The Effects of Hypoxia on Sediment Nitrogen Cycling in the Baltic Sea

Helena Jäntti, Susanna Hietanen

Received: 8 April 2011 / Revised: 21 December 2011 / Accepted: 23 December 2011 / Published online: 14 January 2012

Abstract Primary production in the eutrophic Baltic Sea is limited by nitrogen availability; hence denitrification (natural transformation of nitrate to gaseous N_2) in the sediments is crucial in mitigating the effects of eutrophication. This study shows that dissimilatory nitrate reduction to ammonium (DNRA) process, where nitrogen is not removed but instead recycled in the system, dominates nitrate reduction in low oxygen conditions ($O_2 < 110 \mu M$), which have been persistent in the central Gulf of Finland during the past decade. The nitrogen removal rates measured in this study show that nitrogen removal has decreased in the Gulf of Finland compared to rates measured in mid-1990s and the decrease is most likely caused by the increased bottom water hypoxia.

Keywords Baltic Sea · Sediment · Hypoxia · Nitrogen · Denitrification · DNRA

INTRODUCTION

Baltic Sea is a large brackish water inland sea with a drainage basin inhabited by approximately 80 million people. High anthropogenic pressure has caused severe changes in the Baltic Sea ecosystem and currently Baltic Sea is one of the most polluted marine areas in the world. Baltic Sea has a hypoxic water zone ($O_2 < 2 \text{ ml l}^{-1}$ or $90 \mu M$; Diaz and Rosenberg 1995) that covers up to 20% of the total Baltic Sea area (Conley et al. 2002). Hypoxia has been present in the Baltic Sea since its formation to brackish water basin (approximately 8000 years ago) due to salinity stratification that prevents complete mixing of the water column. However, hypoxia has increased since the industrial revolution in 1850s and the current extent of hypoxia is regarded to be caused by excessive nutrient load from the drainage basin

(Zillén et al. 2008). Hypoxia further fuels eutrophication in the Baltic Sea because hypoxic events enhance phosphorus (P) release from sediment. Increased P availability stimulates primary production, particularly by diazotrophic (nitrogen fixing) cyanobacteria, which in turn causes higher decomposition, depleting oxygen (O_2) even more (Vahtera et al. 2007). Once hypoxia occurs, it is likely to re-occur and the effects of hypoxia become more difficult to reverse (Conley et al. 2009a).

Primary production in the Baltic Sea is limited by nitrogen (N) (Kivi et al. 1993; Tamminen and Andersen 2007) and therefore the natural N removal processes in sediments can counteract eutrophication. N can be removed from the water ecosystem by denitrification and anaerobic ammonium oxidation (anammox) processes, where nitrate (NO_3^-) and nitrite (NO_2^-) are reduced to dinitrogen gas (N_2). Both processes are anoxic, but coupled to NO_3^- producing nitrification process, which requires O_2 . Therefore, anoxia decreases sediment N removal rates. Denitrification dominates N removal in the Baltic Sea sediments and anammox, despite its periodic occurrence, is not regarded a major NO_3^- reduction pathway (Hietanen 2007; Hietanen and Kuparinen 2008; Jäntti et al. 2011). NO_3^- can also be reduced in a dissimilatory nitrate reduction to ammonium (DNRA) process, in which NO_3^- is converted to ammonium (NH_4^+) instead of N_2 (Jørgensen 1989; Christensen et al. 2000). DNRA enhances vicious cycle of eutrophication because N, instead of being removed, is recycled in a bioavailable form.

Denitrification in the Baltic Sea sediments has been found to be tolerant against short-term anoxia (Hietanen and Lukkari 2007), but the effects of prolonged hypoxia and anoxia on N cycling are not known. DNRA has been suggested to be an important process in the Baltic Sea sediments in low O_2 conditions (Kuparinen and Tuominen

2001; Karlson et al. 2005; Hietanen 2007) but direct rate measurements have been lacking. The purpose of this study was to investigate the effect of persistent hypoxia on benthic denitrification and DNRA processes in the Baltic Sea sediments.

MATERIALS AND METHODS

Sampling and Incubations

Samples were collected onboard R/V Aranda (Finnish Environmental Institute) during three cruises in 2008–2009 (Table 1). In May 2008, several of the Gulf of Finland stations sampled in 1990s by Tuominen et al. (1998) were re-sampled. In June 2008, the sampling stations were located along an O₂ gradient in the northern Gulf of Finland. In June 2009, the samples were collected from Gulf of Finland, Baltic Proper and southern Baltic Sea (Fig. 1). Temperature and salinity were recorded using a CTD (SeaBird911plus, Sea-Bird Electronics, Inc., Bellevue, WA). Sediment was collected in acryl plastic cores by using a twin corer with sediment and water in each core. The O₂, NO₃[−], and NH₄⁺ concentrations in the bottom water were measured by withdrawing a water sample from approximately 5 cm above the sediment surface in a core. The loss-on-ignition (LOI%; combustion at 550°C for 3 h), reflecting sediment organic content, was measured from the top 1 cm of the sediment. For the process measurements, each large core was sub-sampled into three or four smaller acryl plastic cores (Ø 26 mm, height 20 cm), so that there was approximately 5 cm of sediment and 13 cm of water in each sub-sample. The remaining volume was filled by caps, leaving no headspace in the cores. The sediment samples from

different casts were randomized for the treatments in which they were enriched with potassium nitrate (K¹⁵NO₃[−], 99% ¹⁵N, Cambridge Isotope Laboratories, Andover, MA) to final concentrations of 40 μM ¹⁵NO₃[−], 80 μM ¹⁵NO₃[−], 120 μM ¹⁵NO₃[−], and 160 μM ¹⁵NO₃[−] in the overlying water (*n* = 4 per concentration). The cores were incubated in dark for 3–4 h at in situ temperature with magnetic stirring bars in the caps. After incubation, the sediment was mixed with the overlying water and allowed to settle for approximately 3 min. A 12 ml water sample for isotopic analysis of N₂ was withdrawn from the top of the core into a gas tight glass vial (Exetainer, Labco Scientific, High Wycombe, UK) containing 0.5 ml ZnCl₂ (100% w/v). The N₂ samples from cruises in 2008 were analyzed with 20–20 isotope ratio mass spectrometer (IRMS) 205 linked to an ABCA-G gas purification module and Gilson autosampler (Europa Scientific Limited, Crewe, Cheshire, UK) in Iso-Analytical laboratories in Crewe, Cheshire, UK, and from cruise in 2009 with RoboPrep G+ in line with Tracer-Mass (Europa Scientific) in the National Environmental Research Institute (NERI), in Silkeborg, Denmark. The remaining core content was centrifuged (1500 rpm, 10 min) and the supernatant was filtered (prewashed 0.8/0.2 μm double filter syringe, Acrodisc, Pall Scientific, UK) and frozen immediately in −20°C for ¹⁵NH₄⁺ analysis.

¹⁵NH₄⁺ Analysis

The ¹⁵NH₄⁺ analysis was modified from Sigman et al. (1997) and Holmes et al. (1998). First, the NH₄⁺ concentration of the samples was determined (Solorzano 1969) to ensure an optimal amount of NH₄⁺ (1–7 μmol N) for the extraction. The samples were then placed in 100 ml bottles, the salinity of the samples was adjusted to 30 with sodium

Table 1 The average and standard error (SE) of DNRA and denitrification rates, percentage of DNRA and denitrification that is based on NO₃[−] from the bottom water, and the physicochemical parameters of the sampling sites

Site	Sampling time	Number of samples (<i>n</i>)	DNRA (SE) (μmol N m ^{−2} day ^{−1})	Denitrification (SE) (μmol N m ^{−2} day ^{−1})	% Based on bottom water NO ₃ [−]	Depth d(m)	O ₂ (μM)	NO ₃ [−] (μM)	NH ₄ ⁺ (μM)	LOI%
LL9	May-08	10	125 (25)	46 (7)	21	65	98	10.7	0.0	4.9
GF 1	May-08	15	604 (92)	49 (16)	4	83	40	1.5	1.3	0.0
GF 2	May-08	14	1060 (148)	89 (12)	11	83	31	5.1	1.5	2.7
XV-1	May-08	14	329 (76)	1619 (413)	24	64	241	8.7	6.7	0.9
St1	Jun-08	14	592 (119)	90 (33)	15	70	45	8.2	6.1	12.6
St2	Jun-08	14	327 (120)	316 (138)	5	64	112	2.0	6.5	10.9
St5	Jun-08	10	487 (66)	387 (174)	7	60	183	1.2	4.2	9.9
HYPERGoF	Jun-09	15	33 (6)	93 (67)	40	58	120	8.4	1.2	n/a
HBP 137	Jun-09	16	33 (8)	121 (8)	27	62	49	8.5	0.2	n/a
LF1	Jun-09	14	13 (2)	61 (14)	10	78	241	2.0	0.1	n/a
HAS	Jun-09	12	23 (8)	38 (10)	46	71	174	3.3	0.2	n/a

Fig. 1 The sampling stations in the Baltic Proper, southern Baltic Sea, and the Gulf of Finland

chloride (NaCl), 1 g of magnesium oxide (MgO)/100 ml of sample was added, and the pH was set to 11 with 10% sodium hydroxide (NaOH). The NH_4^+ was extracted in diffusion packets [an acidified (30 μl 4 N phosphoric acid (H_3PO_4) fiberglass filter (Whatman, GF/D, diameter 5 mm, Whatman, Maidstone, Kent, UK) between membrane filters (Millipore LCWP 02500, Millipore, Billerica, MA)]. After adding a diffusion pack and increasing pH, the bottles were immediately closed and sealed with Parafilm (American Can Company, Greenwich, CT) and polytetrafluoroethylene (PTFE)-coated screw caps. The bottles were incubated upside down for 14 days in room temperature (22°C) on a shaker table (150 rpm). The NH_3 diffusion was done in room temperature to minimize the dissolved organic nitrogen (DON) blank, although it is a compromise with lower NH_4^+ recovery (Holmes et al. 1998). However, in tracer studies, the fractionation caused by incomplete recovery is insignificant (Mathieu et al. 2007) and therefore the lower temperature was chosen for the incubation. After incubation, the diffusion packets were removed, rinsed with 10% hydrochloric acid (HCl) and MilliQ-water (Millipore), and placed on a desiccator under sulfuric acid (H_2SO_4) atmosphere to dry. After 2 days, the packets were disassembled and the fiberglass filters were packed into silver foil cups (Elemental Microanalyses Ltd, Toft, Cambridge, UK). The isotopic ratio of the extracted N was analyzed using Europa Scientific 20–20 EA-IRMS in Iso-Analytical laboratories in Crewe, Cheshire, UK.

Calculations and Statistics

Denitrification rates were calculated by using the isotope pairing technique (IPT), which can differentiate denitrification that is based on NO_3^- available in the bottom water (D_w) from the coupled nitrification–denitrification (D_n) (Nielsen 1992). In IPT, the samples are amended with $^{15}\text{NO}_3^-$ and the production of ^{15}N -labeled dinitrogen gas ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) is measured. The in situ denitrification, based on $^{14}\text{NO}_3^-$ available in the bottom water and produced in nitrification, is calculated assuming random pairing of the added $^{15}\text{NO}_3^-$ and the ambient $^{14}\text{NO}_3^-$ and binomial distribution of the productions of $^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$ (Nielsen 1992). The IPT cannot be used to measure denitrification if anammox is present because anammox produces $^{29}\text{N}_2$ by combining the added $^{15}\text{NO}_3^-$ and ambient $^{14}\text{NH}_4^+$, violating the binomial distribution of $^{28}\text{N}_2$, $^{29}\text{N}_2$, and $^{30}\text{N}_2$ productions. The presence of anammox can be detected and its effect on denitrification calculations removed when $^{15}\text{NO}_3^-$ is added as concentration series in the samples (r-IPT) (Risgaard-Petersen et al. 2003, 2004). In these samples, the in situ denitrification rates showed no concentration dependence, confirming that the

anammox process was not active and the assumptions behind the calculations for denitrification were met. In such case, all the samples are considered replicates despite the different amount of $^{15}\text{NO}_3^-$ added.

The DNRA rates were calculated according Christensen et al. (2000), where the $^{15/14}\text{N}$ ratio of the NO_3^- consumed is assumed to be similar to the $^{15/14}\text{N}$ ratio of N_2 produced by denitrification because DNRA and denitrification occur at the same anoxic sediment layer. This method, similar to the IPT, also differentiates between DNRA that is coupled to nitrification (DNRA_n) and the DNRA based on NO_3^- in the bottom water (DNRA_w). In our data, the in situ DNRA rates showed no concentration dependence and therefore it can be assumed that the NO_3^- consumption by denitrification and DNRA were proportional to each other and the assumptions behind the calculations were met. Similar to the IPT samples, all DNRA samples are considered replicates despite the different amount of $^{15}\text{NO}_3^-$ added.

The correlations between the environmental parameters, DNRA, and denitrification were determined using the Spearman's correlation analysis and deemed significant when $p < 0.05$. The curve describing the correlation between the DNRA/denitrification ratio and O_2 concentration was fitted by using Sigmaplot software (Systat Software Inc. Chicago, IL).

RESULTS

Denitrification and DNRA

Denitrification (38–1619 $\mu\text{mol N m}^{-2} \text{ day}^{-1}$) and DNRA (33–1060 $\mu\text{mol N m}^{-2} \text{ day}^{-1}$) rates exhibited large variability between stations, but there was a clear pattern of high DNRA and low denitrification rates when the O_2 concentration was below 110 μM (Table 1; Fig. 2). 55–95% of the NO_3^- used in denitrification and DNRA was provided by the coupled nitrification (Table 1). No anammox was detected in any of the stations. NH_4^+ accumulation in the bottom water was observed in the Gulf of Finland stations XV-1, ST1, ST2, and ST5 (Table 1). Sulfur bacteria *Beggiatoa* spp. filaments covered sediment surface in stations LL9, GF1, GF2, and HAS.

Correlation of Environmental Parameters with NO_3^- Reduction Rates

Some clear patterns were observed between the process rates and the environmental parameters (Table 2). The total denitrification correlated positively with NH_4^+ and O_2 concentration, while total DNRA correlated positively with NH_4^+ concentration and negatively with O_2 and NO_3^-

Fig. 2 The average and SE of DNRA to denitrification ratio and the O₂ concentration from all samples. Station HBP137, showing different response to O₂ concentration, is *circled*. Due to different response, the rates from station HBP137 were excluded from curve fitting

Table 2 The correlations (*R*) between the NO₃[−] reduction processes and the environmental parameters

	O ₂	NH ₄ ⁺	NO ₃ [−]	LOI%
Total denitrification	0.229	0.427	–	–
<i>D_n</i>	0.183	0.445	–	–
<i>D_w</i>	0.354	–	0.478	–
Total DNRA	−0.420	0.555	−0.283	–
DNRA _n	−0.0437	0.542	−0.307	–
DNRA _w	−0.253	0.517	–	–
DNRA/denitrification	−0.519	–	−0.369	–

Only significant correlations (*p* < 0.05) are shown

concentrations (Table 2). *D_n* correlated positively with O₂ and NH₄⁺ concentrations, and *D_w* correlated positively with O₂ and NO₃[−] concentrations. DNRA_n correlated negatively with O₂ and NO₃[−] concentrations and positively with NH₄⁺ concentration. DNRA_w correlated negatively with O₂ and positively with NH₄⁺ concentrations (Table 2). No correlation was found between the DNRA_w and NO₃[−], and the lack of correlation was probably caused by the low total DNRA rates in stations where the percentage of DNRA based on NO₃[−] from the bottom water was the highest (Table 1). The dominance toward DNRA increased below 110 μM O₂. Although the correlation between the denitrification/DNRA ratio and O₂ appears to exponential (Fig. 2), it was statistically significant (*p* < 0.05) as a linear function (Fig. 2). Only the station HBP137 in southern Baltic Sea differed from this trend and it was excluded from the curve fitting (Fig. 2, circled).

DISCUSSION

Denitrification

Denitrification takes place under anoxic conditions when its substrates, organic carbon and NO₃[−], are available (Kemp et al. 1990; Sloth et al. 1995; Hietanen and Kuoparinen 2008). However, in this study the concentrations of these substrates did not correlate with denitrification rates, suggesting that they do not limit denitrification in the open Gulf of Finland in spring/early summer (Table 2).

A positive correlation between denitrification and NH₄⁺ concentration (Table 2) has been observed also in the coastal Gulf of Finland sediments (Jäntti et al. 2011). High NH₄⁺ availability can indirectly stimulate denitrification by feeding the coupled nitrification process (Jäntti et al. 2011). NH₄⁺ accumulation is also an indicator of increased mineralization that supplies denitrification with labile organic carbon. High NH₄⁺ and O₂ concentrations are likely to be the reason for the extremely high denitrification rate observed in station XV-1 in the eastern Gulf of Finland (Table 1). In addition, the *Marenzelleria* spp. (invasive benthic polychaete) were present for the first time in 2008 in station XV-1 (J. Norkko, pers. comm.), which may have stimulated denitrification by increasing the oxic–anoxic interfaces in the sediment.

The denitrification rates in the Gulf of Finland in May 2008 were, with the exception of station XV-1, lower than the coastal Gulf of Finland values in that same spring (Jäntti et al. 2011) and lower than spring denitrification rates measured in the mid-1990s (Tuominen et al. 1998).

The rates were higher in June, probably because mineralization of the spring bloom had progressed, but even then denitrification in the hypoxic station ST1 remained low (Table 1). The denitrification rate in the hypoxic station HBP137 in the southern Baltic Sea was higher than in the hypoxic Gulf of Finland stations, but lower than in oxidized stations in the area (Deutsch et al. 2010). In the two stations close to each other in the Baltic Proper (LF1 and HAS), both DNRA and denitrification rates were low. The station HAS had *Beggiatoa* spp. and laminations started immediately below sediment surface, indicating prolonged anoxia at the station. Therefore, the detected O_2 must have entered the area only recently, and the NO_3^- reducing bacteria were possibly still adapting to the change in the redox conditions.

Nitrification

In anoxic conditions, nitrification cannot proceed, the coupled nitrification–denitrification pathway is shut down, and NH_4^+ accumulates in the bottom water of the Baltic Sea (Fig. 3c) (Kuparinen and Tuominen 2001). NH_4^+ accumulation begins already before complete anoxia, but it has not been clear whether the NH_4^+ accumulation in hypoxia is caused by DNRA reducing NO_3^- produced by nitrification, or because NH_4^+ cannot be utilized by nitrification due to low O_2 availability. The total nitrification rates were not measured in this study, but active nitrification in the hypoxic stations was indicated by the high coupled nitrification–DNRA rates and presence of NO_3^- in the bottom water despite the low O_2 concentrations (Table 1). In addition, the DNRA rates were high in some stations but no accumulation of NH_4^+ was observed until June when the sediment organic content increased; hence nitrifiers must have actively oxidized NH_4^+ to NO_3^- .

Nitrification rates measured in the coastal Gulf of Finland show a seasonal pattern of highest potential for

nitrification occurring in spring. However, the low availability of NH_4^+ limits nitrification keeping the in situ rates low. The in situ nitrification rates increase during summer, but the potential for nitrification decreases (Jäntti et al. 2011), probably because nitrifiers have to compete for O_2 with heterotrophic bacteria, which are mineralizing the organic matter sedimented from the spring bloom. The competition for O_2 between nitrifiers and heterotrophic bacteria probably intensifies in hypoxic conditions prevailing in the sediments of the open sea, and nitrification can be expected to decrease more than in the coastal sediments that do not suffer from such severe O_2 depletion. Hence, in the open sea sediments, NH_4^+ does not accumulate in spring despite the high DNRA rates, because mineralization rates are low and NH_4^+ is actively oxidized to NO_3^- by nitrification. In summer, the production of NH_4^+ by mineralization increases, and the competition for O_2 between nitrifying and heterotrophic bacteria intensifies, decreasing nitrification. The NH_4^+ accumulation is then caused by the combined effects of DNRA, increased mineralization, and decreased nitrification.

DNRA

The dominance of DNRA over denitrification in NO_3^- reduction clearly increased below the $110 \mu M$ (2.4 ml l^{-1}) O_2 concentration (Fig. 2). DNRA has been shown to increase in high organic carbon concentrations, especially when the availability of NO_3^- is low, and to diminish in relation to denitrification once the organic carbon to NO_3^- ratio decreases (Kelso et al. 1997; Christensen et al. 2000). The DNRA rates in this study were high despite the low sediment organic content and relatively high NO_3^- availability in the bottom water (Table 1). The high rates may be explained by the use of H_2S as an electron donor in DNRA (Sørensen et al. 1980; Brunet and Garcia-Gill 1996). This is also supported by the presence of *Beggiatoa*

Fig. 3 Nitrogen cycling in sediments when the bottom water is oxic (a), hypoxic (b), and anoxic (c). See text for details

spp. filaments at the sediment surface in hypoxic stations. *Beggiatoa* spp. are sediment-bound, continuously gliding filamentous bacteria that can be chemolithoautotrophic or chemolithoheterotrophic, and occupy interfaces between oxic and sulfidic conditions (Schulz and Jørgensen 2001). Although the elemental sulfur (S^0) oxidation with O_2 is a better known pathway for *Beggiatoa* spp., some species of *Beggiatoa* can reduce NO_3^- to NH_4^+ with H_2S (Sayama et al. 2005). *Beggiatoa* spp. were also present in the station HAS in the Baltic Proper, despite the relatively high O_2 concentration. As suggested earlier, the O_2 conditions in that area may have improved only a short time before the sampling, and the low DNRA rates measured at that station may have been caused by O_2 inhibition, since *Beggiatoa* spp. only tolerate very low levels of O_2 (Schulz and Jørgensen 2001).

The Effect of Hypoxia on Nitrogen Cycling

The O_2 concentration in the bottom water and the O_2 consuming processes within the sediment determine the penetration of O_2 into the sediment. When the bottom water is oxic, O_2 penetrates few mm into the sediment and nitrification is converting the NH_4^+ to NO_3^- in the oxic layer. Deeper in the sediment, the oxidized zone, characterized by high concentrations of oxidized inorganic compounds (i.e., NO_3^- , Mn_4^+ , Fe_3^+), is present with denitrification proceeding in this zone (Kristensen 2000). H_2S accumulates only deeper in the reduced sediment (Fig. 3a). In such conditions, short term decreases in bottom water O_2 can even stimulate denitrification by shortening the physical distance between NO_3^- production and reduction (Hietanen and Lukkari 2007). If the bottom water is persistently hypoxic and the O_2 consumption within the sediment surface is high, the oxidized zone, where conditions for denitrification are favorable, is lost (Kristensen 2000). In such conditions nitrification is still proceeding, but DNRA dominates NO_3^- reduction and N remains in the system (Fig. 3b). The coastal areas, which at least in the Gulf of Finland have low DNRA rates (Jäntti et al. 2011), do not establish the nitrification–DNRA cycle, because hypoxia in the coastal areas is typically a seasonal, late summer phenomena, with normal O_2 conditions returning at autumn turnover. In long-term anoxia, the oxidized surface layer is completely lost, benthic nitrification ceases, and NH_4^+ is released from the sediment (Fig. 3c).

The changes in N removal in increasing hypoxia have remained speculative in the Baltic Sea (Conley et al. 2009b). A negative correlation between the dissolved inorganic nitrogen (DIN) pool and the volume of hypoxic water has been found in the Baltic Proper, Gulf of Finland, and Gulf of Riga (Vahtera et al. 2007; Savchuk 2010),

suggesting that N removal intensifies with increasing hypoxia. However, while hypoxia has spread and intensified since the mid-1990s, sediment denitrification rates have not increased but instead decreased in the open Gulf of Finland. Many of the stations where denitrification was measured in the mid-1990s were sulfidic in 2008 and 2009, making re-sampling futile, as no denitrification could take place due to the absence of NO_3^- and inhibition by H_2S . The negative correlation between DIN and volume of hypoxic water cannot be explained by increased coastal denitrification either, because the coastal benthic denitrification rates, measured in oxidized sediments in 2008–2009, also show a decreasing trend when compared to rates measured in 2003–2004 (Hietanen and Kuparinen 2008; Jäntti et al. 2011).

The only explanation for the proposed increased N removal during extensive hypoxia (Vahtera et al. 2007) would be that it takes place in the water column rather than in the sediments. However, the importance of water column N removal in the Baltic Sea is controversial. Some studies show that denitrification in the Baltic Proper water column is not an important sink for N (Shaffer and Rönner 1984; Brettar and Rheinheimer 1992). Other studies have found high denitrification potential (Hannig et al. 2007; Hietanen et al. 2012), but no NO_3^- has been available at the depths where the highest potentials were found. In the Gulf of Finland the water column N removal cannot be important, even if the process could be active below halocline, because the average depth of the Gulf is 38 m and the halocline is located around 60–80 m, leaving very little volume for hypoxic water (Alenius and Hietala 2008). Therefore, the lower N:P ratio caused by more intense N removal in hypoxia does not apply for the Gulf of Finland. Based on the results presented here and in other studies (Lehtoranta 2003), the lowered N:P ratio is caused by massive P release from the sediments, not by increased N removal during hypoxia and anoxia.

Despite the decreased N removal in the sediments and unchanged N load, there has not been accumulation of N in the water column of the Gulf of Finland (HELCOM 2009). Hence, N must be either transported away from the Gulf of Finland or buried in the sediments. Trends in N burial have not been studied in the Gulf of Finland, but organic matter burial in general increases in low O_2 concentrations. In addition, the burial of phosphorus (P) in the Baltic Sea takes place mainly as organic P, with increased burial under hypoxic and anoxic conditions (Mort et al. 2010). It is likely that organic N is similarly increasingly buried, instead of mineralized, when hypoxia and anoxia settle in.

The increase in hypoxia in the Gulf of Finland, which has decreased denitrification rates from mid-1990s, is partially caused by the hydrography of the Baltic Sea. From 1977 to 1992 no major salt water inflow events from the North Sea

occurred, which worsened the O₂ conditions in the main basins of the Baltic Sea. The hypoxic area reached the western parts of Gulf of Finland, but in the central and eastern parts of the Gulf of Finland the halocline weakened, allowing complete mixing of the water column and the sea floor was oxidized annually. In 1994, the major inflow entered the Gulf of Finland, the halocline re-established, and the O₂ conditions in the Gulf of Finland became and have remained poor since. Hence, in addition to seasonal variation, the sediment N removal capacity in the Gulf of Finland also shows inter-annual variation, which is dependent on the long-term O₂ conditions related to the strength of the halocline.

CONCLUSION

The sediment denitrification in the Gulf of Finland has substantially decreased from the rates measured in mid-1990s. Hypoxic stations particularly have low denitrification rates and NO₃[−] reduction is dominated by DNRA process, where N is not removed but instead recycled in a bio-available form. The observed negative correlation between DIN and the volume of hypoxic water in the Baltic Sea cannot be explained by more intense denitrification in the sediments and the only plausible explanation for the negative correlation is more intensive N removal in the water column. In the Gulf of Finland, however, the volume of hypoxic water is small, and therefore the water column denitrification cannot be an important sink for N. As the N concentration in the Gulf of Finland has not increased despite decreased denitrification and unchanged N load, N is either transported away from the Gulf of Finland or, more likely, buried as organic N.

Acknowledgments We acknowledge the funding provided by the Onni Talas Foundation, Academy of Finland (116477), and EU-Bonus projects Assessment and Modeling of Baltic Ecosystem Response (AMBER) and Hypoxia Mitigation for Baltic Sea Ecosystem Restoration (HYPER). We are grateful for the constructive comments provided by Juha Niemistö, Heidi Holmroos, and an anonymous reviewer.

REFERENCES

- Alenius, P., and R. Hietala. 2008. Happitilanne. In *MERI – Report Series of the Finnish Institute of Marine Research No. 64*, ed. M. Raateoja, Helsinki, Finland (In Finnish, English summary).
- Brettar, I., and G. Rheinheimer. 1992. Influence of carbon availability on denitrification in the central Baltic Sea. *Limnology and Oceanography* 37: 1146–1163.
- Brunet, R.C., and L.J. Garcia-Gill. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiology Ecology* 21: 131–138.
- Christensen, P.B., S. Rysgaard, N.P. Sloth, T. Dalsgaard, and S. Schwaerter. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquatic Microbial Ecology* 21: 73–84.
- Conley, D.J., S. Björk, E. Bonsdorf, J. Carstensen, G. Destouni, B.G. Gustafsson, S. Hietanen, M. Kortekaas, et al. 2009a. Hypoxia-related processes in the Baltic Sea. *Environmental Science and Technology* 43: 3412–3420.
- Conley, D.J., J. Carstensen, R. Vaquer-Sunyer, and C.M. Duarte. 2009b. Ecosystem thresholds with hypoxia. *Hydrobiologia* 629: 21–29.
- Conley, D.J., C. Humborg, L. Rahm, O.P. Savchuk, and F. Wulff. 2002. Hypoxia in the Baltic Sea and basin-scale changes in phosphorus biogeochemistry. *Environmental Science and Technology* 36: 5315–5320.
- Deutsch, B., S. Foster, M. Wilhelm, J.W. Dippner, and M. Voß. 2010. Denitrification in sediments as a major nitrogen sink in the Baltic Sea: an extrapolation using sediment characteristics. *Biogeosciences Discussions* 7: 2487–2521.
- Diaz, R.J., and R. Rosenberg. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology* 33: 245–303.
- Hannig, M., G. Lavik, M.M.M. Kuypers, D. Woebken, W. Martens-Habben, and K. Jürgens. 2007. Shift from denitrification to anammox after inflow events in the central Baltic Sea. *Limnology and Oceanography* 52: 1336–1345.
- HELCOM. 2009. Eutrophication in the Baltic Sea. An integrated thematic assessment of the effects of nutrient enrichment in the Baltic Sea region. Baltic Sea Environment Proceedings No. 115B, Helsinki, Finland.
- Hietanen, S. 2007. Anaerobic ammonium oxidation (anammox) in sediments of the Gulf of Finland. *Aquatic Microbial Ecology* 48: 197–205.
- Hietanen, S., and J. Kuparinen. 2008. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea. *Hydrobiologia* 596: 67–77.
- Hietanen, S., and K. Lukkari. 2007. Effects of short-term anoxia on benthic denitrification, nutrient fluxes and phosphorus forms in coastal Baltic sediment. *Aquatic Microbial Ecology* 49: 293–302.
- Hietanen, S., H. Jäntti, C. Buizert, K. Jürgens, M. Labrenz, M. Voss, and J. Kuparinen. 2012. Hypoxia and nitrogen processing in the Baltic Sea water column. *Limnology and Oceanography*. 57: 325–337.
- Holmes, R.M., J.W. McClelland, D.M. Sigman, B. Fry, and B.J. Peterson. 1998. Measuring ¹⁵N-NH₄⁺ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry* 60: 235–243.
- Jäntti, H., C.F. Stange, E. Leskinen, and S. Hietanen. 2011. Seasonal variation in nitrification and nitrate-reduction pathways in coastal sediments in the Gulf of Finland, Baltic Sea. *Aquatic Microbial Ecology* 63: 171–181.
- Jørgensen, K.S. 1989. Annual pattern of denitrification and nitrate ammonification in estuarine sediment. *Applied and Environmental Microbiology* 55: 1841–1847.
- Karlson, K., S. Hulth, K. Ringdahl, and R. Rosenberg. 2005. Experimental recolonisation of Baltic Sea reduced sediments: Survival of benthic macrofauna and effects on nutrient cycling. *Marine Ecology Progress Series* 294: 35–49.
- Kelso, B., R. Smith, R. Laughlin, and D. Lennox. 1997. Dissimilatory nitrate reduction in anaerobic sediments leading to river nitrite accumulation. *Applied and Environmental Microbiology* 63: 4679–4685.

- Kemp, W.M., P. Sampou, J. Caffrey, M. Mayer, K. Henriksen, and W.R. Boynton. 1990. Ammonium recycling versus denitrification in Chesapeake Bay sediments. *Limnology and Oceanography* 35: 1545–1563.
- Kivi, K., S. Kaitala, H. Kuosa, J. Kuparinen, E. Leskinen, R. Lignell, B. Marcussen, and T. Tamminen. 1993. Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnology and Oceanography* 38: 893–905.
- Kristensen, E. 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. *Hydrobiologia* 426: 1–24.
- Kuparinen, J., and L. Tuominen. 2001. Eutrophication and self-purification: Counteractions forced by large-scale cycles and hydrodynamic processes. *Ambio* 30: 190–194.
- Lehtoranta, J. 2003. Dynamics of sediment phosphorus in the brackish Gulf of Finland. Monographs of the Boreal Environment Research 24. PhD Thesis, Helsinki, Finland, University of Helsinki.
- Mathieu, O., J. Leveque, C. Henault, P. Ambus, M. Milloux, and F. Andreux. 2007. Influence of ^{15}N enrichment on the net isotopic fractionation factor during the reduction of nitrate to nitrous oxide in soil. *Rapid Communications in Mass Spectrometry* 21: 1447–1451.
- Mort, H.P., C.P. Slomp, B.G. Gustafsson, and T.J. Andersen. 2010. Phosphorus recycling and burial in Baltic Sea sediments with contrasting redox conditions. *Geochimica et Cosmochimica Acta* 74: 1350–1362.
- Nielsen, L.P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Ecology* 86: 357–362.
- Risgaard-Petersen, N., L.P. Nielsen, S. Rysgaard, T. Dalsgaard, and R.L. Meyer. 2003. Application of the isotope pairing technique in sediments where anammox and denitrification coexist. *Limnology and Oceanography Methods* 1: 63–73.
- Risgaard-Petersen, N., L.P. Nielsen, S. Rysgaard, T. Dalsgaard, and R.L. Meyer. 2004. Errata: Application of the isotope pairing technique in sediments where anammox and denitrification coexist. *Limnology and Oceanography Methods* 2: 315.
- Savchuk, O.P. 2010. Large-scale dynamics of hypoxia in the Baltic Sea. In *Chemical structure of pelagic redox interfaces: Observations and modelling, handbook of environmental chemistry*, ed. E.V. Yakushev. Berlin: Springer.
- Sayama, M., N. Risgaard-Petersen, L.P. Nielsen, H. Fossing, and P.B. Christensen. 2005. Impact of bacterial NO_3^- transport on sediment biogeochemistry. *Applied and Environmental Microbiology* 71: 7575–7577.
- Schulz, H.N., and B.B. Jørgensen. 2001. Big bacteria. *Annual Review of Microbiology* 55: 105–137.
- Shaffer, G., and U. Rønner. 1984. Denitrification in the Baltic Proper deep-water. *Deep Sea Research Part A. Oceanographic Research Papers* 31: 197–220.
- Sigman, D.M., M.A. Altabet, R. Michener, D.C. McCorcle, B. Fry, and R.M. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: An adaptation of the ammonia diffusion method. *Marine Chemistry* 57: 227–242.
- Sloth, N.P., H. Blackburn, L.S. Hansen, N. Risgaard-Petersen, and B.A. Lomstein. 1995. Nitrogen cycling in sediments with different organic loading. *Marine Ecology Progress Series* 116: 163–170.
- Solorzano, L. 1969. Determination of ammonia in natural water by the phenylhypochlorite method. *Limnology and Oceanography* 14: 799–801.
- Sørensen, J., J.M. Tiedje, and R.B. Firestone. 1980. Inhibition by sulfide of nitric and nitrous oxide reduction by denitrifying *Pseudomonas fluorescens*. *Applied and Environmental Microbiology* 39: 105–108.
- Tamminen, T., and T. Andersen. 2007. Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. *Marine Ecology Progress Series* 340: 121–138.
- Tuominen, L., A. Heinänen, J. Kuparinen, and L.P. Nielsen. 1998. Spatial and temporal variability of denitrification in the sediments of the northern Baltic Proper. *Marine Ecology Progress Series* 172: 13–24.
- Vahtera, E., D.J. Conley, B.G. Gustafsson, H. Kuosa, H. Pitkänen, O.P. Savchuk, T. Tamminen, M. Viitasalo, et al. 2007. Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea. *Ambio* 36: 186–194.
- Zillén, L., D. Conley, T. Andrén, E. Andrén, and S. Björck. 2008. Past occurrences of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. *Earth-Science Reviews* 91: 77–92.

AUTHOR BIOGRAPHIES

Helena Jäntti (✉) is a doctoral student in the University of Helsinki. Her research focus is on nitrogen cycling in the Baltic Sea sediments and water column.

Address: Department of Environmental Sciences, Aquatic Sciences, P.O. Box 65, 00014 University of Helsinki, Finland.

Address: Tvärminne Zoological Station, University of Helsinki, J.A. Palménin tie 260, 10900 Hanko, Finland.

e-mail: helena.jantti@helsinki.fi

Susanna Hietanen is university researcher in the University of Helsinki. Her research focus is on nitrogen cycling in water ecosystems.

Address: Department of Environmental Sciences, Aquatic Sciences, P.O. Box 65, 00014 University of Helsinki, Finland.

Address: Tvärminne Zoological Station, University of Helsinki, J.A. Palménin tie 260, 10900 Hanko, Finland.

e-mail: susanna.hietanen@helsinki.fi