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Hypoxia and nitrogen processing in the Baltic Sea water column

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Abstract

Steep redoxclines form between oxic surface water and the stagnant, sulfidic hypolimnion in the eutrophied, brackish water Baltic Sea. Nitrification, denitrification, and anammox were measured at and below the redoxcline to quantify the role of water-column nitrogen processes in the overall magnitude of nitrogen removal in the Baltic Sea. Rates of nitrification were very high (up to 85 nmol N L^{-1} d^{-1}) at the oxic–anoxic interface, but, surprisingly, nitrification was separated from the processes reducing nitrate to N_2 (up to 810 nmol N L^{-1} d^{-1}) by tens of meters in depth. N_2 production was dominated by chemolithotrophic denitrification, with anammox playing only a negligible role, and limited to the water layers in which nitrite or nitrate coexisted with sulfide. The alternating oxygen concentrations in the basin induce irregular bursts of nitrogen removal. Nitrification takes place after mixing of ammonium-rich deep water with oxic water, and denitrification uses the formed nitrite and nitrate once anoxic conditions re-establish. Although removal rates can be high, conditions allowing such rates are likely short-lived. While the sedimentary denitrification rates at the shallower, oxic areas are lower, they are more constant in time, highlighting the need to avoid hypoxia, which would prevent sedimentary denitrification.

The Baltic Sea is a large and shallow brackish inland sea in northern Europe (415,266 km^2, average depth 52 m) that suffers from heavy anthropogenic nutrient loading, and large cyanobacterial blooms are a regular phenomenon. It has been suggested that internal feedbacks sustain the eutrophication (Vahtera et al. 2007). One of the key factors in this feedback mechanism is the effect that increased hypoxia has on the biogeochemical cycles of nutrients. The mechanisms that lead to phosphorus release from the sediments during anoxia are well known, but the effects of hypoxia on nitrogen cycling are still uncertain (Conley et al. 2009), despite the critical role nitrogen has as one of the main nutrients regulating not only the extent but also the species composition of phytoplankton blooms (Smith 1983).

Two naturally occurring microbial processes, denitrification and anammox, have the potential to remove nitrogen from the water via the production of N_2 gas (Codispoti et al. 2001; Kuypers et al. 2005). These anaerobic processes reduce oxidized substrates (nitrate and nitrite), originating from an aerobic nitrification process, to harmless dinitrogen gas. Therefore, nitrification and the processes that mediate the production of N_2 are often spatially tightly coupled and thrive in environments with oxic–anoxic interfaces, such as sediments (Nishio et al. 1983; Jenkins and Kemp 1984). Natural nitrogen removal is important in counteracting the eutrophication process. For example, in the Gulf of Finland, Baltic Sea, about 30% of annual nitrogen loading was removed by denitrification and anammox in sediments in the 1990s and early 2000s, although the efficiency has decreased in the late 2000s (Tuominen et al. 1998; Hietanen and Kuparinen 2008; Jäntti et al. 2011). The removal processes in the Baltic Sea sediments seem to be tolerant against short-term anoxia, but they cease due to lack of substrates if anoxic conditions are prolonged (Tuominen et al. 1998; Hietanen and Lukkari 2007).

Interestingly, a significant negative relationship exists between the total pool of dissolved inorganic nitrogen (DIN) and the volume of hypoxic water in the Baltic Sea (Vahtera et al. 2007), suggesting enhanced nitrogen removal under conditions in which sediments are likely to be anoxic and large water masses above them hypoxic. The decrease in the DIN pool, when anoxia at the bottom prevents nitrification, and thereby also nitrogen removal processes, could be explained by N_2 production taking place in the water column, as the oxic–anoxic interface shifts upwards. In the open oceans, 20–40% of nitrogen removal takes place in the oxygen minimum zones (Gruber and Sarmiento 1997; Codispoti et al. 2001). Nitrogen processing in the water column of the shallow Baltic Sea was studied already in the 1980s, and, using the technology available at that time, it was estimated that significant denitrification could take place also in the water column (Rönner and Sörensson 1985; Brettar and Rheinheimer 1991, 1992). At that time, the other nitrogen-removing process, anammox, still remained to be discovered. Denitrification in the Baltic Sea water column was preliminary suggested (Brettar and Rheinheimer 1991) and later verified to be chemolithoautotrophic, driven by sulfide oxidation (Brettar et al. 2006). The key player was identified as a *Thiomicrospira denitrificans*–like Epsilonproteobacterium that was active at and below oxic–anoxic interface (Brettar et al. 2006). The development of stable isotope technology has advanced nitrogen cycle research considerably within the last decades, and, using the modern technology, Hannig et al. (2007) detected both anammox and denitrification potentials in the Baltic Sea. However,

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the importance of these processes in the water column, compared to those taking place in the sediments in the Baltic Sea, has so far remained unproven. Similarly, little is known about the nitrification process that produces oxidized substrates to fuel denitrification and anammox. Nitrification has so far been measured in two sites in the Baltic Sea, only, and the measured rates have been highly variable, with high activity found only in a very narrow layer at the oxic–anoxic interface (Enoksson 1986; Bauer 2003). The nitrifying population is dominated by a crenarchaeotal subcluster GD2, which is closely related to *Candidatus* Nitrosopumilus maritimus (Labrenz et al. 2010). The archaeal nitrification genes are only expressed in a narrow suboxic layer (Labrenz et al. 2010), confirming the earlier results on the limited occurrence of nitrification.

In this paper, we present data collected on four cruises to the main hypoxic basin of the Baltic Sea between March and September 2009. We measured nitrification potential and calculated in situ rates at the oxic–anoxic interface, and we studied the N_2 production spatially and temporally at and below the hypoxic redoxcline. We aimed at revealing whether N_2 production in the hypoxic water could explain the shown inverse relationship between hypoxic volume and DIN concentration. We measured potential rates and, for the first time for the Baltic Sea, calculated the in situ rates of denitrification and anammox. We then assessed the contribution of the water-column processes to the overall nitrogen removal.

Methods

Water samples for N_2 production measurements were collected in 2009 on cruises in March (R/V *Alkor*), May (R/V *Aranda*), July (R/V *Professor Albrecht Penck*), and September (R/V *Maria S. Merian*), and samples were collected for nitrification measurements on all but the first cruise. Samples were collected from the Baltic proper, covering the main hypoxic basins of the Baltic Sea (Fig. 1). The larger, eastern Gotland Basin (maximum depth 248 m) is connected in the north to the smaller Landsort Deep (maximum depth 450 m, the Baltic Sea maximum). The basins were last ventilated by a large inflow of oxygenated salt water in 2003. Since then, stagnation of the deep water has caused the redoxcline to establish in the water column below the halocline. In the redoxcline, oxygen decreases sharply to zero, and often small nitrite and nitrate maxima can be found at around the point where oxygen disappearance occurs (Fig. 2). Below the redoxcline, sulfide and ammonium accumulate. The conditions at the redoxcline are dynamic, with short-term, even daily fluctuations in oxygen concentrations due to mixing within and across the redoxcline, but the layer in which such fluctuations take place is only some tens of meters deep, below which the water is stagnant.

Before sampling, profiles of temperature, salinity, and oxygen were analyzed from conductivity, temperature, and depth sensor (CTD) casts (Sea-Bird Electronics) and 1–12 sampling depths were selected to represent the interface between oxic and anoxic water layers (Table 1). Two replicate water samples per depth were collected using 5–10-liter Free-Flow bottles attached to a CTD-Rosette system (Hydrobios). Samples for O_2 (Winkler titration, detection limit 0.89 μmol L^{-1}), H_2S, NO_3^-, NO_2^-, and NH_4^+ (Grasshoff 1983; detection limits 0.02 μmol L^{-1}, 0.01 μmol L^{-1}, 0.01 μmol L^{-1}, and 0.3 μmol L^{-1}, respectively) were taken from the first replicate, and samples for process measurements were taken from the second to minimize gas exchange between sample water and atmosphere during sampling.

Denitrification and anammox measurements—Samples were taken in 1-liter glass bottles with threefold overflow, closed, and transported to glove bags immediately. Glove bags were equipped with gas detectors (GW Gas Alert Extreme, Honeywell) and flushed with N_2 so that oxygen concentration stayed below 0.5% of volume over the whole time the samples were exposed to surrounding atmosphere. The water samples were then amended both with $^{15}NO_3^-$ for combined denitrification and anammox measurements ($K^{15}NO_3$, 99 atom%, Cambridge Isotope Laboratories, final concentration 10 μmol L^{-1}), and with $^{14}NO_3^-$ and $^{15}NH_4^+$ (KNO_3, Merck, and $^{15}NH_4Cl$, 99 atom%, Cambridge Isotope Laboratories, final concentrations 10 μmol L^{-1} and 5 μmol L^{-1}, respectively) for anammox measurements. From both treatments, samples were divided into 16 gas-tight glass vials (12-mL Exetainer, Labco). These were incubated at in situ temperature ($\pm 2^{\circ}C$) in the dark. At time intervals of 6–8 h, four replicates were sacrificed by introducing a helium headspace through the septum at the same time as 4-mL aliquots of sample water were withdrawn from each vial. Biological activity in the samples was terminated by adding 100 μL of 7.3 mol L^{-1} $ZnCl_2$ (Merck), and the vials were stored upside down at room temperature until analysis of isotope ratios of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$. The 4-mL water sample was immediately filtered (prewashed syringe tip with 0.2-μm polyethersulfone [PES], VWR International LLC) and frozen for later NO_3^-, NO_2^-, and NH_4^+ analyses. The nutrient measurements from the 4-mL samples were done by downscaling the protocols presented in Solorzano (1969; NH_4^+) and Jones (1984; NO_2^-, NO_3^-) for 1-mL samples.

The isotope ratios of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ were analyzed by gas chromatographic isotope ratio mass spectrometry (GC-IRMS; Thermo Finnigan Delta V plus with ConFlo III, Thermo Fisher Scientific) at the University of Southern Denmark, Odense, Denmark, and the concentrations of labeled products were calculated as excess relative to air. Potential N_2 production (denitrification and anammox) rates were calculated from linear regression of labeled products over time, and the labeling of substrate pools in the samples in which the slopes significantly ($p < 0.05$) differed from zero (Thamdrup and Dalsgaard 2002; Thamdrup et al. 2006). The in situ rates were calculated assuming Michaelis–Menten kinetics, with measured rates representing maximum rates (V_{max}), and using in situ NO_x concentration and a Michaelis constant (K_m) value of 2.9 μmol L^{-1}, estimated for water-column chemolithotrophic denitrifiers in Mariager Fjord, Denmark (Jensen et al. 2009).

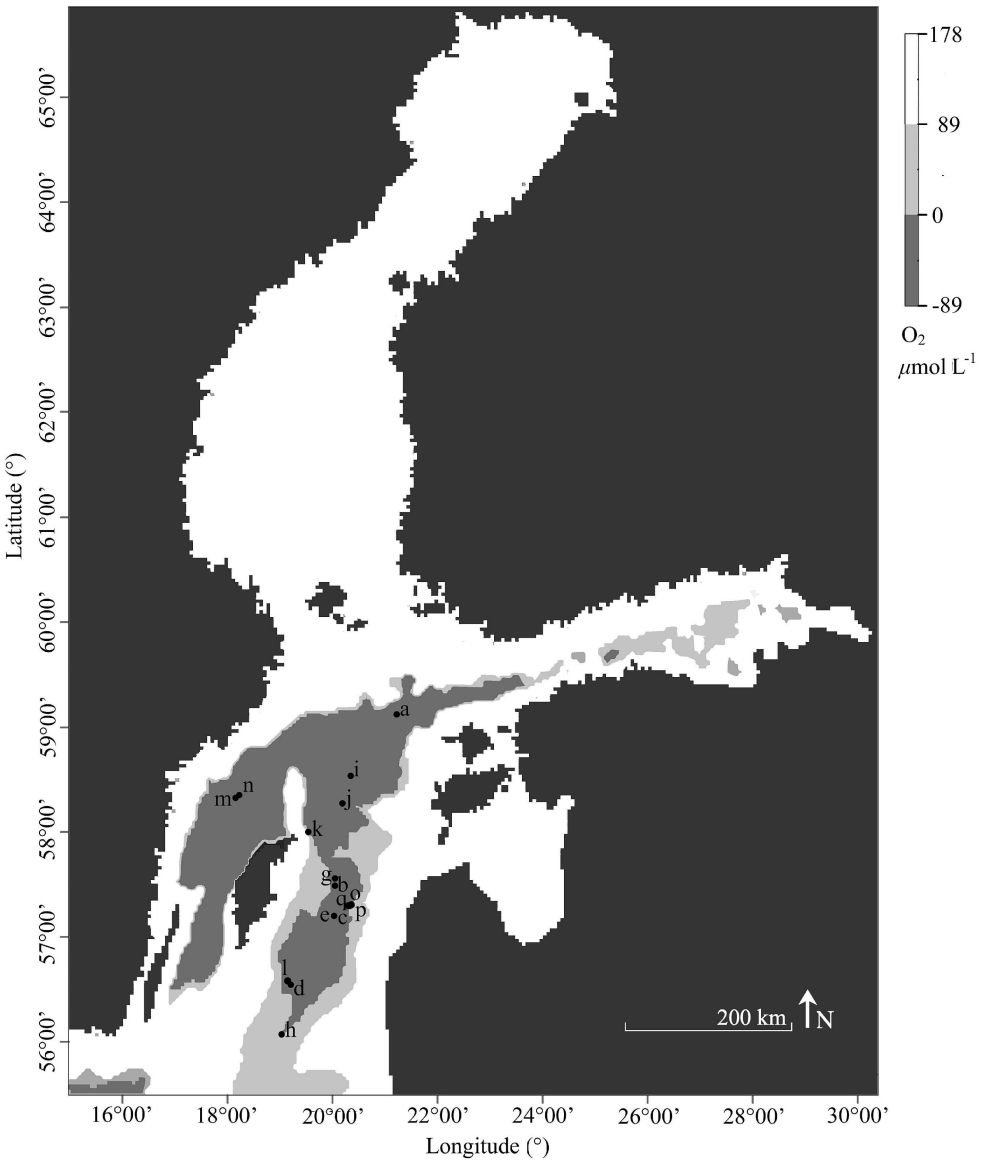

Fig. 1. Map of the extent of the hypoxic and anoxic areas of the Baltic Sea in May 2009 and the sampling stations. For station codes, *see* Table 1.

Nitrification measurements—Samples for nitrification measurements were collected and handled in a glove bag similar to the ^{15}N-N_2 production experiments. The samples were amended with $^{15}NH_4^+$ ($^{15}NH_4Cl$, 99 atom%, Cambridge Isotope Laboratories, final concentration 5 μmol L^{-1}) and divided into 12 gas-tight vials (12-mL Exetainer, Labco). The rest of the $^{15}NH_4^+$-amended sample was filtered (prewashed syringe tip with 0.2-μm PES, VWR International LLC) and immediately frozen (−20°C) for zero time $^{15}NO_x^-$ ($^{15}NO_2^-$ + $^{15}NO_3^-$) concentration measurements. Samples were incubated at in situ temperature (±2°C) in the dark. At 4-h intervals, four replicates were filtered (prewashed syringe tip with 0.2-μm PES, VWR International LLC) and immediately frozen for later $^{15}NO_3^-$ analyses. $^{15}NO_3^-$ produced by nitrification was analyzed using the denitrifier method (Sigman et al. 2001), with small modifications. *Pseudomonas chlororaphis* (American Type Culture Collection [ATCC] No. 43928) was grown on tryptic soy agar (Fluka Analytical [Sigma-Aldrich]) amended with 10 mmol L^{-1} KNO_3, and after two 3–4-d platings, one bacterial colony was transferred into a 400-mL liquid culture (tryptic soy broth [Fluka Analytical], 10 mmol L^{-1} KNO_3, 1 mmol L^{-1} ammonium sulfate [$\{NH_4\}_2SO_4$], and 1 mL L^{-1} antifoaming agent [Dow Corning Antifoam RD emulsion, VWR International LLC]). The bacteria were grown on a shaker table (100 rounds per minute) for 7 d in the dark at room temperature. The bacterial culture was concentrated 10 times by dividing 40-mL aliquots into 50-mL centrifuge tubes (Sarstedt) and centrifuging them for 10 min at 7500 rpm and 21°C. The supernatant was decanted from the tubes with the bacteria pellet remaining at the bottom. Four-milliliter aliquots of the decanted supernatant were pipetted back into the tubes to resuspend the pellet. The

Table 1. Sampling dates, stations, depths, temperature, salinity, oxygen, hydrogen sulfide, nutrients, and statistically significant ($p < 0.05$) nitrogen processing rates (nmol N L^{-1} d^{-1}). V_{max}, process potential; in situ rate was calculated assuming Michaelis–Menten kinetics, using K_m value of 2.9 μmol L^{-1} NO_x^- for denitrifiers (Jensen et al. 2009) and 0.27 μmol L^{-1} NH_4^+ for nitrifiers (Bauer 2003). Empty space, not significant process rate; n/a, not analyzed; —, missing data.

Date	Station name	Map code	Depth (m)		T (°C)	Salinity	O_2 (mL L^{-1})	H_2S (μmol L^{-1})	NO_3^- (μmol L^{-1})	NO_2^- (μmol L^{-1})	NH_4^+ (μmol L^{-1})	Denitrification				Anammox		Nitrification			
			Bottom	Sample								V_{max}	SD	In situ	SD	V_{max}	SD	V_{max}	SD	In situ	SD
05 Mar	3020	a	131	90	5.6	10.1	0.38	0.08	3.60	0.01	1.24					10.6	12.8	n/a	n/a	n/a	n/a
				100	5.6	10.3	0.04	0.16	0.003	0.09	—							n/a	n/a	n/a	n/a
05 Mar	3017	b	144	105	6.1	11.1	0.08	0.00	2.68	0.01	1.70					12.2	13.6	n/a	n/a	n/a	n/a
				115	6.2	11.4	0.00	2.38	0.003	0.01	0.48					17.7	12.8	n/a	n/a	n/a	n/a
06 Mar	GD	c	244	100	5.9	10.8	0.17	0.00	3.59	0.01	0.11							n/a	n/a	n/a	n/a
				120	6.2	11.4	0.05	0.35	0.03	0.00	1.03							n/a	n/a	n/a	n/a
				130	6.3	11.7	0.00	6.61	0.00	0.01	3.59	440.3	316.8	1.5	1.1			n/a	n/a	n/a	n/a
				140	6.4	12.0	0.00	20.71	0.02	0.01	7.99	9318.3	2242.3	95.4	23.0			n/a	n/a	n/a	n/a
				160	6.3	12.3	0.00	51.47	0.39	0.01	13.91	6660.9	952.4	807.4	115.4			n/a	n/a	n/a	n/a
				180	6.3	12.4	0.00	61.80	0.40	0.00	16.96	2303.0	182.3	279.2	22.1			n/a	n/a	n/a	n/a
07 Mar	3015	d	146	120	5.8	11.6	0.29	0.13	2.32	0.11	0.22							n/a	n/a	n/a	n/a
				135	6.4	11.9	0.00	20.81	0.00	0.01	7.87	7714.5	877.2	26.5	3.0			n/a	n/a	n/a	n/a
07 Mar	Grid3	e	245	138	6.7	11.9	0.00	8.05	0.00	0.05	3.28							n/a	n/a	n/a	n/a
08 Mar	3010	f	110	90	7.6	11.5	0.69	0.00	7.66	0.00	0.16							n/a	n/a	n/a	n/a
29 May	LF5	g	135	96	5.7	10.3	0.08	0.12	5.98	0.13	0.09							50.2	7.4	10.3	1.5
				98	—	—	0.06	0.00	5.50	0.26	0.07							57.7	11.9	11.9	2.4
				102	5.8	10.7	0.00	0.00	0.21	0.67	0.04							50.2	7.4	10.3	1.5
08 Jul	Inflow2	h	130	85	6.2	10.7	0.11	0.00	6.19	0.01	0.31							n/a	n/a	n/a	n/a
11 Jul	Inflow 7	i	168	85	5.7	10.2	0.07	0.00	0.08	0.01	1.11							29.3	2.4	23.6	1.9
				95	5.8	10.5	0.00	4.43	0.07	0.01	1.83							17.6	0.8	15.3	0.7
				105	5.9	10.7	0.00	4.74	0.09	0.00	2.14							n/a	n/a	n/a	n/a
11 Jul	TF285	j	124	90	5.6	10.2	0.00	0.00	1.56	0.05	1.16							n/a	n/a	n/a	n/a
				97	5.8	10.4	0.00	2.66	0.07	0.00	1.94							n/a	n/a	n/a	n/a
11 Jul	TF286	k	197	80	5.1	9.8	2.04	0.00	5.81	0.21	1.30							14.3	1.4	11.8	1.1
				90	5.7	10.1	0.09	0.00	4.57	0.06	0.74							18.9	0.7	13.9	0.5
				100	5.9	10.6	0.00	2.10	0.07	0.00	0.96							n/a	n/a	n/a	n/a
12 Jul	GD	c	251	80	5.4	9.5	1.23	0.00	5.82	0.16	1.80							18.4	1.6	16.0	1.4
				95	6.0	10.5	0.11	0.16	6.07	0.24	0.33							47.4	5.5	11.5	15.1
				110	6.5	11.1	0.00	0.10	4.28	0.09	0.75							n/a	n/a	n/a	n/a
13 Jul	Inflow3	l	174	110	6.7	11.2	0.75	0.00	7.09	0.02	0.31							8.4	1.1	4.5	0.6
				117	6.6	11.5	0.00	0.16	2.25	0.00	0.50							39.3	11.3	25.5	7.4
				125	6.6	11.7	0.00	4.51	0.15	0.00	1.99							n/a	n/a	n/a	n/a
14 Sep	LD	m	442	67	4.8	8.9	2.00	0.00	4.76	0.02	0.14							9.5	2.8	3.2	1.0
				69	5.1	9.2	1.44	0.00	4.35	0.01	0.10							16.1	0.2	4.3	0.1
13 Sep				71	5.2	9.6	0.44	0.10	2.94	0.03	1.38							99.1	12.2	82.9	10.2
				73	5.4	9.7	0.25	0.13	2.83	0.06	0.31							156.5	6.6	83.6	3.5
				75	5.4	9.8	0.00	0.15	0.00	0.06	0.38							122.7	38.5	75.8	112.8
				77	5.4	9.9	0.00	0.21	0.00	0.11	1.16							n/a	n/a	n/a	n/a
12 Sep				80	5.5	10.1	0.00	6.13	0.00	0.04	4.06							n/a	n/a	n/a	n/a
				85	5.5	10.1	0.00	7.44	0.00	0.02	5.22							n/a	n/a	n/a	n/a
				90	5.6	10.3	0.00	11.66	0.00	0.02	4.07							n/a	n/a	n/a	n/a
				95	5.7	10.5	0.00	10.80	0.00	0.01	3.89							n/a	n/a	n/a	n/a
11 Sep				100	5.7	10.5	0.00	11.55	0.00	0.00	3.35	1209.0	504.6	0	0			n/a	n/a	n/a	n/a
				105	5.7	10.5	0.00	11.70	0.00	0.04	3.47	1033.3	646.5	14.1	8.8			n/a	n/a	n/a	n/a

Table 1. Continued.

Date	Station name	Map code	Depth (m) Bottom	Depth (m) Sample	T (°C)	Salinity	O_2 (mL L^{-1})	H_2S (μmol L^{-1})	NO_3^- (μmol L^{-1})	NO_2^- (μmol L^{-1})	NH_4^+ (μmol L^{-1})	Denitrification V_{max}	Denitrification SD	Denitrification In situ	Denitrification In situ SD	Anammox V_{max}	Anammox SD	Nitrification V_{max}	Nitrification SD	Nitrification In situ	Nitrification In situ SD
16 Sep	LD3	n	430	77	5.3	9.6	0.32	0.23	3.31	0.02	0.13							82.9	36.3	4.3	1.9
				79	5.4	9.7	0.00	0.21	0.00	0.10	0.26							27.8	5.8	0.0	0.0
19 Sep	GD	c	244	85	5.7	10.0	0.92	0.37	7.11	0.00	0.00							86.1	6.5	2.9	0.2
				90	5.9	10.3	0.14	0.08	3.68	0.04	0.17							4.3	4.0	1.8	1.7
				95	6.0	10.6	0.01	3.47	0.10	0.04	2.12							4.2	3.1	1.3	1.0
				100	6.1	10.7	0.01	1.06	0.00	0.05	1.56							n/a	n/a	n/a	n/a
				108	6.3	10.8	0.00	2.31	0.00	0.04	1.48							n/a	n/a	n/a	n/a
				110	6.5	10.9	0.00	2.10	0.00	0.03	1.64							35.6	1.4	4.1	0.2
18 Sep				108	6.5	11.1	0.05	0.00	3.15	0.05	0.58							22.0	3.2	1.4	0.2
				110	6.5	11.1	0.04	0.00	2.96	0.03	0.31							4.0	0.6	0.5	0.1
				115	6.7	11.3	0.19	0.47	3.40	0.02	0.58							n/a	n/a	n/a	n/a
				120	6.7	11.4	0.00	0.18	0.07	0.02	0.31							n/a	n/a	n/a	n/a
				125	6.8	11.6	0.00	0.00	0.03	0.01	1.19	103.9	91.6	1.4	1.3			n/a	n/a	n/a	n/a
				130	6.8	11.8	0.00	5.90	0.00	0.01	2.20							n/a	n/a	n/a	n/a
19 Sep	Transect 1	o	88	72	5.6	10.0	0.03	0.00	1.58	0.12	1.03							n/a	n/a	n/a	n/a
				75	5.8	10.2	0.04	0.00	0.09	0.05	1.46							n/a	n/a	n/a	n/a
19 Sep	Transect 3	p	128	80	5.8	10.3	0.07	0.00	0.00	0.00	1.16							n/a	n/a	n/a	n/a
				85	6.0	10.6	0.04	0.00	0.18	0.10	0.74							n/a	n/a	n/a	n/a
19 Sep	Transect 4	q	148	80	5.8	10.3	0.02	0.00	0.28	0.00	1.57							n/a	n/a	n/a	n/a
				100	6.5	11.1	0.15	0.00	3.76	0.02	0.11							n/a	n/a	n/a	n/a

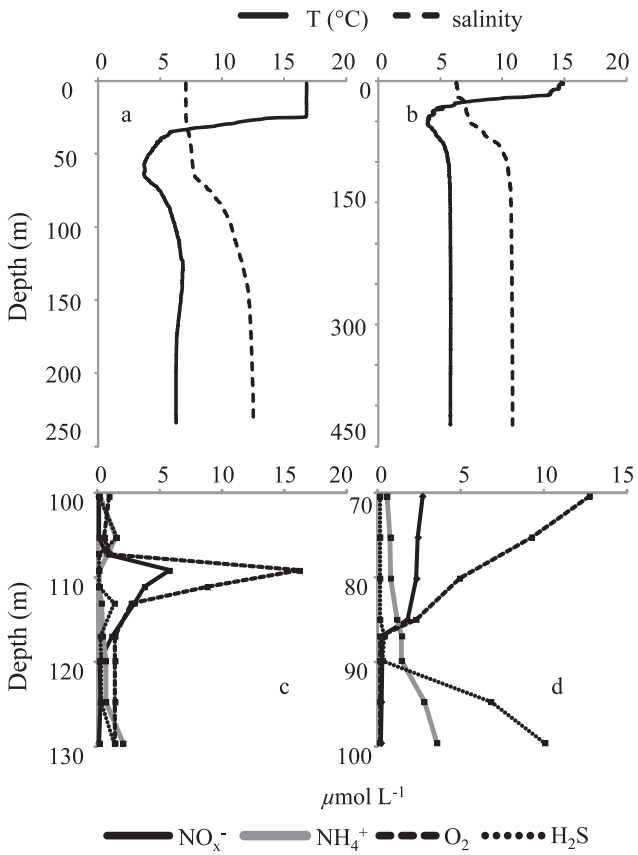

Fig. 2. Examples of biogeochemical profiles at the main basins in September 2009. (a, c) Gotland Deep (Sta. c), (b, d) Landsort Deep (Sta. m). (a, b) temperature (T, °C) and salinity in the whole water column; (c, d) NO_x (NO_2^- + NO_3^-), NH_4^+, O_2, and H_2S (μmol L^{-1}) in the redoxcline.

concentrated culture was divided into 2-mL aliquots in 20-mL gas-tight glass vials (TraceClear, VWR International LLC). The vials were closed tightly with screw caps and 3-mm septa, and purged with N_2 flowing at 10–20 mL min^{-1} for 4–5 h. Thereafter, using the zero-time NO_x^- concentration, the optimal sample volume for analysis was calculated so that the samples (incubated with $^{15}NH_4^+$) contained about 8 nmol NO_x^-. The calculated sample volume was injected into the glass vial containing the bacteria suspension. After overnight incubation in the dark, 0.1-mL aliquots of 10 mol NaOH L^{-1} were injected into the glass vials to lyse the bacteria and strip the carbon dioxide (CO_2) from the headspace to the liquid. The ^{15}N label in the N_2O produced was analyzed with a GC-IRMS system (Thermo Finnigan Delta V plus with ConFlo IV, Thermo Fisher Scientific) using a trace gas preconcentrator (PreCon, Thermo Fisher Scientific) at the Centre for Ice and Climate, University of Copenhagen, Denmark.

Potential nitrification rate was calculated from a linear regression of $^{15}NO_x^-$ production over time and the labeling of the NH_4^+ pool in the samples in which the slopes significantly ($p < 0.05$) differed from zero. The in situ nitrification rates were calculated assuming that the measured rates represented V_{max} rates, using in situ NH_4^+ concentration and the K_m value of 0.27 μmol L^{-1}

estimated for Baltic Sea redoxcline nitrifiers by Bauer (2003).

Results

Biogeochemical profiles at the Gotland Deep—In March, the halocline was located at 60–75-m depth in the Gotland Deep. No oxygen could be detected below 128 m (Winkler titration every 2 m at the interface), and sulfide started to accumulate at 116 m. Ammonium started to increase below 100 m, which was the depth of the highest nitrate peak as well. Nitrate quickly decreased to zero at 120 m, but a sharp nitrate peak (1.25 μmol L^{-1}) coincided with a small peak in nitrite (0.05 μmol L^{-1}) at 125 m. Below that, nitrate and nitrite were very low but detectable at all depths sampled, down to 180 m (Table 1). In July, the halocline at the deep station had moved down to 75–110-m depth, and the redoxcline had moved up, with oxygen disappearing at 100 m and sulfide starting to accumulate at 95 m. Plenty of nitrate and nitrite were available at the depths sampled at the interface. In September, the halocline was located at 65–95-m depth (Fig. 2). During our 3-d stay at the station, the oxic–anoxic interface fluctuated at around 115 m. On 17 September, there was a distinct 15-m layer of hypoxic water between 100- and 115-m depths that diminished to less than 10 m by next day and was practically gone on the third sampling day (Fig. 3). Sulfide was present from 115 m downward on the first 2 d but disappeared from depths of 115–120 m by 19 September. Nitrate and nitrite followed the oxygen dynamics; their concentrations dropped sharply below 115 m on the first 2 d and disappeared below 90 m on the third.

Biogeochemical profiles at the Landsort Deep—The other major deep basin, Landsort Deep, was only sampled in September. The halocline was located at 45–60-m depth (Fig. 2), and, like at the Gotland Deep, the redoxcline fluctuated from day to day. The depth at which oxygen disappeared decreased from 85 m on 10 September to 75 m on 13 September and increased back to 83 m by 15 September, with nitrate concentrations declining sharply at 85 m on the first day, and closely following the fluctuation of oxygen on the subsequent days. Traces of nitrite could still be measured at 105 m. At the same time, the depth of sulfide appearance decreased from 85 m to 70 m and increased back to 79 m.

Production of N_2 gas by denitrification and anammox—Both denitrification and anammox potential were detected in the Eastern Gotland Basin and the Landsort Deep (Fig. 4; Table 1), but their activity was highly variable both spatially and temporally. Only the Gotland Deep revealed any significant N_2 production, and this was dominated by denitrification, with anammox occurring at very low rates and only in the spring samples. It is, however, noteworthy that only anammox was detected at the two northern stations. Interestingly, anammox was active in both oxic (up to 1.7 μmol O_2 L^{-1}) and sulfidic (2.38 H_2S μmol L^{-1}) conditions, with highest rate measured at the sulfidic depth. In all the cases, when labeled nitrate disappeared from the samples, all of it was recovered as labeled N_2, indicating complete denitrification as the reducing process instead of partial nitrate reduction to nitrite or dissimilatory nitrate reduction to ammonium.

Denitrification in the Gotland Basin—The 244-m-deep station (Gotland Deep) at the Eastern Gotland Basin was sampled in March, July, and September 2009. No denitrification took place at the hypoxic depths studied, but the process was active in anoxic water layers.

In March, denitrification was found in all of the four depths studied between 130 and 180 m (Fig. 5; Table 1). Denitrification potential peaked at 140 m, with an extremely high rate of 9.3 μmol N L^{-1} d^{-1}, almost double to that measured at the same site in 128-m depth in July 2002 (5.4 μmol N L^{-1} d^{-1}; Hannig et al. 2007). The denitrification potential started to diminish below 140 m and had dropped by 75% by 180 m. The in situ rates, based on the potential rates, NO_x^- concentrations, and Km value, varied from 1.5 to 807 nmol N L^{-1} d^{-1}, with the highest rate at 160-m depth. Extrapolation of the in situ rates from the measured depths to the whole layer in which denitrification was active (130–180 m) gave a rate of 21 mmol N m^{-2} d^{-1} in this area in March. No anammox was detected at any of the depths studied using either $^{15}NO_3^-$ (six depths) or $^{15}NH_4^+$ + $^{14}NO_3^-$ addition (two depths).

In March, two other stations within the Gotland Basin were studied, 55 km north and south of the main station (Fig. 1). At the northern station, very low levels of anammox and no denitrification were detected. At the southern station, no anammox was detected, but denitrification (26.5 nmol N L^{-1} d^{-1}) was found at 135-m depth, but not at 120-m depth (Table 1). At the station less than 2 km west of the main station, no nitrogen removal potential was detected at 138-m depth, which was chosen for sampling due to a peak in the turbidity profile, indicating active sulfide oxidation.

In July, no nitrogen removal could be found at the 80-, 90-, and 100-m hypoxic depths studied at the deep station or at the oxic–anoxic interface on any of the five other stations studied within the basin (Fig. 1; Table 1).

In September, no active nitrogen removal processes could be found in the depths sampled (85–130 m) despite the dynamic oxygen conditions at the interface, with the exception of very low rates of denitrification at 125-m depth (1 nmol N L^{-1} d^{-1}). Nitrogen removal was additionally measured on a transect 55 km east of the deep basin, with samples taken at three adjacent stations with increasing depth. No nitrogen removal could be detected at the oxic–anoxic interface of these stations either (Table 1).

Denitrification at the Landsort Deep—The Landsort Deep was sampled in September. No nitrogen removal could be found at the dynamic interface, but denitrification potential of 1 μmol N L^{-1} d^{-1} was found at the two greatest depths studied (100 and 105 m), with only traces of nitrite and no nitrate left, giving in situ rates of 0–14 nmol N L^{-1} d^{-1}. At the station 11 km northeast of the

Landsort Deep main station, no nitrogen removal was detected at the interface (Table 1).

Nitrification—Nitrification was measured in May, July, and September at the oxic–anoxic interface, and just like the removal processes, nitrification showed large spatial and temporal variation. Nitrification and N_2-producing processes never appeared in the same depth.

In May, two depths were studied at the northern part of the Gotland basin (Table 1). The nitrification potentials (nitrification based on added $^{15}NH_4^+$ and the ambient $^{14}NH_4^+$) varied between 50 and 58 nmol N L^{-1} d^{-1}. The in situ nitrification rates were only one fifth of the potential rates due to low ammonium availability.

In July, a north–south transect in the Gotland Basin was sampled (Table 1). The nitrification potentials varied between 8 and 47 nmol N L^{-1} d^{-1}, and in all stations, the nitrification potential was higher in the sampling depth where the O_2 concentration was lower, unless sulfide was present in high concentration (4 μmol L^{-1}). However, low concentrations of sulfide (< 0.16 μmol L^{-1}) did not inhibit nitrification. There were no latitudinal trends, and nitrification potentials varied nearly as much between the sampling depths as between the stations. The in situ nitrification rates remained low, but they were higher than the rates measured in May.

In September, the vertical profiles of nitrification were studied around the oxic–anoxic interface with samples from five to eight depths at the Gotland Deep and two stations at the Landsort Deep (Table 1). Nitrification potentials were high at the Gotland Deep, varying between 5 and 89 nmol N L^{-1} d^{-1}. The highest rates were found at the depth with low oxygen concentration (< 6.7 μmol L^{-1}), decreasing ammonium concentration, and increasing nitrate concentration. At the first station at the Landsort Deep, the nitrification potential increased from less than 10 nmol N L^{-1} d^{-1} to $\sim$ 160 nmol N L^{-1} d^{-1} within 6 m, demonstrating the rapid change in nitrification potential within only a few meters change in depth. The highest in situ rates were measured at hypoxic conditions with high availability of ammonium (1.38 μmol NH_4^+ L^{-1}). At the other station in the Landsort Deep, only the deeper of the two depths sampled, separated by 2 m, showed nitrification potential. Despite the presence of sulfide, the potential was high (83 nmol N L^{-1} d^{-1}), but the actual nitrification rate was low.

Discussion

Vertical and horizontal distribution of anammox and denitrification—The magnitude of hypoxic water volume correlates negatively with the amount of dissolved inorganic nitrogen in the Baltic Sea (Vahtera et al. 2007). This suggests that N_2 production co-occurs with nitrification at water layers with traces of oxygen left. Indeed, denitrification tolerates concentrations of up to 11 μmol O_2 L^{-1} in the Baltic Sea (Rönner and Sörensson 1985) and up to 15 μmol O_2 L^{-1} in a stratified, anoxic Mariager Fjord in Denmark (Jensen et al. 2009). Similarly, anammox tolerates up to 13 μmol O_2 L^{-1} in the Black Sea (Jensen et al. 2008).

Contrary to this theory and findings from earlier studies (Brettar and Rheinheimer 1991; Brettar et al. 2006; Hannig et al. 2007), in this study neither anammox nor denitrification potentials were found around the oxic–anoxic interfaces. The only exception was in March, when low levels of anammox could be detected in the redoxclines of the two stations located at the northern, shallower end of Gotland Basin. The redoxclines at the rim of the basin exhibit more dynamic conditions than do the more stagnant central parts. The co-occurrence of oxygen and sulfide in the same water layer at the time of sampling indicated that mixing of different water layers had taken place recently. Mixing across the redoxcline increases the availability of oxygen and ammonium, enhancing nitrification. When oxygen is again used up, the nitrate accumulated is reduced by denitrification and anammox. Such seasonal hydrological processes create conditions resembling those found after major inflows. However, the conditions at the oxic–anoxic interface are probably too dynamic for the slow-growing anammox bacteria, as later in the year no anammox could be found at any station sampled.

Our findings, together with those of Hannig et al. (2007), support the theory of anammox being active only in hypoxic, sulfide-free conditions (Jensen et al. 2008). Chemolithotrophic denitrification starts to dominate the N_2 production as stagnation sets in and sulfide diffuses to the anoxic water layer (Hannig et al. 2007; Jensen et al. 2009). In the Black Sea, anammox was completely inhibited at 13.5 μmol O_2 L^{-1}, as well as by 1.5 μmol H_2S L^{-1} (Jensen et al. 2008). In this study, however, the low levels of anammox potential were found in both oxic (up to 17 μmol O_2 L^{-1}) and sulfidic (2.38 H_2S μmol L^{-1}) layers, with the highest rate measured in the presence of sulfide. The potential anammox rates we measured (10–17 nmol N L^{-1} d^{-1}) in March 2009 were at the lower end of the range of those measured in May 2005 after the inflow event (10–100 nmol N L^{-1} d^{-1}; Hannig et al. 2007), and very low compared to the denitrification potentials measured at the greater depths in the Gotland Deep in March.

Instead of active N_2 production at the redoxcline, potential was found at the greater, often sulfidic depths, supporting the theory of the chemolithotrophic nature of the process (Brettar et al. 2006; Jensen et al. 2009). Denitrification was the only process detected both at the Gotland Deep and at the Landsort Deep, and the activity was always located in the deeper layers sampled, well below the dynamic interface. The potential rates we measured were high, up to 9 μmol L^{-1} d^{-1}, and exhibited surprisingly high seasonal variability. Similar or even higher potentials have, however, been measured in other locations (Table 2).

Denitrification in other open-ocean oxygen minimum zones is thought to be mainly heterotrophic and regulated by availability of nitrate and organic carbon (Ward et al. 2008). Less is known about autotrophic, sulfide-driven chemolithotrophic denitrification, which is suggested to be responsible for the nitrogen removal in the Baltic Sea and

Table 2. The highest denitrification and anammox potential and in situ rates reported from different areas (nmol N L^{-1} d^{-1}).

	Denitrification		Anammox	
Location	V_{max}	In situ	V_{max}	Reference
Baltic Sea				
Northern Baltic			18	This study
Landsort Deep	2418	28		This study
Gotland Deep	9318	808		This study
Gotland Deep		110		Brettar and Rheinheimer (1991)
Gotland Deep	5400		50	Hannig et al. (2007)
Eastern tropical South Pacific				
Off Chile			34	Thamdrup et al. (2006)
Off Peru			328	Hamersley et al. (2007)
Off Peru			280	Lam et al. (2009)
Benguela upwelling system				
Off Namibia			360	Kuypers et al. (2005)
Off Namibia	560		140	Lavik et al. (2009)
Black Sea			22	Jensen et al. (2008)
Golfo Dulce, Costa Rica	5200		1200	Dalsgaard et al. (2003)
Arabian Sea	50		8	Ward et al. (2009)
Mariager Fjord, Denmark	37200	11800		Jensen et al. (2009)
Lake Tanganyika	180		20	Schubert et al. (2006)

the Mariager Fjord, Denmark (Brettar et al. 2006; Jensen et al. 2009). The data available so far cannot fully explain the lack of potential below the redoxcline in the Baltic Sea, nor the seasonal variation in the potentials. The lack of removal potential in the anoxic water layers right below the oxic–anoxic interface may reflect oxygen conditions too dynamic for the denitrifiers, or too strict substrate limitation, as only traces of oxidized nitrogen species and sulfide were detected in these upper anoxic depths. The increase in potential in the greater depths may be related to more stable conditions in terms of oxygen and sulfide, the energy source for chemolithoautotrophic denitrification, despite the extremely low availability of nitrate and nitrite. In those few samples where significant activity could be found, the potential was not correlated with nitrite and nitrate concentrations, likely due to co-limitation by sulfide. The potential first increased with increasing sulfide concentration, with the highest potentials found at about 20 μmol L^{-1} for sulfide, and then there was a decrease in activity at the 50 μmol L^{-1} and 60 μmol L^{-1} concentrations, in which NO_x peaked (0.4 μmol L^{-1}). Jost et al. (2010) have shown that the dark CO_2 fixation (autotrophic activity) in the Baltic Sea anoxic water column decreases at sulfide concentrations above 20 μmol L^{-1}, which could explain the decrease in rates with increasing depth in March, despite increasing NO_x availability. However, similar to our direct measurements of N_2 production after nitrate addition, nitrate addition had no effect on the dark CO_2 fixation rate at the oxic–anoxic interface (1.9 μmol H_2S L^{-1}), or at 10 m below it (10.2 μmol H_2S L^{-1}), but it increased at 20 m below the interface (48 μmol H_2S L^{-1}) (Jost et al. 2010). This, too, demonstrates the spatial gap between nitrification at the interface and nitrate processing 20 m below.

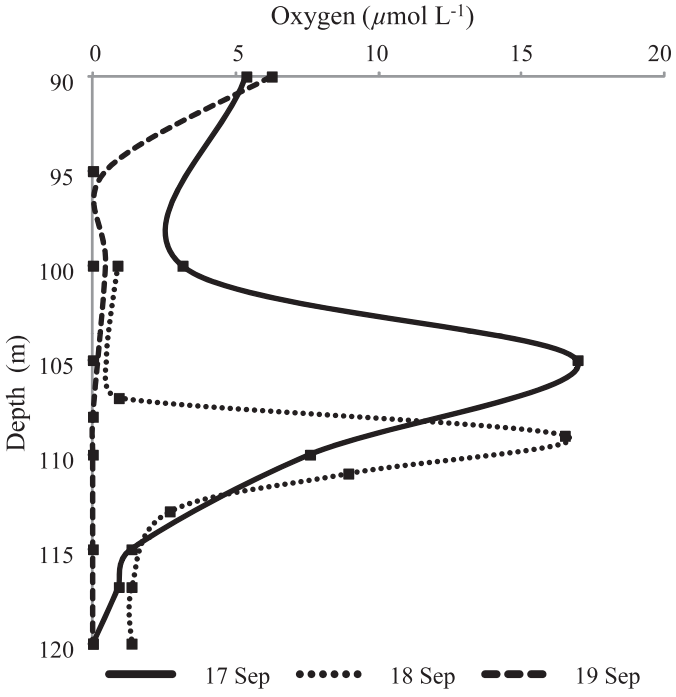

Fig. 3. Oxygen dynamics at the redoxcline of the Gotland Deep (Sta. c), 17–19 September 2009.

The in situ removal rates in this study were calculated assuming Michaelis–Menten kinetics for the substrate and a K_m value for nitrate-based denitrification estimated in an anoxic Mariager Fjord (Jensen et al. 2009), because of the lack of such an estimate for the Baltic Sea. Both the potential and in situ rates in the fjord were remarkably high compared to those measured in the Baltic Sea earlier (Brettar and Rheinheimer 1991; Hannig et al. 2007) and also in this study, which may be related to the steeper

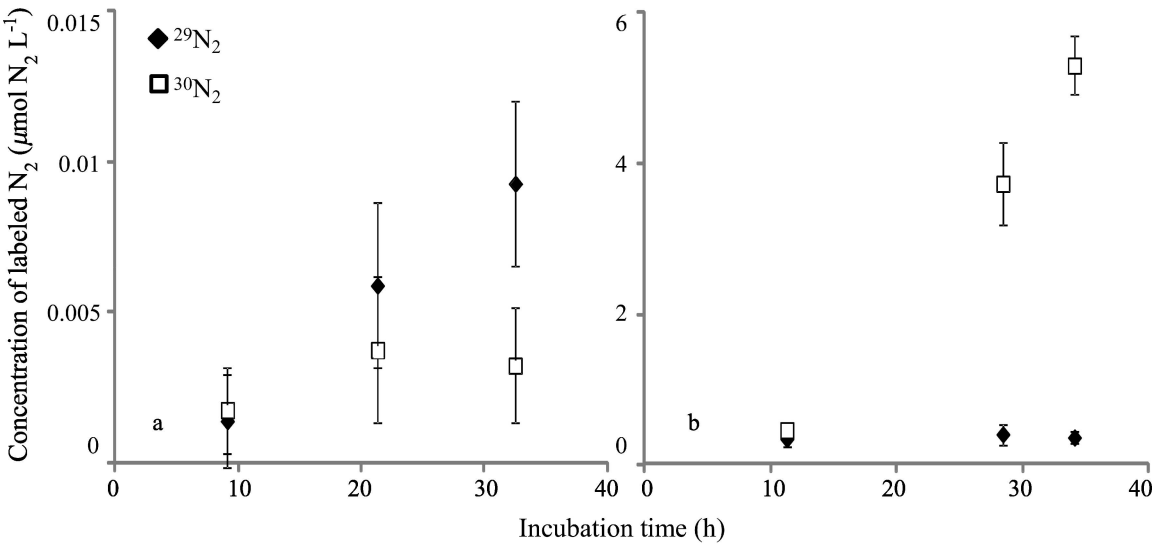

Fig. 4. Production of $^{29}N_2$ and $^{30}N_2$ over time on (a) 05 March 2009 at Northern Gotland Basin (Sta. b) at 115-m depth indicating anammox potential ($^{15}NH_4^+$ + $^{14}NO_3^-$ incubation) and on (b) 06 March 2009 at Gotland Deep (Sta. c), at 140-m depth indicating denitrification potential ($^{15}NO_3^-$ incubation).

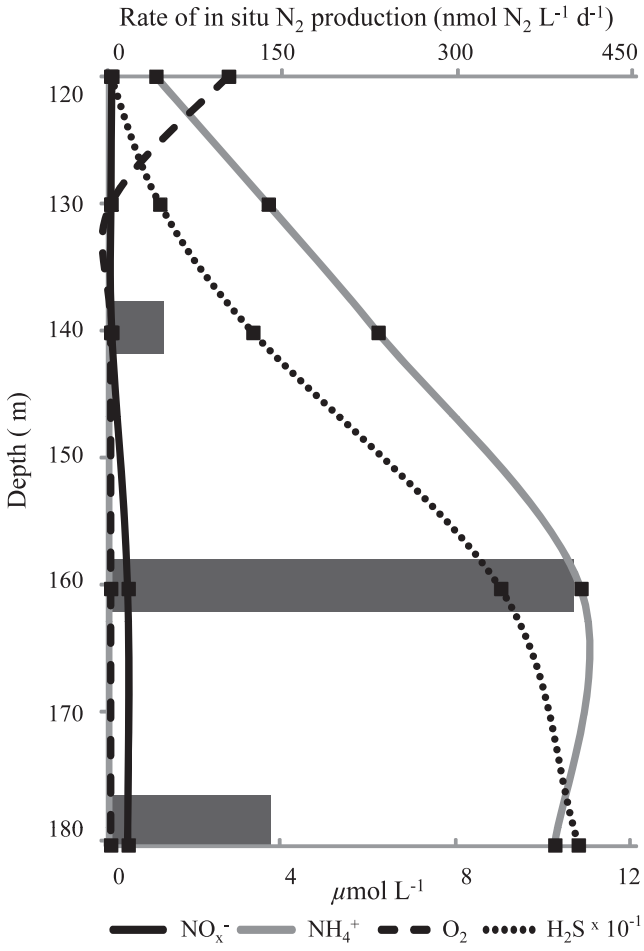

Fig. 5. In situ N_2 production rates at the Gotland Deep (Sta. c) on 06 March 2009 (nmol N_2 L^{-1} d^{-1}) and concentrations of NO_x^- (NO_2^- + NO_3^-), NH_4^+, O_2, and H_2S (μmol L^{-1} H_2S × 10^{-1}) in the water column.

gradients of nitrate and sulfide in the Mariager Fjord (Jensen et al. 2009). If the chemolithotrophic denitrifiers in the Baltic Sea have a lower K_m than the denitrifiers in the Mariager Fjord, the in situ rates are even higher than the ones calculated here. Still, low availability of nitrite and nitrate at the depths in which the greatest denitrification potential was located limits the magnitude of nitrogen removal in the deep basins.

Nitrification at the oxic–anoxic interface—Nitrification is active at the oxic–anoxic interface in the water column of the Baltic Sea (Enoksson 1986; Bauer 2003) and in the oxygen minimum zones of the oceans (Lam et al. 2007). The nitrification rates measured in this study, up to 80 nmol N L^{-1} d^{-1}, are similar to rates measured in the Gulf of California (Beman et al. 2008) but higher than those measured in the oxygen minimum zones of the Black Sea (Lam et al. 2007) and off the Peruvian coast (Lam et al. 2009). Nitrification rates higher than those found in this study have only been measured previously in the Baltic Sea (Enoksson 1986; Bauer 2003) (Table 3). The activity at the redoxcline in the Gotland Deep significantly declined from nearly 400 nmol N L^{-1} d^{-1} in March 1998 to only 56 nmol N L^{-1} d^{-1} in May 1999, which Bauer (2003) suggested is related to the changes is physico-chemical conditions at the interface. No significant trend could be found in the ammonium and oxygen concentrations, but it is possible that during the sampling period, more sulfide may have diffused to the interface from below, limiting nitrifier activity (Joye and Hollibaugh 1995).

In our study, nitrification was active even in depths where oxygen concentrations were below detection limit (0.89 μmol L^{-1}), and the highest activities were always measured at oxygen concentrations below 22 μmol L^{-1} (Fig. 6). In pure cultures, nitrification rates are highest at oxygen concentrations $<$ 232 μmol L^{-1} for ammonia-oxidizing bacteria (AOB) (Gundersen 1966), and at $<$

Table 3. The highest nitrification potential and in situ rates reported from different areas (nmol N L^{-1} d^{-1}).

Location	V_{max}	In situ	Reference
Baltic Sea			
Northern Baltic Sea	57	12	This study
Landsort Deep	157	84	This study
Gotland Deep	89	34	This study
Gotland Deep		202	Bauer (2003)
Gotland Deep	280		Enoksson (1986)
Gulf of California	93		Beman et al. (2008)
Eastern tropical South Pacific			
Off Peru	144		Lam et al. (2009)
Black Sea	13		Lam et al. (2007)

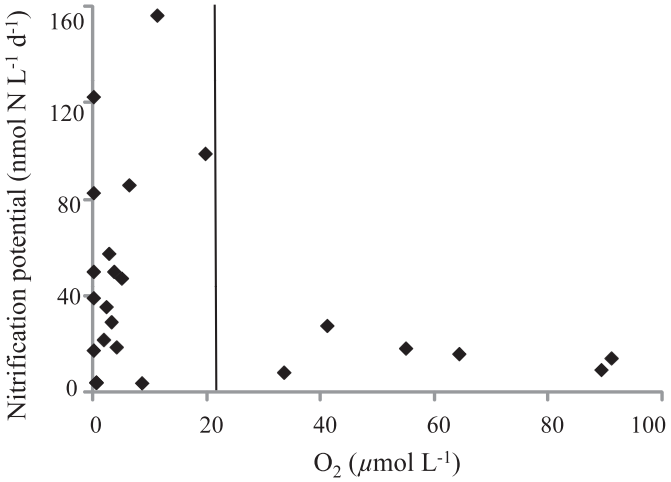

Fig. 6. Nitrification potential (nmol N L^{-1} d^{-1}) at different oxygen concentrations measured in this study. Higher than 20 nmol N L^{-1} d^{-1} rates were only measured at concentrations below 22 μmol O_2 L^{-1} (vertical line).

201 μmol L^{-1} for ammonia-oxidizing archaea (AOA) (Hatzenpichler et al. 2008). The AOA have also been detected from the water column of the eastern tropical North Pacific at oxygen concentration $<$ 3.1 μmol L^{-1} (Francis et al. 2005) and from the Black Sea at $<$ 0.89 μmol L^{-1} (Coolen et al. 2007). The required minimum concentration for nitrification, while not known for the Baltic Sea nitrifiers, is therefore also likely to be very low.

Bauer (2003) suggested that the nitrifying community of the Baltic Sea redoxcline consists of Betaproteobacteria that are phylogenetically related to *Nitrosomonas* and *Nitrospira*. Later, Labrenz et al. (2010) found that the crenarchaeotal subcluster GD2 was expressing amoA in the Baltic Sea redoxcline. The AOB excel in environments with high ammonium and oxygen availabilities and no sulfide, whereas the AOA are dominant in low ammonium, oxygen, and sulfide conditions (Erguder et al. 2009), and may also tolerate up to 30 μmol L^{-1} sulfide concentrations (Coolen et al. 2007). If the Baltic nitrifier community is dominated by the AOA, as suggested by the findings of Labrenz et al. (2010), the active nitrification layer can possibly reach deeper suboxic, even sulfidic, depths, where nitrification was previously considered impossible.

Because the conditions at the redoxcline are highly dynamic, the actively nitrifying microorganisms and the nitrification rates are constantly changing, and nitrification proceeds more as pulses than at steady rates, which makes estimation of in situ nitrification rates challenging. In this study, the ambient ammonium availability was in most cases lower than the saturation concentration (3 μmol L^{-1}; Bauer 2003), suggesting that nitrification was limited by ammonium availability. Extrapolating the in situ rates calculated for the depths sampled in September, 67–75 m at the Landsort Deep (8-m layer) and 85–115 m at the Gotland Deep (30-m layer), nitrate was produced at a rate of 0.11 and 0.06 mmol N m^{-2} d^{-1}, respectively. As the measured removal rates, tens of meters below the nitrifying layer, were 14.1 and 1.4 mmol N m^{-2} d^{-1} in September, it is clear that also the removal processes were substrate limited, demonstrated also by the high potential compared to in situ denitrification rates and by the nutrient profiles in which hardly any oxidized nitrogen compounds could be found in the anoxic depths, indicating tight coupling between nitrification and denitrification.

Annual nitrogen removal in the central basin—Models (Schaffer and Rönner 1984; Savchuk 2005; Yakushev et al. 2007), budget calculations (Schneider et al. 2002; Gustafsson and Stigebrandt 2007; Vahtera et al. 2007), isotope signatures of nitrate (Voss et al. 2005), and nutrient profiles (Vahtera et al. 2007), as well as direct process measurements (Rönner and Sörensson 1985; Brettar and Rheinheimer 1991; Hannig et al. 2007) all indicate active nitrogen removal in the deep water of the Baltic proper. The rate estimates range from 0.04 mmol N m^{-2} d^{-1}, assuming most of the removal takes place in the sediments (Schaffer and Rönner 1984), to 2.2 mmol N m^{-2} d^{-1} (Brettar and Rheinheimer 1991), approximately balancing the annual inputs of nitrogen to the Baltic proper or at the very least the input by N fixation (Schneider et al. 2002; Voss et al. 2005).

Extrapolating the in situ rates, calculated in the present study, to the 50-m-thick water layer in which denitrification potential was detected and nitrite or nitrate could be measured at the Gotland Deep in March gives a N removal rate of 21 mmol N m^{-2} d^{-1}. It is also possible that denitrification took place even deeper than we sampled, although at low rates due to limited availability of nitrite and nitrate and the inhibiting effect of high concentrations of sulfide at the greater depths. The calculated rate is 10–20 times higher than the previous, modeled estimate. Considering the large variability in the rates in time and space, it is, however, not unrealistically high. The nitrate and nitrite measured in the denitrifying depths in March were likely a result of a recent vertical mixing, and the stores of oxidized nitrogen species in these depths are not replenished regularly. At the rates measured, these would have been used up within a few days, demonstrating the ephemeral, batch-like nature of the nitrogen removal several meters

below the redoxcline. Much less activity was found during the other sampling times. Still, if nitrogen removal at a rate measured on March would take place for 10–14 d, only, within a year, it would already account for the amount of nitrogen modeled to be removed in a whole year (Schneider et al. 2002; Savchuk 2005). However, this calculation is based on results from one station, and hardly any N_2 production could be measured nearby, despite availability of both sulfide and nitrite. It is likely that the distributions of oxygen, sulfide, ammonium, nitrite, and nitrate are very patchy in early spring, after winter storms, and create transient hot spots of activity that disappear once the calmer summer season becomes established.

In the deep, almost permanently anoxic parts of the central Baltic proper, only the water-column removal processes are active, because lack of nitrite or nitrate precludes denitrification in the sediments. Compared to sedimentary denitrification in the shallower, oxic areas of the Baltic Sea, however, the water-column nitrogen removal is of small importance. Denitrification rates ranging from 10 μmol N m^{-2} d^{-1} to 670 μmol N m^{-2} d^{-1} have been measured in the Baltic proper sediments, with the lowest rates in sediments experiencing hypoxia at the seafloor (Tuominen et al. 1998; Deutsch et al. 2010). While these rates are clearly lower than those measured in March in the water column, they are more constant in time, in the end removing more nitrogen from the Baltic. In the shallower parts of the Baltic Sea, such as the Gulf of Finland, hardly any water-column nitrogen removal can be expected to occur due to small volume of water below the halocline. Still, increased hypoxia below the halocline has already more than halved the sedimentary denitrification rate from 39,000,000 kg N in the early 2000s (Hietanen and Kuparinen 2008) to 16,000,000 kg N in the late 2000s (Jäntti et al. 2011). Similarly, Deutsch et al. (2010) showed by modeling that a rise of the halocline in the Baltic proper from 100-m to 80-m depth, increasing the anoxic area, would decrease the nitrogen removal in the sediments by 30%.

Hypoxia and DIN removal in the Baltic Sea—The results presented here clearly show that the notion of inorganic nitrogen pool diminishing together with increasing hypoxia (Vahtera et al. 2007) should be interpreted with caution. The alternating oxygen concentrations, caused by both large-scale events such as inflows and upwelling, and by smaller-scale eddy diffusion and turbulence across the redoxcline, create conditions similar to those used in wastewater treatment plants to remove nitrogen: In oxic conditions, ammonium, the end product of organic matter mineralization, is nitrified to nitrite and nitrate, to be removed by denitrification and anammox when hypoxia and anoxia settle in. The negative correlation between the hypoxic water volume and the amount of dissolved inorganic nitrogen can therefore not be seen as evidence of ever-increasing nitrogen removal capacity with increasing hypoxia, without considering the need for the aerobic substrate-producing process preceding nitrogen removal. Kuparinen and Tuominen (2001) demonstrated accumulation of ammonium in sediments during anoxic conditions, followed by nitrification and subsequent denitrification after oxic conditions had been reestablished by large inflow events. Gustafsson and Stigebrandt (2007) showed that while nitrate is lost, ammonia and total nitrogen concentrations increase during stagnation. Increase in hypoxic volume is coupled to increase in the area of anoxic seafloor, which has the effect of shutting down the sedimentary denitrification. In the light of the results presented here, hypoxia-enhanced nitrogen removal cannot be expected to counteract increases in nitrogen loading, or to replace sedimentary denitrification. Since hypoxia unarguably also has other negative effects on the water ecosystem, fighting the anthropogenic eutrophication-driven hypoxia in the Baltic Sea, by reducing nutrient loading to the basin, is of utmost importance for a better future for the sea.

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