed rates in the Gotland Deep. FISH analysis with
specific oligonucleotide probes in 2005 confirmed the
presence of anammox bacteria (Fig. 5B,C). Bacteria of
the Candidatus Scalindua sorokinii and Candidatus S.
wagneri clade represented ~1.5% of the total DAPI cell
counts. This approximates $2.2 \times 10^4$ anammox cells mL$^{-1}$
(Fig. 5A,C), which would correspond to a cell-specific rate
of ~2.3 fmol d$^{-1}$ NH$_4^+$.

**Potential cause for a shift from denitrification to anam-
nox**—Intrusions of oxic North Sea water masses in 2002–
2003 ventilated the sulfidic zone, removed the biogeochem-
ical stratification of the water column, and caused an
apparent change in the processes responsible for the N loss
in the water column of the Gotland Basin. Previous studies
indicated that the inflow of oxygenated North Sea water
resulted in the oxidation of dissolved manganese in anoxic
waters and in subsequent precipitation of MnO$_x$, which
leaves the Gotland Basin waters depleted of manganese
(Heiser et al. 2001). The following development of a
renewed anoxia and sulfidic bottom-water conditions
were demonstrated to facilitate the release of reduced
manganese from the sediment. Recirculation of Mn(II)
back into the water column from the fluffy surface
sediments might be accelerated by sequent intrusions
and near-bottom currents (Heiser et al. 2001). This recycling
of Mn from the surface sediments after re-establishment of
the anoxic bottom-water conditions seems to be supported
by our results where the total integrated amount of Mn in the
entire water column is similar in the Gotland Basin before
and after the ventilation events in 2002–2003 (~0.5 ±
0.1 mol m$^{-2}$ in 2002, 2004, and 2005).

During the August 2003 cruise, no H$_2$S was found in the
water column (Fig. 2B), whereas in 2004 there were high
H$_2$S concentrations as well as high amounts of Mn(II) and
MnO$_x$ in the bottom water. Maximum concentrations of
oxidized and reduced manganese were up to 3–13-fold
higher than in 2002, before the ventilation (Fig. 2). We
propose that the enhanced flux of manganese led to
increased oxidation of reduced S compounds at the
redoxcline after the re-established anoxic conditions in
2004. During this process, Mn(III/IV)-reducing bacteria
rapidly oxidize H$_2$S to SO$_4^{2-}$ or sulfate by using MnO$_x$
(Canfield et al. 2005). From the 2002 cruise it was estimated
that settling MnO$_x$, formed upon Mn(II) oxidation in low-
oxgen waters, accounts for up to 30% of H$_2$S oxidation in
the Gotland Deep (Neretin et al. 2003). Lateral intrusions
of oxygenated waters in the Black Sea were demonstrated
by Schippers et al. (2005) to be of importance for the
oxidation of Mn(II) to MnO$_x$. Recent investigations
verified that the flux of O$_2$ significantly intensified the
formation of MnO$_x$ and thus also the consumption of H$_2$S
at the Black Sea’s oxic–anoxic interface (Konovolov et al.
2003).

Our results suggest that spatial separation of NO$_3^-$ and
H$_2$S, resulting from H$_2$S oxidation by MnO$_x$, favored
the establishment of a substantial anammox population. In
2004, anammox activity was only measured in one sample,
just at the transition between measurable \( \text{NO}_3^- \) and \( \text{H}_2\text{S} \) concentrations (Figs. 2C, 3B). In the extended suboxic zone of 2005, with inorganic N concentrations at the detection limit, anammox activity was measured at 202 m and at 206 m (Fig. 2D). A similar zone of very low inorganic N concentrations was found in the wide suboxic zone of the Black Sea where anammox activity is the dominant sink for inorganic N (Kuyper et al. 2003). There is evidence from other systems that manganese, via its interactions with electron donors used in denitrification, may indirectly affect the distribution of anammox. The occurrence of high MnO\(_x\) amounts in marine sediments was suggested to favor anammox more than denitrification (Engstr\ö m et al. 2005).

The authors attributed this to the oxidation of organic matter by Mn and Fe oxides, rendering it unavailable for denitrification, which creates favorable conditions for anammox bacteria. Analogously to the oxidation of organic matter in sediments, we deduce that in our study the oxidation of \( \text{H}_2\text{S} \) at the Gotland Deep’s redoxcline is influenced also by MnO\(_x\). This may have led to the disappearance of the \( \text{NO}_3^- \)-\( \text{H}_2\text{S} \) interface and resulted in an environment more favorable for anammox bacteria than for chemolithotrophic denitrifiers. The fact that we found higher anammox rates in 2005 despite decreased MnO\(_x\) concentrations compared with 2004 might be due to the previous consumption of MnO\(_x\) by redox processes. However, our study cannot resolve the temporal dynamics of manganese in the Gotland Deep and there might be other factors (e.g., lateral intrusions) that also cause the development of an extended suboxic, sulfide-free zone.

\( ^{15}\text{N} \) incubations and supplementary data from nutrient profiles imply a dependency of the N-loss processes in the Gotland Deep on the dynamic development of the biogeochemical gradients across the suboxic–sulﬁdic interface. The absence of direct \( \text{NO}_3^- \) conversion to \( \text{N}_2 \) by denitrifiers in suboxic water layers suggests that heterotrophic denitrification plays a negligible role in the production of \( \text{N}_2 \). Instead, our \( ^{15}\text{N} \) incubation experiments indicate that chemolithotrophic denitrifiers and anaerobic ammonium oxidizers were responsible for N loss from the Gotland Basin waters. High \( \text{N}_2 \) production rates reconfirmed the importance of denitrification at the \( \text{NO}_3^- \text{-H}_2\text{S} \) interface, which was attributed to chemolithotrophic denitrifiers in earlier studies. This study reports the first observations of active anammox bacteria in the water column of the Baltic Sea. The onset of the activity of anammox bacteria may have been promoted by the temporal changes in the water column chemistry at the redoxcline resulting from the inflow of oxygenated North Sea water masses.

References


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