The roles of nitrification and nitrate reduction pathways in nitrogen cycling of Baltic Sea

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Academic dissertation

To be presented
for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Auditorium 2041, Biocenter 2, Viikki Campus on January 20th 2012 at 12 noon.

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ISBN 978-952-10-7381-6 (Paperback)
ISBN 978-952-10-7382-3 (PDF)
http://ethesis.helsinki.fi
Helsinki University Print
Helsinki 2012

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This thesis is compiled from the information given in the following publications. Each publication is referred in the text by its respective roman number.

I) H. Jäntti, E. Leskinen, C. Stange & S. Hietanen. Measuring nitrification in sediments – comparison of two techniques and three $^{15}NO_3^-$ analysis methods (In press in *Isotopes in Environmental and Health Studies*).
The data collection and experimental design were done by H. Jäntti and S. Hietanen. The data was partially analyzed in collaboration with C. Stange with H. Jäntti having the main responsibility of the analyses. The manuscript was written by H. Jäntti (corresponding author) and commented by S. Hietanen and E. Leskinen.

II) H. Jäntti, C. Stange, E. Leskinen & 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.
The data collection and experimental design were done by H. Jäntti and S. Hietanen. The nitrification data analyses were done in collaboration with C. Stange. The rest of the data analyses were done by H. Jäntti and with S. Hietanen assisting with the calculations. The manuscript was written by H. Jäntti (corresponding author) and commented on by S. Hietanen and E. Leskinen.

III) H. Jäntti & S. Hietanen. The effects of hypoxia on sediment nitrogen cycling in the Baltic Sea (In press in *AMBIO*).
The data collection and experimental design were carried out by H. Jäntti and S. Hietanen. Data were analyzed by H. Jäntti, with S. Hietanen assisting with the calculations. The manuscript was written by H. Jäntti (corresponding author) and commented by S. Hietanen.

IV) S. Hietanen, H. Jäntti, C. Buizert, K. Jürgens, M. Labrenz, J. Kuparinen. Hypoxia and nitrogen processing in the Baltic Sea water column (In press in *Limnology and Oceanography*).
The experimental design for the nitrification measurements were taken by H. Jäntti. The nitrification samples were analyzed by H. Jäntti and C. Buizert. The sample collections were taken by S. Hietanen and H. Jäntti. H. Jäntti is responsible of writing the sections on nitrification, with S. Hietanen being the corresponding author of the manuscript.

ABSTRACT

The Baltic Sea is one of the largest brackish water bodies in the world. Primary production in the Baltic Sea is limited by nitrogen (N) availability with the exception of river outlets and the northernmost phosphorus limited basin. The excess human induced N load from the drainage basin has caused severe eutrophication of the sea. The excess N loads can be mitigated by microbe mediated natural N removal processes that are found in the oxic-anoxic interfaces in sediments and water column redoxclines. Such interfaces allow the close coupling between the oxic nitrification process, and anoxic denitrification and anaerobic ammonium oxidation (anammox) processes that lead to the formation of molecular nitrogen gas. These processes are governed by various environmental parameters. The effects of these parameters on N processes were investigated in the northern Baltic Sea sediments. During summer months when the sediment organic content is at its highest, nitrification and denitrification reach their maximum rates. However, nitrification had no excess potential, which was probably because of high competition for molecular oxygen (O_2) between heterotrophic and nitrification microbes. Subsequently, the limited nitrate (NO_3^-) availability inhibited denitrification. In fall, winter and spring, nitrification was limited by ammonium availability and denitrification limited by the availability of organic carbon and occasionally by NO_3^-. Anammox was not an important N removal process in the northern Baltic Sea. Modeling studies suggest that when hypoxia expands in the Baltic Sea, N removal intensifies. However, the results of this study suggest the opposite because bottom water hypoxia ($O_2 <$ 2 ml l^{-1}) decreased the denitrification rates in sediments. Moreover, N was recycled by the dissimilatory nitrate reduction to ammonium (DNRA) process instead of being removed from the water ecosystem. High N removal potentials were found in the anoxic water column in the deep basins of the Baltic Proper. However, the N removal in the water column appeared to be limited by low substrate availability, because the water at the depths at which the substrate producing nitrification process occurred, rarely mix with the water at the depths at which N removal processes were found. Overall, the natural N removal capacity of the northern Baltic Sea decreased compared to values measured in mid 1990s and early 2000. The reason for this appears to be increasing hypoxia.

LIST OF ABBREVATIONS

Anammox	Anaerobic ammonium oxidation
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
ATCC	American Type Culture Collection
ATU	Allylthiourea
ClO_3^-	Chlorate
CTD	Conductivity-temperature-density probe
D_n	Denitrification coupled to nitrification
$D_n(A)$	$^{15}N_2$ production from the $^{15}NH_4^+$ amended samples
D_w	Denitrification based on NO_3^- in the bottom water
DIN	Dissolved inorganic nitrogen
DNRA	Dissimilatory nitrate reduction to ammonium
DON	Dissolved organic nitrogen
GC	Gas chromatograph
H_2S	Hydrogen sulfide
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HELCOM	Helsinki Commission
IPT	Isotope pairing technique
IRMS	Isotopic ratio mass spectrometer
MgO	Magnesium oxide
MnO_x	Manganese oxide (Mn(III)/(IV))
N	Nitrogen
N_2	Molecular nitrogen gas
N_2O	Nitrous oxide
NaOH	Sodium hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
O_2	Molecular oxygen
OMZ	Oxygen minimum zone

P	Phosphorus
PON	Particulate organic nitrogen
QMS	Quadrupole mass spectrometer
r-IPT	Revised isotope pairing technique
SANBALTS	Simple as necessary Baltic long-term large-scale model
SPINMAS	Sample preparation unit for inorganic nitrogen coupled to aquadrupole mass spectrometer
V_{max}	Maximum nitrification rate of the organism
$V(III)Cl_3$	Vanadium trichloride

1. Introduction

1.1 The Baltic Sea

The Baltic Sea (Figure 1) is one of the largest brackish water basins in the world with a surface area of 415 200 km^2 (Helsinki Commission; HELCOM 2009). It is a semi-enclosed basin that is connected to the North Sea through shallow sills in the Danish Straights. The Danish Straights have nearly marine conditions. However, the salinity decreases towards the north because of the sills between the basins, which restrict the flow of denser saline water in the deep bottom layers. Therefore, the northernmost parts of the Baltic Sea have almost completely freshwater conditions (Granéli et al. 1990). The southern Baltic Sea has a mild temperate climate with a heavily populated drainage basin. The northern parts of the Baltic Sea have a cooler climate with extensive ice cover during the winter months. The population density decreases in the north, and the northernmost Bothnian Bay is considered to be the most pristine area of the sea (HELCOM 2009).

The Baltic Sea has a positive water balance that is caused by surplus freshwater entering from a large drainage basin, which covers over three times the area of the sea. The freshwater from the drainage basin and the saline water that originates from the North Sea cause stratification to occur, where the surface and bottom waters are separated by a halocline that prevents the complete mixing of the water column. Because of this, the deepest waters of the Baltic Sea are depleted in molecular oxygen (O_2) and have accumulating hydrogen sulfide (H_2S) (HELCOM 2009). The stagnant, O_2 depleted bottom water is replaced in the deep basins during inflow events. These inflows occur when the prevailing wind from the east turns to the west in the Danish Straights, and large amounts of saline water from the North Sea enters the Baltic Sea. The salt water inflows were a frequent phenomenon in the 1950s but the frequency decreased in late 70s to a nearly decadal occurrence (Zillen et al. 2008). The reason for the low frequency of inflows is unknown, but it has caused a long-term stagnation (no significant inflow of salt water from North Sea) of the deepest bottom waters. Consequently, large hypoxic (O_2 <2 ml l^{-1}) water volumes are currently present in the Baltic Sea. In addition to the decreased frequency of inflow events, the current extent of hypoxia is regarded to be caused by excessive nutrient load discharge from the drainage basin (Zillén et al. 2008).

The Gulf of Finland is a northern Baltic Sea sub-basin that is surrounded by Finland in the north, Russia in the east and Estonia in the south (Figure 1). It is directly connected to the Baltic Proper in the west, and ends to the Neva delta in the east. During inflow events, when the deep basins in the Baltic Proper are ventilated, the old stagnant water from the deep basins is pushed over to the Gulf of Finland. Therefore, when the O_2 conditions improve in the Baltic Proper, the halocline typically strengthens, and the deep waters in the central Gulf of Finland become O_2 depleted. The Gulf of Finland is considered to be one of most severely eutrophicated parts of the Baltic Sea. However, the nutrient loading into the gulf has decreased since the early 90s due to improved wastewater treatment and economic changes in Russia and Estonia (Pitkänen et al. 2001). Despite this, the Gulf of Finland has the highest nitrogen (N) and phosphorus (P) loads of the Baltic Sea sub-basins (HELCOM 2009).

1.2 Nitrogen cycle in aquatic environments

N is a component of DNA and proteins and therefore one of the key elements that sustain life. N is constantly recycled in microbe mediated processes (Arrigo 2005)(Figure 2), in which micro-organisms gain energy by reducing and oxidizing N compounds. The largest pool of N on earth is molecular nitrogen (N_2) gas, which accounts for 78% of the atmosphere. However, N_2 gas is available only to diazotrophic (N fixing) bacteria that can reduce N_2 gas to ammonia (NH_3; in neutral aqueous solution ammonium, NH_4^+). The N fixation process is energy demanding due to the triple bond between the N atoms of the N_2. Therefore, N fixation occurs only when no fixed forms of N are already available (Postgate 1978). Some of the best known diazotrophic organisms in aquatic environments are the cyanobacteria that form mass blooms during late summer when the easily available N reserves have been depleted. N fixation can also occur in non-biological processes such as by lightning, or industrially through the Haber-Bosch process, or by combustion.

The dissolved inorganic nitrogen (DIN) that either originates from non-biological sources or is fixed by diazotrophs, is assimilated by organisms to form particulate organic nitrogen (PON). Eventually PON containing organisms die and begin to sink. During sedimentation PON is broken down into dissolved organic nitrogen (DON), which is later ammonificated to NH_4^+. NH_4^+ rarely accumulates under oxic conditions, because it is incorporated into primary

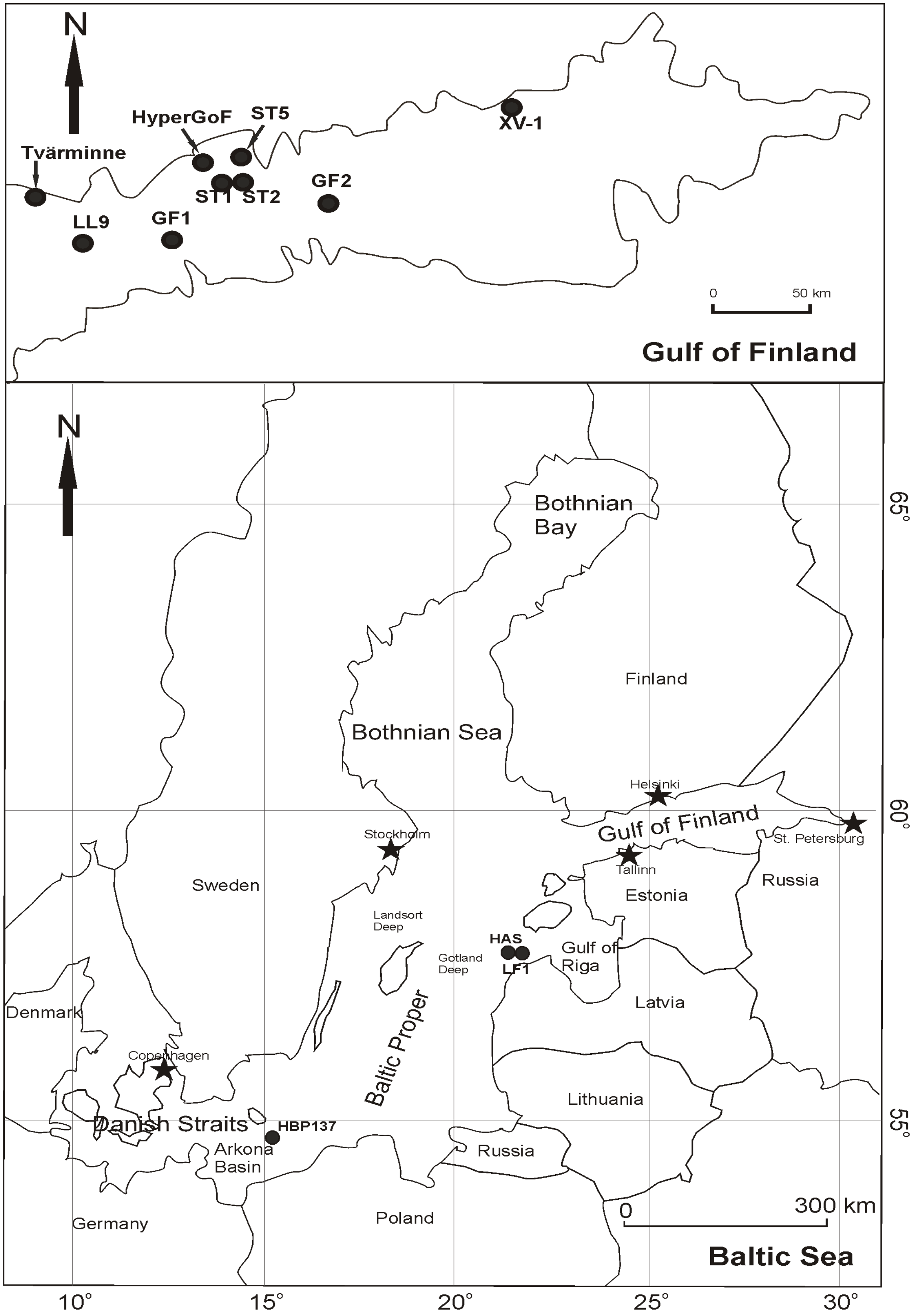

Figure 1. The Baltic Sea. The sampling sites of this study are indicated by black circles. Figure modified from publication III.

producers to form PON, or taken up by nitrification process in which NH_4^+ is oxidized by O_2 to nitrite (NO_2^-) and nitrate (NO_3^-) (Figure 2). In general, nitrification is an obligate aerobic chemolithoautotrophic process, although some alternative pathways, such as nitrous oxide (N_2O) production, have also been recognized in very low O_2 concentrations (Ward 1996). In the first step of nitrification, NH_4^+ is oxidized to NO_2^- by ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA). The AOB have relatively limited diversity and the two main lines of AOB descent from ß- and γ-proteobacteria (Ward 1996). The AOA were discovered less than a decade ago and belong to the phylum *Crenarchaeota* (Könneke et al. 2005). The importance of AOA in the N cycle is not yet well understood but AOA have been found in nearly every environment in the world, and may contribute significantly to nitrification particularly in those environments where NH_4^+ and O_2 availabilities are low (Walker et al. 2010). NH_4^+ oxidation is usually tightly coupled to NO_2^- oxidation, which leads to the formation of NO_3^- (Figure 2). The nitrite oxidizing bacteria are much less studied than ammonia oxidizing microbes, but are known to have limited diversity and to descend from the bacterial lineages of α-, γ-, and δ-proteobacteria (Koops & Pommerening-Röser 2001). Active nitrification in sediments is typically restricted to a very thin surface layer where both NH_4^+ and O_2 are available. Nitrification can proceed at surprisingly low O_2 concentrations and can even tolerate low levels of H_2S (Caffrey et al. 2010). Benthic animals (Kristensen 2000) and plant roots (Bodelier et al. 1996) stimulate nitrification by creating burrows that increase the area of the oxic surfaces. Sediment nitrification is inhibited by the growth of microalgae (Rysgaard et al. 1995, Risgaard-Petersen et al. 2004b) and heterotrophic bacteria (Verhagen & Laanbroek 1991, Caffrey et al. 1993, Sloth et al. 1995, Strauss & Lamberti 2000, Starry et al. 2005), because nitrification microbes are relatively poor competitors for NH_4^+ and O_2 due to their slow growth rates (Herbert 1999). Nitrification can also occur throughout the water column. However, the highest nitrification rates occur in dark conditions close to zero O_2 concentrations (Ward 1996). Therefore, redoxclines that contain high volumes of O_2 deficient water are usually the sites where the highest water column nitrification rates are found.

The NO_3^- ion can be taken up by primary producers or can diffuse deeper into the anoxic layers to be reduced to N_2 gas by denitrification process (Figure 2). Denitrification bacteria are facultative anaerobes, and denitrification can only occur in anaerobic conditions. Nitrification and denitrification are frequently tightly coupled, particularly in sediments (Jenkins & Kemp 1984). Therefore, the factors that stimulate nitrification often also stimulate the coupled denitrification. The potential for denitrification is widely spread, and has been detected, in addition to traditional denitrification sites such as sediments and anoxic water columns, in cyanobacterial aggregates (Tuomainen et al. 2003), sea ice (Kaartokallio et al. 2001) and foramins (eukarya) (Piña-Ochoa et al. 2010). Despite the widespread potential, denitrification is limited only to certain anaerobic environments. Denitrification can be either a heterotrophic or a chemolithoautotrophic process. Heterotrophic denitrification, that is commonly found in sediments and also in some water columns is controlled by the availability of organic carbon in addition to the NO_3^- availability (Tuominen et al. 1998, Hietanen & Kuparinen 2008, Ward et al. 2008). In contrast, the controlling factors for chemolithoautotrophic denitrification are not as clear, but it has been found to be an important N loss pathway in anoxic watercolumns, in which H_2S is available as an electron donor (Walsh et al. 2009). Water column denitrification has been suggested to be largely responsible for the N loss occurring in some oxygen minimum zones (OMZs) found in some oceans (Ward et al. 2009, Bulow et al. 2010). However, this finding was later challenged because the measurement technique used in these estimates can be disrupted by the presence of the dissimilatory nitrate reduction to ammonium (DNRA) process, which is also found in the OMZs (Jensen et al. 2011).

Anaerobic ammonium oxidation (anammox) oxidizes NH_4^+ with NO_2^- and also forms N_2 (Figure 2). Anammox is an anoxic chemolithoautotrophic process, and the diversity of bacteria capable of anammox is limited to the slowly growing phylum of *Planctomycetes* (Strous et al. 1999). It was discovered in the mid-90s in a wastewater treatment plant (Mulder et al. 1995), and when sought in natural environments, it was first found in sediments (Thamdrup & Dalsgaard 2002) and then later also in water columns (Dalsgraad et al. 2003, Kuypers et al. 2003). Anammox can constitute over half of the N_2 production in some sediments (Thamdrup et al. 2002). It was also found to be an important N loss process in the OMZs off Peru, Chile and Namibia (Lam et al. 2009). However, anammox is not as universally found as denitrification.

In addition to the NO_3^- reduction processes that result in N being lost from the system, NO_3^- can also be reduced in DNRA, where NO_3^- is converted to NH_4^+. DNRA is one of the least understood N processes, but it has been found in highly reducing environments, such as high organic content sediments (Christensen et al. 2001, An & Gardner et al. 2002) and anoxic water columns (Lam et al. 2009). DNRA uses NO_3^- as an electron acceptor in fermentation or in the oxidation of sulfur (Tiedje et al. 1982). The capability to DNRA might be more commonplace than previously thought, because in addition to bacteria, capability to DNRA has also been found among diatoms (Kamp et al. 2011). DNRA is an energy gaining process, but it has also been suggested to be a mechanism for bacteria to rid themselves of toxic NO_2^- (Canfield et al. 2005). Moreover, DNRA in diatoms has been suggested to be a long-term survival mechanism of resting stages in anoxic conditions (Kamp et al. 2011). The importance of DNRA in the water column N cycling is still widely unknown, but in the Oman Shelf DNRA was found to be an important mineralization pathway for organic matter (Jensen et al. 2011).

Before industrialization, N fixation was the only pathway that introduced N for primary production. This changed when the use of combustion engines and commercial fertilizers began (Vitousek et al. 1997). The increased N availability has stimulated primary production in aquatic ecosystems and their eutrophication has become a serious problem (Nixon 1995). However, the role of N in coastal eutrophication is still debated. Schindler et al. (2007) showed strong evidence that P is the limiting nutrient for primary production, and that reductions only in P loading are required to reduce eutrophication. Moreover, these authors suggested that reductions in N load would favor growth of the N fixing cyanobacteria as they are not dependent on fixed N. This suggestion was later challenged by Conley et al. (2009a) who argued that reductions of both, N and P, are prerequisites to reduce eutrophication, because sedimentation of the N limited spring blooms is the main reason for late summer hypoxia. They also argued that the results of Schindler et al. (2007) cannot be applied to marine coastal waters, because cyanobacterial N_2 fixation has not been observed at salinities greater than 8–10. The excess N loads can be mitigated by denitrification and anammox processes that convert DIN back to N_2 gas. However, hypoxia, which is one of the consequences of eutrophication (Diaz 2001) has been shown to reduce denitrification rates (Seitzinger 1988). Therefore, once hypoxia occurs, the effects of eutrophication become more difficult to reverse and hypoxia is more likely to re-occur (Conley et al. 2009b).

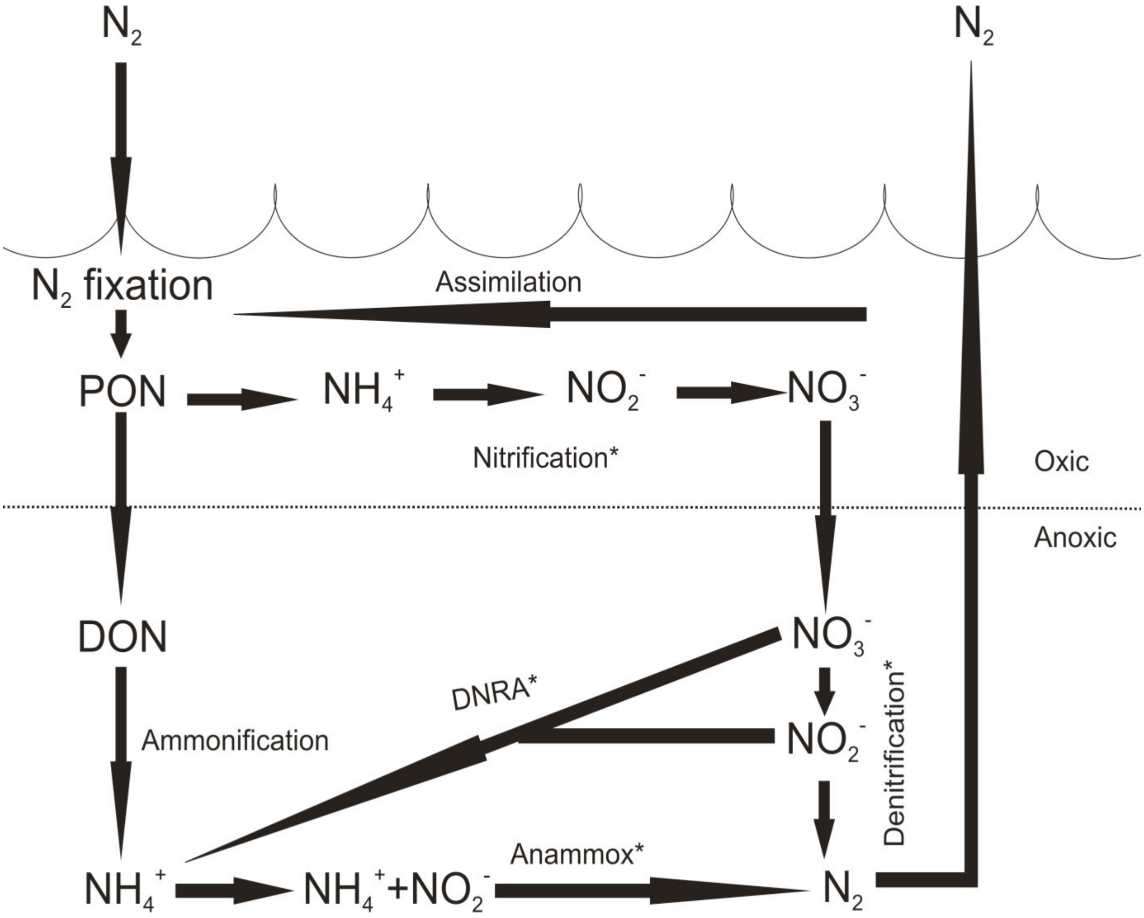

Figure 2. Nitrogen cycling in aquatic environments (modified from Arrigo 2005). PON = particulate organic nitrogen, DON = dissolved organic nitrogen, DNRA = dissimilatory nitrate reduction to ammonium, Anammox = anaerobic ammonium oxidation. The oxic-anoxic interface can be in the water column or in the sediment. *Processes measured in this study.

1.3 Nitrogen cycling measurements

N cycling rates are difficult to measure, because the processes components are fast and interconnected. N cycling rates can be measured by using inhibitors such as acetylene (C_2H_2) (Sørensen 1978), chlorate (ClO_3^-) (Belser & Mays 1980), allylthiourea (ATU) (Hall 1984), or N-serve (Billen 1976) (Table 1). The inhibitors block the stepwise N processes to a level at which the end product is easy to measure. For instance, ClO_3^- prevents the second step of nitrification, which causes accumulation of NO_2^-, whose precise concentrations can be easily measured. Accumulation rate of NO_2^- in the presence of ClO_3^- is regarded as the nitrification rate (Belser & Mays 1980) (Table 1). However, inhibitor based techniques are biased by indirect rate estimates and incomplete blockages. For example, in the ClO_3^- inhibition technique low NO_2^- accumulation rate can also be caused by NO_2- consumption by denitrification and not necessarily by low nitrification rate. Moreover, not all organisms are sensitive to the inhibitors (Table 1). For instance, ATU, a commonly used inhibitor for NH_4^+ oxidation has been found to incompletely inhibit NH_4^+ oxidation by AOA (Santoro et al. 2010).

Because inhibitor based techniques have so many flaws, the nitrification rates in this study were measured by using the stable isotope of nitrogen (^{15}N) as a tracer (Table 1). ^{15}N is naturally found in the environment (0.366% of the total N pool), is not discriminated by organisms, and it is not hazardous for the environment or for humans unlike radioisotopes (Peterson & Fry 1987). The use of ^{15}N in N cycling studies has increased during the recent decades due to the improvement in stable isotope mass spectrometry methods and decreasing analysis costs. Despite the improved ^{15}N analyses and well established measurement techniques, the *in situ* process rate measurements are still challenging, particularly in sediments that have strong concentration and density gradients that can prevent even mixing of the tracers (Steingruber et al. 2001). Therefore, the applicability of the ^{15}N based measurement techniques can vary spatially and in time. Moreover, the suitability of the technique should always be verified for the conditions that prevail in the sampling area.

Two commonly used ^{15}N based nitrification measurement techniques are the $^{15}NO_3^-$ pool dilution technique (Koike & Hattori 1978)(Table 1) and the $^{15}NH_4^+$ oxidation technique (Nishio et al. 1983, Jenkins & Kemp 1984) (Table 1). In the $^{15}NO_3^-$ pool dilution technique, the sample is amended with excess $^{15}NO_3^-$, and the dilution of the ^{15}N labeling of the total NO_3^- pool by the $^{14}NO_3^-$ produced by *in situ* nitrification over the incubation time is the measure of nitrification (Koike & Hattori 1978). This technique measures only uncoupled nitrification, by which NO_3^- is not consumed by denitrification, but diffuses to the water overlying the sediment. Nitrification in sediments is often tightly coupled to NO_3^- reduction (Figure 2), thus the NO_3^- reduction processes also have to be quantified for accurate estimate of nitrification. This can be done in the same $^{15}NO_3^-$ enriched cores by using the isotope pairing technique (IPT; Nielsen 1992) and by measuring $^{15}NH_4^+$ production (Table 1). The IPT was developed for denitrification measurements, and the technique can distinguish between denitrification based on the NO_3^- diffusing from the bottom water (D_w) from the denitrification coupled with nitrification in the sediment (D_n) (Nielsen 1992). When using IPT, the sample is amended with $^{15}NO_3^-$ and the production of $^{15}N_2$ is measured. The *in situ* denitrification is estimated by assuming random pairing of the added $^{15}NO_3^-$ and the ambient $^{14}NO_3^-$. The denitrification that is based on ambient $^{14}NO_3^-$ is calculated from the binomial distribution of the productions of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$. The binomial distribution is skewed in the presence of anammox, because anammox pairs the added $^{15}NO_3^-$ with ambient $^{14}NH_4^+$ and produces more $^{29}N_2$ than denitrification would *per se*. Consequently, the IPT was later revised (r-IPT) to also account for anammox (Risgaard-Petersen et al. 2003, 2004a) (Table 1). In addition to denitrification and anammox, NO_3^- can also be reduced in the DNRA process. The proportion of DNRA in NO_3^- reduction can be estimated from the same $^{15}NO_3^-$ amended cores by measuring the production of $^{15}NH_4^+$ (Christensen et al. 2000, Dong et al. 2009) (Table 1). By measuring the denitrification, anammox, and DNRA rates, all processes that can be coupled to nitrification are measured; hence the total nitrification rate is quantified.

In the $^{15}NH_4^+$ oxidation technique, the uncoupled nitrification produces NO_3^- in the bottom water and the nitrification that is coupled to denitrification can be estimated from the same sample treatment (Nishio et al. 1983, Jenkins & Kemp 1984). However, anammox and DNRA processes cannot be directly quantified by using this technique (I). In the technique, the sample is amended with excess $^{15}NH_4^+$, and the productions of $^{15}NO_3^-$ (uncoupled nitrification) and the $^{15}N_2$ (coupled nitrification denitrification) over incubation time are the measures of nitrification. Because more substrate is added than is naturally available, this technique measures only nitrification potential, when nitrification is NH_4^+ limited. There are also certain assumptions that have to be met in order to estimate the nitrification rate with the $^{15}NH_4^+$ oxidation technique: 1) the ^{15}N label measured in the NO_3^- and N_2 produced must have undergone the nitrification pathway, 2) the reaction must follow first-order kinetics by which the nitrification rate is only dependent on the availability of NH_4^+, and 3) the added $^{15}NH_4^+$ must be the only substrate available for nitrification. The first assumption is violated in the presence of anammox, and therefore anammox has to be measured parallel to the nitrification measurements. The second assumption is not met when the incubation time is long because other factors than NH_4^+ availability, such as decreasing O_2 concentrations, begin to inhibit nitrification. In order to avoid such confounding artifacts, the incubation times have to be kept as short as possible. The third assumption is met when the NH_4^+ concentration in the sediment surface is low.

1.4 Nitrogen in the Baltic Sea

Approximately 5000 000 tonnes of N internally recycles in the Baltic Sea (Granéli & Granéli 2008) (Figure 3). This N is the balance between the N removal processes and the N load. The N load into the Baltic Sea is 1 009 000 tonnes per year of which 25% is composed of atmospheric deposition and 75% as waterborne inputs (HELCOM 2005) (Figure 3). Regardless the high N load, primary production in the pelagic Baltic Sea is limited by N availability because massive amounts of P are released from anoxic sediments (Lehtoranta 2003). The N limitation enhances the growth of diazotrophic cyanobacterial blooms, which are an annual late summer phenomena in the Baltic Sea. The N input from cyanobacterial N fixation adds to the N load, on top of the atmospheric deposition and waterborne N, approximately 370 000 tonnes per year (Wasmund et al. 2001). However, the 370 000 tonnes is a conservative estimate, because some calculations suggest that the contribution of N fixation to the total N load could be as high as 855 000 tonnes of N per year (Voss et al. 2005a) (Figure 3). It has been estimated that between 380 000-899 000 tonnes of N per year is removed by denitrification in sediments (Deutsch et al. 2010), 300 000 tonnes of N per year are permanently buried into the sediment, and 150 000 tonnes of N per year are exported to the Kattegat and Skagerrak (Granéli & Granéli 2008) (Figure 3). Coastal denitrification in the southern Baltic Sea has been suggested to efficiently reduce the amount of N that enters the pelagic Baltic Proper (Voss et al. 2005a; 2005b). In the northern Baltic Sea, coastal denitrification removes only a small percentage of the riverine N loads (Silvennoinen et al. 2007). Only a few studies have measured anammox in the Baltic Sea sediments, and they show that anammox does not contribute substantially to the annual N removal (Hietanen 2007, Hietanen & Kuparinen 2008). To date, there are no direct measurements of sediment nitrification or DNRA processes, or their importance in the N cycle in the Baltic Sea. Moreover, the genetic diversity of microbes mediating N processes in the sediment is almost completely unknown.

Table 1. Inhibitor and ^{15}N based techniques that can be used in nitrification, denitrification, and DNRA measurements, and the publications where the techniques were applied 1) technique was applied to sediment samples in this study 2) technique was applied to water samples in this study.

	Process measured	Compound added	Process inhibited	Product measured	Advantage	Disadvantage	Reference	Publication
Inhibitor techniques								
Acetylene (C_2H_2)	Nitrification, denitrification	C_2H_2	Ammonia oxidation, final step of denitrification	NH_4^+ (ammonia oxidation), N_2O (denitrification)	Inexpensive	Cannot detect denitrification coupled to nitrification, disturbed by H_2S, indirect rate estimate, the effect on AOA unknown	Sørensen (1978)	-
Chlorate (ClO_3^-)	Nitrification	ClO_3^-	Nitrite oxidation	NO_2^-	Inexpensive	Indirect rate estimate, the effect on AOA unknown	Belser & Mays (1980)	-
Allylthiourea (ATU, $C_4H_8N_2S$)	Nitrification	$C_4H_8N_2S$	Ammonia oxidation	NH_4^+	Inexpensive	Indirect rate estimate, does not inhibit AOA	Hall (1984)	-
N-serve sensitive [^{14}C]bicarbonate incorporation ($H^{14}CO_3^-$)	Nitrification	N-serve + $H^{14}CO_3^-$	Ammonia oxidation	$H^{14}CO_3^-$ incorporation with and without ammonia oxidation inhibitor	Inexpensive, sensitive analysis for $H^{14}CO_3^-$ incorporation	Requires an conversion factor for CO_2^- consumed/ NH_4^+ oxidized, N-serve has to be dissolved to acetone, the effect on AOA unknown	Billen (1976)	-
^{15}N techniques								
$^{1)}$ Isotope pairing (IPT)	Denitrification	$^{15}NO_3^-$	-	$^{29}N_2$, $^{30}N_2$	Can detect D_n and D_w	Distorted by anammox, works only if denitrification is NO_3^- limited	Nielsen (1992)	I, II, III
$^{1)}$Revised isotope pairing (r-IPT)	Denitrification + anammox	$^{15}NO_3^-$	-	$^{29}N_2$, $^{30}N_2$	Can detect D_n and D_w + anammox	Works only if denitrification/ anammox is NO_3^- limited	Risgaard-Petersen et al. (2003, 2004)	I, II, III
$^{1)15}NH_4^+$ production in IPT samples	DNRA	$^{15}NO_3^-$	-	$^{15}NH_4^+$	The only technique for DNRA measurements	Works only if denitrification/ anammox and DNRA are NO_3^- limited	Christensen et al. (2000)	II, III
$^{1,2)15}NH_4^+$ oxidation	Nitrification	$^{15}NH_4^+$	-	$^{15}NO_3^-$, $^{29}N_2$, $^{30}N_2$	Sensitive, direct estimate of nitrification	Extrapolation of rates to *in situ* NH_4^+ concentration difficult	Nishio et al. (1983)	I, II, IV
$^{1)15}NO_3^-$ pool dilution	Nitrification uncoupled from denitrification	$^{15}NO_3^-$	-	$^{15}NO_3^-$	Can detect *in situ* nitrification, direct estimate of nitrification	Cannot be used for low nitrification rates	Koike & Hattori (1978)	I

There is a negative relationship between DIN pool and the volume of hypoxic water in the Baltic Proper and it has been proposed that N removal intensifies when the hypoxia expands (Vahtera et al. 2007, Savchuck 2010). Because nitrogen removal rates in sediments decrease in hypoxia (Seitzinger 1988), it has been suggested that when the volume of hypoxic water expands, nitrogen removal intensifies in the water column. The older rate estimates do not support this, because they show that water column denitrification is not an important N sink compared to denitrification in the sediments (Rönner & Sörensen 1985, Brettar & Rheinheimer 1991). However, the inhibitor based measurement techniques used in these studies may have underestimated the denitrification rates. Re-

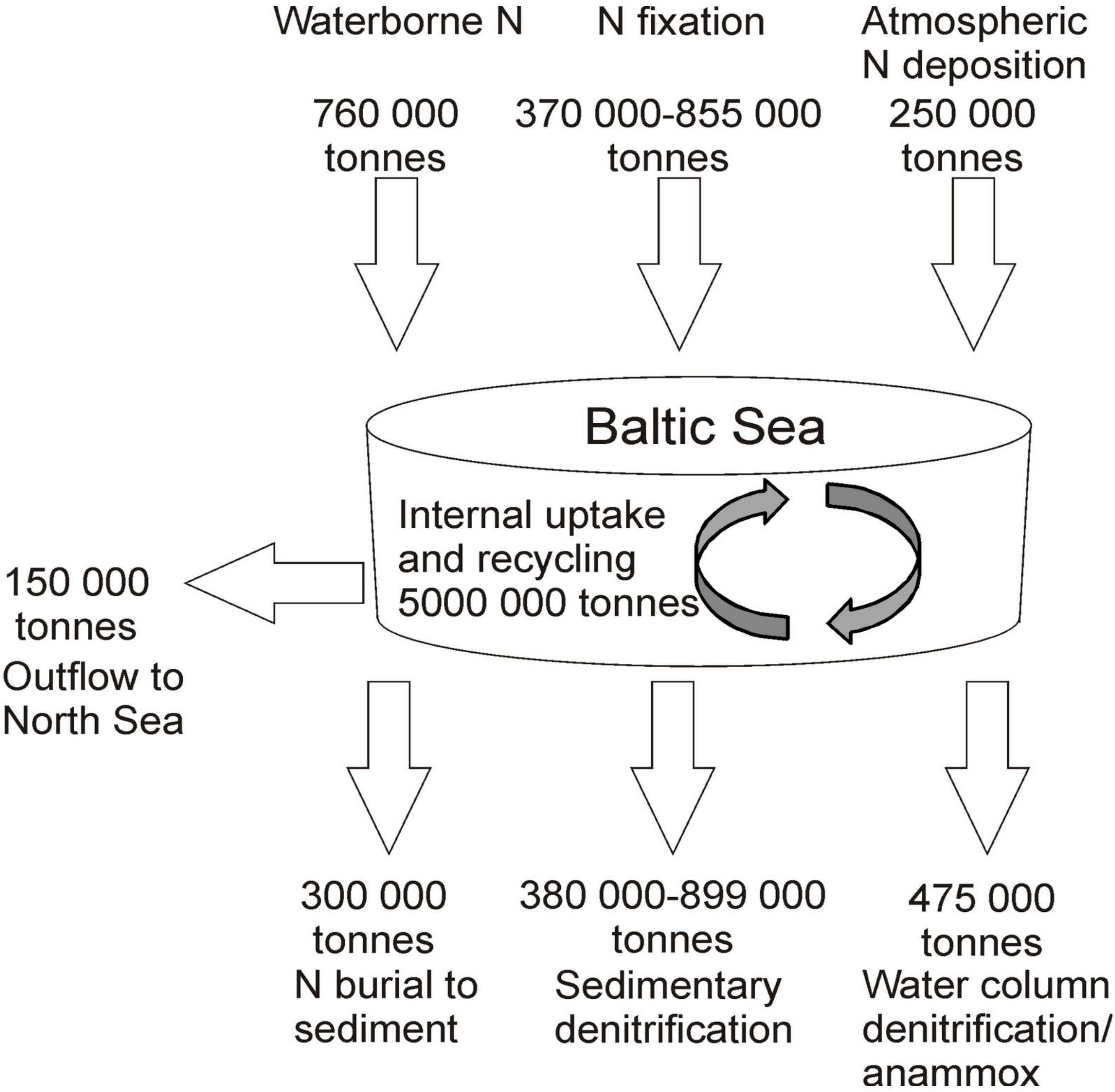

Figure 3. The N budget of the Baltic Sea.

cent studies, that used novel ^{15}N stable isotope based techniques, found high N removal potential from the water column (Hannig et al. 2007). However, the *in situ* denitrification rates were not quantified. The molecular studies of Baltic Sea water column denitrification bacteria suggest that denitrification in the Baltic Sea water column is chemolithoautotrophic process, driven by H_2S (Labrenz et al. 2005, Brettar et al. 2006, Hannig et al. 2007). Anammox has also been detected in the Baltic Sea water column (Hannig et al. 2007), but its importance as a N sink is unknown. Data about nitrification in the Baltic Sea water column are also sparse. High nitrification rates have been measured in the redoxcline, but the rates vary depending on the measurement technique used (Enoksson 1986, Bauer 2003). Moreover, the depth distribution of nitrification activity has not been verified. Both AOB and AOA have been detected in the Baltic Sea redoxcline (Bauer 2003), but the active ammonia oxidizer community has been suggested to consist solely of one crenarchaeotal subcluster GD2 (Labrenz et al. 2010).

2. The aims of the thesis

The aims of this thesis were to carry out the following objectives: **1) Optimize the ^{15}N stable isotope based nitrification measurement technique for the environmental conditions prevailing in the northern Baltic Sea**. A ^{15}N based nitrification measurement technique was first optimized for coastal Gulf of Finland sediments. Nitrification has a crucial role as a controlling factor for N removal, but has only been measured in a very few sediment studies. This is because measuring nitrification from intact cores by using ^{15}N tracers is difficult due to the tight coupling between nitrification and the NO_3^- reduction processes, and the difficulty in labeling the N pool. The optimization was done by comparing $^{15}NO_3^-$ pool dilution technique (Koike & Hattori 1978) combined with the IPT (Nielsen 1992) to $^{15}NH_4^+$ oxidation technique (Nishio et al. 1983) in a mesocosm set up (I). Both techniques required an analysis of $^{15}NO_3^-$, and the $^{15}NO_3^-$ analysis methods of ammonia diffusion (Sigman et al. 1997), denitrifier (Sigman et al. 2001), and SPINMAS (Stange et al. 2007) were also compared (I).

2) Determine the temporal and spatial variation of nitrification and NO_3^- reduction rates in the Baltic Sea with main focus on the Gulf of Finland. After determining the optimal nitrification measurement technique, it was applied to the coastal sediments in the Tvärminne area (Figure 1), where in addition to nitrification, denitrification, anammox, and

DNRA were measured three times per year for two consecutive years (II). The denitrification and DNRA rates in the open Gulf of Finland were also measured and at some stations in the southern Baltic Sea during three cruises in 2008-2009 (III) (Figure 1). Nitrification measurements were also attempted from the open sea sediment samples, but neither of the methods tested was suitable for the conditions found in the open sea (data not shown). Nitrification was also measured in the water column in the Baltic Proper to determine the water depths where nitrification occurs (IV).

3) Determine the controlling factors for nitrification and NO_3^- reduction rates in the Baltic Sea. There was high seasonal variation in the environmental parameters in the coastal Gulf of Finland, and the relationship between these factors and the N cycling rates were determined (II). Much emphasis was put on determining the effect of hypoxia on sediment N cycling (II, III). Lastly, the controlling factors for water column nitrification and their role as a substrate producing process for the N removal processes were also determined from the redoxclines around the Gotland deep and the Landsort deep (IV).

3. Materials and methods

3.1 Study areas

This study mainly focuses on sediments taken from the Gulf of Finland in the northern Baltic Sea (Figure 1). The Gulf of Finland sediments are high in organic matter and nutrients which have been transported from the drainage basin. The average depth in the gulf is only 38 m and the halocline lies at between 60-80 m. Although hypoxia is a common phenomenon in the Gulf of Finland, the volume of hypoxic water is not high due to shallow water depth of the gulf. Due to the low volume of hypoxic water in the Gulf of Finland, the water column nitrification rates were determined in the Baltic Proper. In the Baltic Proper the volume of hypoxic water is much higher, which allows coupling of nitrification and NO_3^- reduction processes.

3.1.1 The Coastal Gulf of Finland (I, II)

The sediment for the mesocosm (I) was collected from Storfjärden (59°51.31N, 23°15.81E). The sediment samples for the experiment that investigated seasonal variation in nitrification and nitrate-reduction pathways (Table 2, II) were collected from Storfjärden and also Muncken (59°51.14N, 23°14.70E). Both stations are located in the north-western Gulf of Finland, near the Tvärminne

Zoological Station (Figure 1). The Storfjärden site represents a typical outer archipelago accumulation basin (Niemi 1975), which is 33 m in depth and has soft mud sediment. Muncken is located approximately 1 km from Storfjärden close to Pojo Bay. It is a more shallow (11 m) transportation area, in which the sediment consists of soft mud and fine-grained sand.

3.1.2 The open sea (III, IV)

The open sea sediment samples were collected during three cruises 2008-2009 (Figure 1, Table 2; III). In the first cruise, several of the Gulf of Finland stations that were sampled in the 90s by Tuominen et al. (1998) for sediment denitrification were re-sampled. Attempts were made to sample more stations but several of the stations were completely anoxic in 2008. In the second cruise samples were collected along an O_2 gradient outside of Helsinki. On the third cruise, the sediment samples were collected from the Gulf of Finland and also from three stations in the southern Baltic Sea (III). For investigating nitrification in the redoxcline, water column samples were collected during three cruises from the deepest hypoxic basins of the Baltic Sea (IV). The sampling focused in the Gotland Deep and in the Landsort Deep (Figure 1).

3.2 Sampling and incubations

3.2.1 Sediment collection and set up for the mesocosm (I, II, III)

The sediment for the mesocosm was constructed from the surface sediment (0 – 1 cm) and from sediment collected below the surface (1–15 cm). The deeper sediment was collected using a box corer, and the surface sediment was collected by using an Ockelman sledge. The deeper sediment was sieved through a 1-mm sieve, whereas the surface sediment was passed through a 0.5-mm sieve to remove macrofauna. The sediment was divided into containers (15 cm x 15 cm x 10 cm) with 6 cm of the deep sediment being placed on the bottom and 1.5 cm surface sediment put on the top. The containers were submerged in an aquarium (300 L) with nutrient free artificial seawater at salinity 2 and a temperature 4 °C that mimic typical *in situ* conditions in the sampling area. The sediment was allowed to settle for six weeks after which the subsamples for the incubations were collected as described below.

The coastal and open sea sediment samples were collected in acrylic cores, by using a Gemini (diameter 80 mm, height 80 cm) or Gemax (diameter 90 mm, height 80 cm) twin corer with sediment and water taken in each core. In all sediment samples, the O_2, NO_3^-, and NH_4^+ concentrations in the bottom water were measured in a water sample that was taken from approximately 5 cm above the sediment surface in a core. The sediment organic content was determined from the topmost sediment as a loss on ignition (LOI%). The porosity of the topmost sediment was also determined. Each large core (coastal and open sea samples) and sediment container (mesocosm) was subsampled into smaller acrylic plastic cores (diameter 26 mm, height 20 cm), so that there was approximately 5 cm of sediment and 13 cm of water in each subsample. The remaining volume in the core was filled by caps, which left no air in the cores. The subsamples were treated further within 2 h after being collected.

3.2.2 Sediment incubations (I,II,III)

Intact sediment cores were taken for measuring denitrification, anammox, DNRA, and nitrification by using the $^{15}NO_3^-$ pool dilution technique. Subsequently, these sediment cores were enriched with potassium nitrate ($K^{15}NO_3^-$, 99% ^{15}N, Cambridge Isotope Laboratories) to final concentrations of 40 µM $^{15}NO_3^-$ (not in the mesocosm), 80 µM $^{15}NO_3^-$, 120 µM $^{15}NO_3^-$, and 160 µM $^{15}NO_3^-$ in the overlying water (n = 4 per concentration)(I,II, III). Intact sediment cores that were taken for measuring nitrification using the $^{15}NH_4^+$ oxidation technique involved the enrichment with ammonium chloride ($^{15}NH_4$ Cl, 99% ^{15}N, Cambridge Isotope Laboratories) to final concentrations of 100 µM $^{15}NH_4^+$, 200 µM $^{15}NH_4^+$, and 300 µM $^{15}NH_4^+$ in the overlying water (n = 4 per concentration) (I,II). All cores were incubated in the dark for 3-4 h in the *in situ* temperature with mixing provided by magnetic stirring bars in the caps. After incubation, the water from the $^{15}NO_3^-$ pool dilution samples was filtered (prewashed 0.2-µm syringe filter, Acrodisc, Pall Corporation). The sediment was mixed with the overlying water for the rest of samples, and the cores were submerged in ice-cold water and allowed to settle for approximately 3 min. A 12-ml water sample was withdrawn from the top of the core into a gastight glass vial for isotopic analysis of N_2 (Exetainer, Labco Scientific). These gas tight vials contained 0.5 ml zinc chloride ($ZnCl_2$) (100% w/v) to terminate the microbial activity. The remainder of the core content was centrifuged (1500 rpm, 10 min) and the supernatant was filtered (prewashed 0.2-µm syringe filter, Acrodisc, Pall Corporation) and frozen immediately at -20 °C for $^{15}NH_4^+$ analysis (DNRA samples, II, III) and $^{15}NO_3^-$ analysis ($^{15}NH_4^+$ oxidation samples, I, II).

3.2.3 Collection and incubation of water samples for nitrification measurements (IV)

Before sampling, profiles of temperature, salinity and O_2 from conductivity-temperature-density (CTD) –casts were analyzed. The sampling depths were selected to represent the interface between oxic and anoxic water layers. Two replicate samples were collected by using Free-Flow bottles attached to a CTD–rosette system (Hydro-Bios, Hydro-Bios Apparatebau GmbH). Samples for the process measurements were taken from the first replicate bottle and samples for O_2, H_2S, NO_3^-, NO_2^- and NH_4^+ analyses were taken from the second bottle to avoid O_2 contamination in the process measurement samples.

Samples for the process measurements were collected into 1 l glass bottles with threefold overflow of the water prior taking the final water sample. The bottles were closed and transported to glove bags immediately. The glove bags were equipped with gas detectors (GW Gas Alert Extreme, Honeywell) and flushed with N_2 so that O_2 concentration stayed below 0.5% of volume the whole time the samples were exposed to surrounding atmosphere. In the glove bag $^{15}NH_4^+$ (final concentration 5 µM) was added, the sample was mixed and divided into aliquot by putting it into gas tight vials. The rest of the $^{15}NH_4^+$ -labeled sample was filtered (pre-washed syringe tip 0.2 µm PES, VWR International) and immediately frozen at – 20°C for zero time $^{15}NO_3^-$ concentration measurements. Usually two different depths were treated as described above at the same time and handling of the samples never took longer than 15 minutes from sampling to closing the gas-tight vials. The samples were incubated at in situ temperature (±2 degrees) in the dark. Four replicate samples were allocated at time intervals of 4 hours by filtering (pre-washed syringe tip 0.2 µm PES, VWR International) the sample and immediately freezing (-20°C) for later $^{15}NO_3^-$, NO_3^-, NO_2^- and NH_4^+ analyses.

3.3 ^{15}N analyses

The ^{15}N isotopic composition of sediment denitrification N_2 samples (I, II, III) were analyzed by either using a 20-20 isotopic ratio mass spectrometer (IRMS) 205 linked to an ABCA-G gas purification module and Gilson autosampler (Europa Scientific Limited), or by using a gas chromatographic (GC) column coupled to a triple-collector (RoboPrep G+ in line with Tracer-Mass, Europa Scientific).

The $^{15}NH_4^+$ analysis for the sediment DNRA samples was a modified method of that described by Sigman et al. (1997) and Holmes et al. (1998) and the protocol is described in detail in manuscripts II and III. Briefly, a sample containing 1-7 µmol of NH_4^+ was added to 100 ml bottles, salinity was adjusted to 30, 1g of magnesium oxide (MgO) per 100 ml of sample was added, and the pH was adjusted to 11 by adding 10% sodium hydroxide (NaOH). The NH_4^+ was extracted in diffusion packets (an acidified fiberglass filter between membrane filters) by incubating the bottles with the diffusion packets pack in them upside down at room temperature (22 °C) on a shaker table (150 rpm) for 14 days. After incubation, the diffusion packets were removed, rinsed with 10% hydrochloric acid (HCl) and MilliQ-water (Millipore), and placed on a desiccator in a sulfuric acid (H_2SO_4) atmosphere to dry. After two days, the packets were disassembled and the fiberglass filters were packed into silver foil cups (Elemental Microanalyses Ltd). The isotopic ratio of the extracted N was analyzed using the Europa Scientific 20-20 elemental analysis-IRMS.

The $^{15}NO_3^-$ analysis with the ammonia diffusion method (I) was very similar to the $^{15}NH_4^+$ analysis method described above. The only difference to the protocol described above was that the ambient NH_4^+ and DON in the sample were first removed by increasing the pH with MgO and 10% NaOH, and heating the sample at 60˚C for five days. Thereafter, NO_3^- was converted to NH_4^+ by using 75 mg of Devarda's alloy (DA) /100 ml per sample (Sigman et al. 1997). The resulting NH_4^+ was extracted to diffusion packets by incubating the samples at 37°C for five days. The ^{15}N from the filters was analyzed as described above.

The $^{15}NO_3^-$ analysis with the SPIN-MAS method (I, II) followed the protocol described by Stange et al. (2007). Briefly, 1 ml of sample was mixed in a reaction vial (22 ml, capped with a 2-mm septum) with 3 ml vanadium trichloride ($V(III)Cl_3$) solution (15.7 g VCl_3 and 160 ml 32% HCl in 1000 ml) at 85 ˚C for 1 min to form nitric oxide (NO). The NO produced was transported with helium as a carrier gas (10 ml min^{-1}) to the inlet capillary (open split) of quadrupole mass spectrometer (GAM 400, InProcess Instruments GmbH) where the ^{15}N abundance of the NO was analyzed. Cryotrap (-120˚C) was used to remove H_2O and CO_2 prior to the ^{15}N analysis.

The sample preparation for the $^{15}NO_3^-$ analysis with the denitrifer method (I, IV) followed the protocol presented in Sigman et al. (2001). The sample water was incubated with concentrated *Pseudomonas chlororaphis* (American Type Culture Collection (ATCC) # 43928) culture, which converted the NO_3^- in the sample to N_2O in anoxic conditions. The ^{15}N label of the resulting N_2O was analyzed using continuous flow-IRMS (Finnigan MAT delta plus, Thermo Finnigan) fitted with a trace gas pre concentrator (PreCon, Thermo Finnigan) or fitted with a GC-IRMS system (Thermo Finnigan Delta V plus with ConFlo IV) with PreCon.

3.4 Calculations

The nitrification rates that were measured using the $^{15}NO_3^-$ pool dilution technique (I) were calculated according to Jensen et al. (1996). The nitrification rates measured by the $^{15}NH_4^+$ oxidation technique (I,II) were estimated from the $^{15}NO_3^-$ and $^{15}N_2$ produced by the $^{15}NH_4^+$ that was amended to the sample cores. The *in situ* rates of the $^{15}NH_4^+$ oxidation samples were calculated by extrapolating the rates to the NH_4^+ concentration present in the bottom water (II). The denitrification rates were calculated by using the IPT (Nielsen 1992). When denitrification is measured by using the IPT or r-IPT, denitrification should be NO_3^- limited. Hence, the total denitrification rates should increase with NO_3^- availability (Nielsen 1992). This was not always the case as found in publications I and II. Consequently, the coupled nitrification denitrification had to be estimated from the $^{15}NH_4^+$ oxidation samples. This was done by assuming that the NO_3^- originating from benthic nitrification diffuses as similar $^{15}N/^{14}N$ ratios to those of the bottom water and the denitrification layer deeper in the sediment thus:

1)coupled nitrification denitrification

$$= \frac{D_n(A)}{r}$$

where D_n (A) is the $^{15}N_2$ production from the $^{15}NH_4^+$ amended samples and r is the $^{15}N/^{14}N$ ratio of the NO_3^- that diffuses out from the sediment.

The DNRA rates of the coastal Gulf of Finland sediment samples were calculated according to Dong et al. (2009). In this calculation scheme the $^{15}N/^{14}N$ ratio of the NO_3^- consumed by DNRA is simply the ratio of the ambient $^{14}NO_3^-$ to the added $^{15}NO_3^-$. By assuming this, the *in situ* DNRA rates (based on the ambient $^{14}NO_3^-$) can be calculated by multiplying the $^{15}NH_4^+$ production by the ratio of $^{15}NO_3^-/^{14}NO_3^-$ present in the bottom water. The DNRA rates of the open sea samples were calculated according to Christensen et al. (2000). In this calculation scheme the *in situ* DNRA rate was estimated from the production ratio of $^{14}N_2/^{15}N_2$ that was obtained from the IPT calculations. In order to estimate DNRA rates accurately by using the calculation schemes of Christensen et al. (2000), the $^{15}NH_4^+$ production should increase in parallel with the $^{15}N_2$ production. This criterion was met in the open sea samples, but not in the coastal samples, and therefore two different calculation schemes were used.

The nitrification potential in the $^{15}NH_4^+$ amended water column samples was calculated from the linear regression of $^{15}NO_3^-$ production of over time and by the labeling of the substrate pools in the samples, in which the slopes significantly ($p<0.05$) differed from zero. The $^{15}N_2$ production in the $^{15}NH_4^+$ amended samples was not measured because no $^{15}N_2$ production was detected in those samples for which $^{15}NO_3^-$ had been added (data not shown). Nitrification in the oxic-anoxic interface of the Baltic Sea water column has been found to be substrate saturated at approximately 3 µM NH_4^+ (Bauer 2003), therefore the rates were assumed to represent the maximum nitrification rate of the organism (V_{max}). The *in situ* nitrification rates in the water column samples were calculated by using the *in situ* NH_4^+ concentration and half saturation constant value of 0.27 µM, estimated for Baltic Sea redoxcline nitrification bacteria by Bauer (2003).

4. Results and discussion

4.1 Optimal ^{15}N stable isotope based nitrification measurement technique for the environmental conditions prevailing in the northern Baltic Sea

For both the $^{15}NO_3^-$ pool dilution technique and the $^{15}NH_4^+$ oxidation technique, the nitrification rate is estimated from the change in the ^{15}N atom% of the total NO_3^- pool. The ^{15}N atom% is the proportion of $^{15}NO_3^-$ in the total NO_3^- pool. The major difference between the two techniques is that nitrification decreases the ^{15}N atom% of NO_3^- pool for the $^{15}NO_3^-$ pool dilution technique. In contrast, it increases it in the $^{15}NH_4^+$ oxidation technique. Since the total NO_3^- concentration increases in the $^{15}NO_3^-$ pool dilution technique, a relatively higher nitrification rate (increase in the $^{14}NO_3^-$) is required to cause an equal change in the ^{15}N atom% when compared to the $^{15}NH_4^+$ oxidation technique. For example, if the amount of ambient NO_3^- in the core is 0.5 µmol and the amount of NO_3^- nitrification produces is 0.2 µmol, the change in the ^{15}N atom% is 8.3% in the $^{15}NO_3^-$ pool dilution technique (assuming 0.5 µmol $^{15}NO_3^-$ addition = 50 atom% enrichment) and 40% in the $^{15}NH_4^+$ oxidation technique. Therefore, the sensitivity of the $^{15}NO_3^-$ pool dilution technique is lower compared to the $^{15}NH_4^+$ oxidation technique. If the $^{15}NO_3^-$ pool dilution technique is applied, there should also be a detectable amount of NO_3^- in the bottom water so that approximately 50% ^{15}N enrichment could be prepared. With this enrichment level, the change in the atom% is the easiest to detect because the relative change in the atom% is the highest (Figure 4).

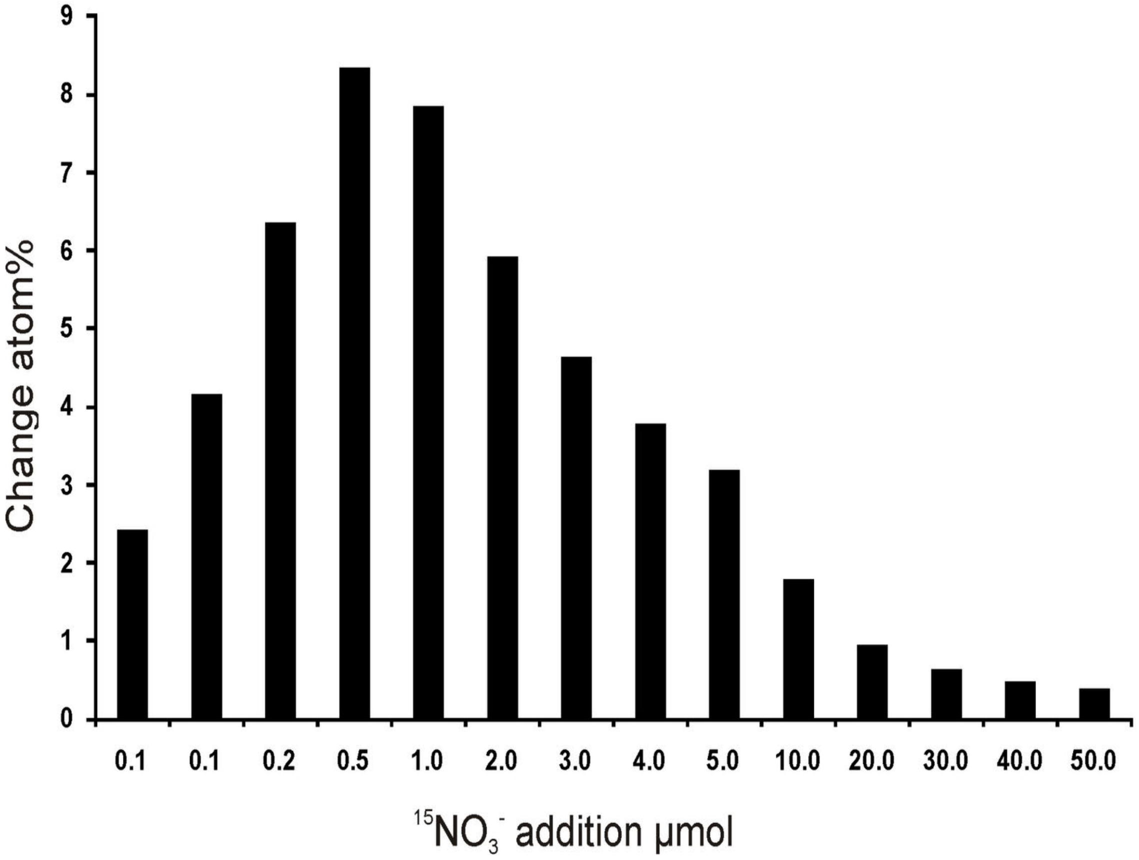

Figure 4. Change in the atom% in different ^{15}N enrichment levels in the $^{15}NO_3^-$ pool dilution technique when the amount of ambient $^{14}NO_3^-$ was assumed to be 0.5 µmol, and the increase in the $^{14}NO_3^-$ over the incubation period is 0.2 µmol.

The $^{15}NO_3^-$ pool dilution technique was only sensitive enough for the mesocosm sediments for which the mean nitrification rate was 100-fold and the NO_3^- concentration was approximately 10-fold higher that of the natural coastal sediments (I, II). In the natural coastal sediments, nitrification had to be measured by using the $^{15}NH_4^+$ oxidation technique (II). However, the $^{15}NH_4^+$ oxidation technique has several flaws that should be considered when interpreting nitrification rates measured using this technique. Sediments have sharp concentration and density gradients, and the complete labeling of the NH_4^+ pool with $^{15}NH_4^+$ in the nitrification layer is challenging. This is particularly difficult when NH_4^+ has accumulated within the sediment surface. Accumulation of NH_4^+ in the sediment surface can be caused by high mineralization rates, as was the case in the mesoscosm and coastal Gulf of Finland in August 2008 (I, II), or by high DNRA rates that occurred in the open Gulf of Finland (III). Under these conditions the $^{15}NH_4^+$ oxidation technique was not applicable.

Nitrification can also be NH_4^+ limited. In this case, the $^{15}NH_4^+$ additions should be done as a concentration series and the rates should then be extrapolated to the ambient NH_4^+ availability to estimate nitrification rates under *in situ* conditions (II). An estimation of the ambient NH_4^+ concentration cannot be measured with the known porewater sampling methods because in muddy sediments the nitrification layer typically lies a few mm below the sediment surface (I). Moreover, even if it could be measured, the NH_4^+ concentration does not necessarily represent the actual NH_4^+ availability because NH_4^+ is constantly diffusing into the nitrification from layers that are deeper within the sediment and at the same time NH_4^+ is consumed by nitrification (Ward 1996). Therefore, the NH_4^+ concentration immediately above the sediment surface was used as an estimate of the ambient NH_4^+ availability.

Overall, the $^{15}NH_4^+$ oxidation technique is a poor method for measuring the nitrification rates in the sediments. This technique tends to underestimate the nitrification rates due to incomplete labeling of the substrate pool and due to the difficulty in extrapolating the potentials to the *in situ* NH_4^+ concentration (I, II). In addition, the effects of anammox and DNRA on the nitrification rates cannot be verified by using this technique solely (I). The anammox and DNRA rates were low in the sediments used in this study (Table 2), and therefore the effects of anammox and DNRA on the coupled nitrification denitrification rates can also be expected to be low. However, the effects cannot be estimated precisely. To date there are no better techniques available for measuring low nitrification rates in sediments, thus despite its flaws, this approach was the only option to estimate nitrification.

The most convenient method for measuring $^{15}NO_3^-$ of the nitrification samples at the tracer level was the SPINMAS method. The denitrifier method gave similar results to the SPINMAS, but the sample processing required considerably more time and effort. The ammonia diffusion method not only gave lower atom% values due to blanks associated with DON, but was also time-consuming and labor-intensive (Figure 5). The disadvantage of using the SPINMAS method was that it cannot be used to measure very low NO_3^- concentrations and low ^{15}N enrichments (Stange et al. 2007). Hence, for the low NO_3^- concentrations and the low ^{15}N enrichments found in the water column nitrification measurements, the denitrifier method was the more appropriate method to use (IV).

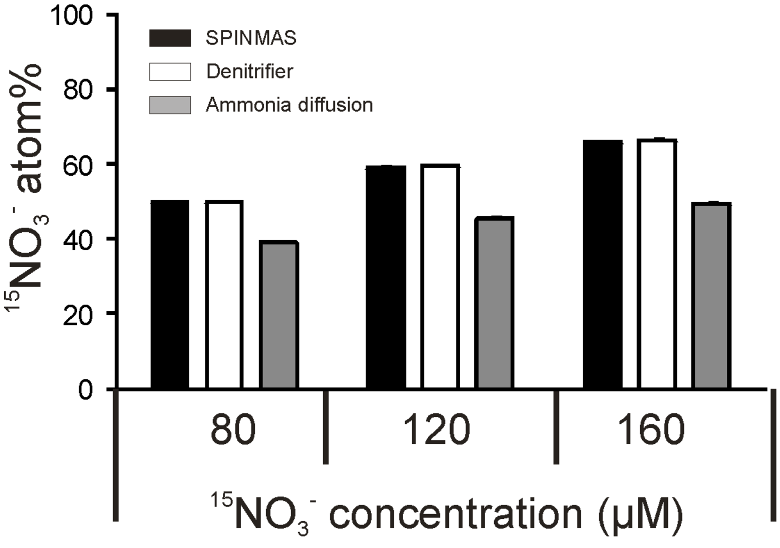

Figure 5. The atom% values measured in the same samples by using three different methods (I).

4.2 The temporal and spatial variation of nitrification and NO_3^- reduction rates in the Baltic Sea with main focus on the Gulf of Finland

4.2.1 Temporal variation of nitrification and NO_3^- reduction pathways in the sediment

Nitrification and the NO_3^- reduction rates varied temporally in the sediments of coastal Gulf of Finland. The highest nitrification and denitrificaton rates under *in situ* conditions were measured in late summer, when the rates were approximately double those of the values measured in spring and late fall (Table 2). The denitrification potential was also the highest in late summer, but the nitrification potential was often the highest in spring and late fall (II). Only low anammox rates were found in late fall when the overall N_2 production rates were low. Therefore, the anammox contributed only little in the total N_2 production (Table 2). The DNRA rates had no clear temporal variation as both high and low rates were found in same seasons (Table 2).

4.2.2 Spatial variation of nitrification and NO_3^- reduction pathways in the sediment

The nitrification rate measurements from the open sea did not produce reliable rates (data not shown). However, active nitrification was indicated in the open sea samples by the relatively high NO_3^- concentrations in the bottom water and a lack of NH_4^+ accumulations despite the low O_2 concentration (Table 2). There were no exact nitrification rates available in samples from the open Gulf of Finland, thus only the coastal sites can be compared for spatial variation of nitrification rates. The nitrification rates under *in situ* conditions were usually higher in Storfjärden, which is the deeper station in the accumulation basin. However, the nitrification potential was often higher in Muncken, which is the shallow transportation area (II). This suggest that the conditions are often more favorable for nitrification in shallow transportation areas in which the organic matter does not accumulate. However, due to low amounts of organic matter, only small amounts of NH_4^+ were available for nitrification and the *in situ* nitrification rates remained low.

There were no data available for direct comparison of nitrification rates in the Baltic Sea sediments. Overall, only a few nitrification measurements have been determined in intact sediment cores. Of the studies that were, partly, comparable in terms of measurement technique, the nitrification rates measured at the Danish coasts were at least 10-fold higher than those of this study (Henriksen et al. 1981, Sloth et al. 1992). Furthermore, at the coast of Island of Sylt, Germany (Jensen et al. 1996) and at a Danish lake (Rysgaard et al. 1993) nitrification rates were approximately 2-fold higher than those measured in this study. At Chesapeake Bay, USA, the rates were nearly 20-fold higher in spring, but decreased to zero during the summer months (Jenkins & Kemp 1984). For the most part, the nitrification rates in the coastal Gulf of Finland are low, compared with rates measured in more southerly locations.

The denitrification rates in the northern Baltic Sea had high spatial variability, but due to different sampling times direct comparison of rates between areas was not possible. Of the coastal stations, the highest denitrification rates were measured in the deeper accumulation basin (Table 2). When all the stations in the Gulf of Finland were compared for denitrification rates measured in spring and early summer, the highest N removal rate was measured in station XV-1 in the open Gulf of Finland in May 2008, and the lowest rate in Muncken in the coastal Gulf of Finland in April 2009. In general, the denitrification rates were higher in the open sea stations compared to the coastal stations. However, the difference in N removal rates in the spring/early summer between the open sea and coastal Gulf of Finland stations found in this study may have been caused by the one to two month difference in the sampling times. The coastal stations were sampled in April at which time mineralization of spring bloom has not started. The open sea stations were sampled after May when most of the organic matter that originated from spring bloom had sedimented and mineralization of the organic matter was underway (Tallberg & Heiskanen 1998). The denitrification rates in the southern Baltic Sea stations were similar to the rates measured in the Gulf of Finland (Table 2).

Denitrification has been studied more extensively than nitrification and more data are available for comparison. When compared to those of more southerly locations the denitrification rates in the Gulf of Finland are low. For example, the rates were 5% those measured in the Colne estuary, UK (Dong et al 2000, 2009), and approximately 1% those of the Rowley River estuary, USA (Tobias et al. 2003), Humber estuary, UK (Barnes & Owens 1999) and Thames estuary, UK (Trimmer 2000). Therefore, it is surprising that the denitrification rates in the southern Baltic Sea were not higher when compared to the Gulf of Finland (Table 2). The low denitrification rates in the southern Baltic Sea may have been caused by the early summer sampling time and relatively poor O_2 conditions prevailing in two of the stations (Table 2). When the Gulf of Finland denitrification rates were compared to sites at the same latitude, the rates measured in this study were lower than the denitrification rates in sediments in the Archipelago Sea (Silvennoinen et al. 2007), but higher than in the eastern Gulf of Finland (Gran & Pitkänen 1999) and in the Gulf of Bothnia (Stockenberg & Jonstone 1997). However, the Gulf of Bothnia denitrification rates had been measured by using the C_2H_2 inhibition technique. This technique underestimates denitrification rates because it does not account for denitrification that is coupled to nitrification (Seitzinger et al. 1993) (Table 1).

4.2.3 The significance of sedimentary NO_3^- reduction processes in N removal

The results of publications II and III show that the natural N removal capacity found in sediments has shown a decreasing trend both in the coastal and open waters of the Gulf of Finland. The coastal denitrification rates measured in 2008-2009 were nearly 50% that of the denitrification rates measured in the early 2000s (Hietanen & Kuparinen 2008). In the open Gulf of Finland, the spring denitrification rates had decreased to less than half the values measured in the mid-90s (Tuominen et al. 1998). Coastal denitrification was estimated to remove approximately 2-3% of the N load entering the area (II). Since all of the N removal rates in the open Gulf of Finland sediments were measured in spring and early summer, we have no temporal coverage of the N removal in the open sea. By extrapolating the coastal rates for the entire Gulf of Finland, it can be estimated that denitrification removes only 10% of the annual N load (II). This is substantially less than those estimated by Tuominen et al. (1998), Kiirikki et al. (2006), and Hietanen & Kuparinen (2008) who predicted 30% removal of the annual N load by denitrification. Even the 10% estimate may be an exaggeration, as the seasonal dynamics of the N removal in the open sea are probably different from the coastal areas due to poorer O_2 conditions. The O_2 concentrations were very low in spring and early summer in the open sea, therefore it can be reasoned that the bottom water becomes completely anoxic in late summer when the mineralization consumes all O_2 of the bottom water. Therefore, it is likely that the N removal is completely impeded in late summer in the open sea, whereas in the coastal areas it is the season when the highest N removal rates occur. If this is the case, the N removal rates measured in spring and early summer represent the annual high in the open sea, with lower N removal rates for the rest of year.

Anammox was only found at the coastal stations. The lack of anammox in the open sea may have been caused by the sampling times of the open sea samples. Anammox was found in the coastal stations in the late fall, when no open sea samples were collected. Anammox has been previously found in the Gulf of Finland by Hietanen (2007) and Hietanen & Kuparinen (2008) and the rates measured in those studies are slightly higher compared to those of the present study. However, similar to the present study, anammox did not to contribute to N_2 production for all seasons. In this study (II), anammox contributed 18% and 26% of the N_2 production when it occurred, which are intermediate percentages compared to the values reported in the literature (Dalsgaard et al. 2005). Overall, the contribution of anammox to NO_3^- reduction is spatially highly variable. Anammox appears to be favored in arctic latitudes and deep waters where competition for NO_3^- is not as intense as that found of warmer waters (Dalsgaard et al. 2005). However, despite the fact that the Gulf of Finland is located at northern latitudes, the results of this and previous studies suggest that anammox is not an important NO_3^- reduction pathway in this region. This is probably because the other controlling factors, such as sediment organic content and water depth, are not favorable for anammox to occur in the northern Baltic Sea.

In contrast to anammox, DNRA was detected in nearly all stations and in all seasons, and the rates had high spatial variability. The DNRA rates were low in the two coastal stations and in the southern Baltic Sea stations, whereas approximately 10-fold higher DNRA rates were measured in several of the open Gulf of Finland stations (Table 2). The low DNRA rates measured in the coastal stations and the southern Baltic Sea fall into the same range as those measured in a lowland lake (Nizzoli et al. 2010) and a wetland (Scott et al. 2008). The open Gulf of Finland DNRA rates are slightly higher than those measured in salt marsh sediments (Koop-Jakobsen & Giblin 2010), and similar to the DNRA rates in the Colne estuary, UK (Dong et al. 2009). However, they were lower compared to those in the sediments under fish cages in Horsens Fjord in Denmark (Christensen et al. 2000) and tropical Cisadane estuary in Indonesia (Dong et al. 2011). DNRA does not have clear latitudinal trends as in the cases of anammox and denitrification: instead high DNRA rates are restricted to some specific highly reduced conditions.

4.2.4 Nitrification in the water column

In the water column, potential nitrification rates between 14-157 µM d^{-1} were measured in approximately 10 meters thick water layer in the redoxcline (IV). The estimation of the *in situ* nitrification rates in the water column was accomplished by extrapolating the V_{max} rates to the ambient NH_4^+ concentration by using Michaelis–Menten kinetics. By doing this, the *in situ* rates were usually low (3-84 µM d^{-1}) because the ambient NH_4^+ concentrations were correspondingly low (IV). However, it can be expected that the NH_4^+ availability for nitrification is higher than the ambient NH_4^+ concentration as NH_4^+ does not necessarily accumulate due to constant NH_4^+ consumption by nitrification (Ward 1996). In order to estimate the *in situ* nitrification rates more reliably, additional information is required from the oscillations of water masses in the redoxcline that is probably the most important pathway that transports NH_4^+ and O_2 for nitrification (IV).

The water column nitrification rates measured in the Black Sea OMZ (Lam et al. 2007) and Peruvian OMZ (Lam et al. 2009) fall in to the lower end of the rates measured in this study. Similar rates to this study have been found in the Gulf of California (Beman et al. 2008). Higher nitrification rates than those found in this study have only been measured previously in the Baltic Sea (Enoksson 1986, Bauer 2003). The high nitrification rates measured in the Baltic Sea water column may be explained by the large amount of nutrients discharged into the system from the drainage basin. A high nutrient load stimulates primary production, of which mineralization causes high NH_4^+ availability for nitrification. Furthermore, the position of the redoxcline in the Baltic Sea is dynamic (IV) and its oscillations can bring large amounts of NH_4^+ and O_2 available for nitrification.

4.2.5 Sediment versus water column as a site for N removal processes

When sediment surface is well oxidized, N removal occurs in the sediment. If O_2 is depleted in the bottom water, the oxic-anoxic interface, at which N processes occur, migrates from the sediments to the water column (III, IV) (Figure 2). Because the nitrogen cycling rates are much lower in the water column compared to the sediments (II, III, IV), a substantial hypoxic water volume is required to compensate for the loss of N removal capacity in the sediments. Consequently, in shallow estuarine and coastal areas, such as those found in the Gulf of Finland, sediments are always the most important sites for N removal. In deeper areas with high volumes of hypoxic water, the water column can potentially be an important site for N removal.

Modeling studies suggest that in the Baltic Sea approximately 40% of the total nitrogen removal occurs within the hypoxic water column (Savchuk & Wulff 2007) (Figure 3). However, the importance of the water column N removal in the Baltic Sea is controversial because there are only a few N removal rate estimates that could confirm these values. Some studies show that denitrification in the water column of the Baltic Proper is not an important sink for N (Shaffer & Rönner 1984, Brettar & Rheinheimer 1992). Other studies have found a high denitrification potential (Hannig et al. 2007, IV), but no NO_3^- has been available at the depths in which the highest potentials were found. Theoretically nitrification and denitrification have the same O_2 tolerance ranges (Lam & Kuypers 2011). Moreover, nitrification in the Baltic Sea redoxcline was not sensitive to low amounts of H_2S (IV) therefore nitrification and chemolithoautotrophic denitrification could occur within the same layer. However, direct coupling of nitrification and denitrification was never observed in study IV.

High anammox rates in the Baltic Sea water column have been found parallel with high MnO_x concentrations after a major inflow event (Hannig et al. 2007). The occurrence of anammox in high MnO_x concentrations was explained by H_2S oxidation with MnO_x. When H_2S is oxidized by MnO_x, denitrification does not have electron donors, which allows anammox to dominate NO_3^- reduction. There are indications that nitrification could use MnO_x as an electron acceptor (Luther et al. 1997, Hulth et al. 1999), therefore presence of MnO_x

in the anoxic depths could allow the coupling of nitrification and anammox. However, conditions that are favorable for coupled nitrification anammox probably occur rarely. In study IV, only on one occasion were low levels of anammox measured in the redoxcline of the northern, shallower end of the Gotland Basin. This occurred after intense mixing of the water column, which may have created conditions resembling those found after major inflow events.

The N removal in the redoxcline may also proceed as pulses, by which the redoxcline oscillates and brings NO_3^- for denitrification. However, this is not supported by the modeling studies that indicate the depths at which the denitrification potentials were found may be stagnant for several years (Stigebrandt & Wulff 1987). Therefore, it is likely that the denitrification potentials found in the water column are substantially higher than the *in situ* denitrification rates.

4.4 The controlling factors for nitrogen cycling in the Baltic Sea

4.4.1 Sediment organic content

The seasonality of the sediment N cycling rates can be explained by the high variation in the availability of the organic matter. In the Gulf of Finland, approximately 80% of carbon is sedimented after the spring bloom in May with low sedimentation rates for the rest of the year (Tallberg & Heiskanen 1998). Although the material that is sedimented in May is slowly mineralized and the availability of organic carbon for heterotrophic bacteria can be high up to late fall (Hietanen & Kuparinen 2008), the quality (lability or degradability) of organic matter for denitrification appeared to decrease within a few months. This is because the denitrification rates decreased from the maximum rates in August to approximately one fifth in November. Moreover, in the more shallow station where the sediment organic content was particularly low, the addition of NH_4^+ increased nitrification in late fall but the denitrification was not sufficient to reduce the NO_3^- produced by nitrification and NO_3^- diffused into the watercolumn (II). The lack of capability of the denitrification process to reduce the NO_3^- was probably caused by a lack of organic carbon.

The highest nitrification and denitrification rates under *in situ* conditions in the coastal Gulf of Finland were measured in late summer, when the sediment organic content was at its highest. At this time, denitrification was limited by the low NO_3^- availability (data not shown) and nitrification reached its maximum potential in NH_4^+ oxidation (II). The reason why NH_4^+ additions did not stimulate nitrification in high organic content sediments may have been due to the large numbers of heterotrophic bacteria found in the sediment. Laboratory experiments have shown that heterotrophic bacteria have a much higher affinity for O_2 when compared to nitrification bacteria, and that the heterotrophic bacteria outcompetes nitrification bacteria for O_2 uptake (Herbert 1999). This phenomenon is also supported by the increased nitrification potential found in the coastal stations in spring and late fall when the sediment organic content was low (II). The NO_3^- concentrations found in the open Gulf of Finland bottom water indicate active nitrification (Table 2). Thus it appears that if the sediment organic content is low, as was the case in the open Gulf of Finland, only very small amounts of O_2 would be required for nitrification. Therefore, the availability of the organic matter, which regulates the competition between heterotrophic and nitrification microbes, seems to be a more important controlling factor for nitrification than the concentration of O_2, as long as O_2 is available in very low concentrations.

Although the competition for O_2 was not as intense in low organic content sediments, the NH_4^+ availability was low and nitrification was correspondingly limited by the availability of NH_4^+. This was indicated by the stimulation of nitrification when NH_4^+ was added into the low organic content sediment cores (II). Stimulation of nitrification by NH_4^+ addition also explains the positive correlation between the NH_4^+ concentration in the bottom water and denitrification rates. When nitrification increases, the NO_3^- concentration increases and denitrification is also stimulated (II,III). The correlation between NH_4^+ concentration in the bottom water and denitrification can also be caused NH_4^+ accumulation being a sign of high mineralization rates (Herbert 1999). If the mineralization rates are high, usually also the sediment organic content is high, and consequently denitrification is stimulated by the high availability of labile organic carbon.

Anammox is a chemolithoautotrophic process, and it is not dependent on organic carbon. Instead anammox is favored in low organic content sediments in which denitrification as a heterotrophic process has very low carbon availability, and therefore the competition for NO_3^- between denitrification and anammox microbes is not as intense as in high organic content sediments (Thamdrup and Dalsgaard 2002, Dalsgaard et al. 2005, Engström et al. 2005). The results of this study are well in line with this phenomenon, because anammox occurred in late fall when the sediment organic content had decreased from the late summer values.

DNRA can be both heterotrophic and chemolithoautotrophic process. However, heterotrophy of DNRA in the Gulf of Finland is not supported by the high DNRA rates found in the open sea stations where the sediment organic content was low (Table 2). This is because fermentative DNRA is favored in conditions where the availability of organic carbon is high (Bonin 1996, Nijburg et al. 1997). Therefore, it is more likely that DNRA is a chemolithoautotrophic process that uses H_2S as an electron donor. Chemolithoautotrophic DNRA in the open Baltic Sea sediments is also supported by the presence of *Beggiatoa* spp. in those stations where the highest DNRA rates were measured (III). *Beggiatoa* spp. are large gliding sulfur bacteria that form white mats on anoxic sediment surfaces. Although most known *Beggiatoa* spp. strains use O_2 as an electron acceptor, some strains have been found to be capable respiring NO_3^-, hence to carry out DNRA (Preisler et al. 2007). Moreover, the low DNRA rates found in the coastal stations also support chemolithoautotrophy of DNRA. In the coastal stations the surface layer remained well oxidized throughout the sampling season (II), which caused no H_2S accumulation near sediment surface. If the H_2S were deeper in the sediment, then the substrates for DNRA did not co-exist, and the DNRA rates remained low.

4.4.2 Temperature

The effect of temperature on the N cycling rates in the open Gulf of Finland sediments is insubstantial, because during the strong halocline the bottom waters do not mix with the surface layer. Mixing of the open sea water column can occur during a weak halocline, but only in late fall when the surface water temperatures have decreased. Therefore, the bottom water temperatures remain relatively stable in the open sea throughout the year. However, in the coastal areas fluctuations in the temperature cause considerable seasonal variability in the N cycling rates. The temperatures in the coastal stations varied between 2-16°C and the highest nitrification rates under *in situ* conditions were measured during late summer when the temperatures were high (Table 2). This can be expected because nitrification is stimulated by warm temperatures (Focht & Verstraete 1977). However, the highest nitrification potentials in this study were found at low temperatures (II). Stimulation of nitrification in cooler temperatures can be explained by the negative effect that temperature has on the activity of the heterotrophic bacteria that compete for O_2 with nitrification microbes. Furthermore, in cooler water temperatures the solubility of O_2 increases, which can stimulate nitrification even further (Herbert 1999). Hence, in organic rich sediments found in the sampling area, the competition for O_2 between heterotrophic and nitrification microbes, is the factor that controls nitrification, rather than temperature *per se*.

The highest denitrification rates were also measured when the temperatures were at their annual high (Table 2) and the positive correlation between denitrification and temperature has been showed in several studies (Nowicki 1994). On the other hand, anammox has been found to be stimulated in cooler temperatures when the denitrification rates decrease and the competition for NO_3^- between denitrification and anammox bacteria ceases (Rysgaard et al. 2004). The stimulation of anammox in cooler temperatures was also observed in this study. However, it is not clear whether the stimulation was caused by colder temperatures or because of decreased organic content in sediments, which occurred parallel with the temperature decrease.

The effect of temperature on DNRA rates remained unclear because both high and low DNRA rates were found in similar temperatures (Table 2). However, a chemostat study with isolates obtained from estuarine sediments showed that in temperatures of around 20°C DNRA bacteria had a higher affinity for NO_3^- than denitrification bacteria. In lower temperatures (5°C), denitrification bacteria were found to be better scavengers for NO_3^- (Ogilvie et al. 1997). Moreover, DNRA is strongly regulated by the O_2 concentration in the bottom water, therefore it can be expected that the high O_2 consumption by mineralization and low solubility of O_2 in high temperatures stimulates DNRA. Support for this suggestion was provided when DNRA stimulated during warm water temperatures in estuarine sediments (Jørgensen 1989). Similarly, DNRA dominated NO_3^- reduction was reported in a tropical estuary where water temperatures were always above 24 °C (Dong et al. 2011).

4.4.3 O_2

O_2 availability is one of the key factors that control nitrification. In this study the highest nitrification rates under *in situ* conditions were found in the in late summer when the O_2 concentrations were at their annual lowest (Table 2). Moreover, NO_3^- was available in the bottom water in the open sea samples despite the low O_2 concentration (Table 2), thus it appears that nitrification was not inhibited by low O_2 availability. Many nitrification bacteria prefer low O_2 concentrations. However, the O_2 optimum appears to be location specific and nitrification bacteria can adapt to varying O_2 concentrations (Canfield et al. 2005). Nitrification bacteria can even survive periodic anoxia and, although the physiological basis is not well understood, it seems that nitrification bacteria are capable of heterotrophic growth during anoxic periods. Nonetheless, they recover nitrification capacity immediately after O_2 re-appears (Jensen et al. 1993, Bodelier et al. 1996).

Bottom water O_2 concentration also controls denitrification. In the coastal sites, no correlation was found between O_2 and N removal rates. However, in the open sea where much lower O_2 concentrations were found, denitrification correlated positively with O_2 concentration (III). This suggests that N removal does not cease until a certain threshold O_2 concentration is attained, which is below the O_2 concentrations measured at the coastal sites. The denitrification rates measured in the open Gulf of Finland were not substantially lower than the denitrification rates measured in the coastal sites in early spring, although the O_2 concentrations were much lower than those measured at the coastal sites (Table 2). However, when the open sea denitrification rates are compared to denitrification rates measured in early 90s, the rates had decreased by almost 50% (Tuominen et al. 1998). The major difference between the early 90s and 2008 is the decreased O_2 concentration in the bottom water. Therefore it appears that the low O_2 conditions that were prevailing in 2008 had decreased the N removal rates. In addition, when the O_2 concentrations decreased below approximately 2 ml l^{-1}, NO_3^- was reduced by DNRA instead of denitrification (III).

DNRA rates were also highly regulated by the O_2 concentration in the bottom water, and the highest DNRA rates were found in the lowest O_2 concentrations (Table 2, III). This may be because low O_2 concentrations in the bottom water decrease the penetration of O_2 by diffusion into the sediment, which allows the accumulation of H_2S near the sediment surface. DNRA has also been found to be stimulated in such conditions (Gardner et al. 2006, McCarthy et al. 2008). It has also been shown that H_2S inhibits denitrification, and when H_2S accumulates near sediment surface, DNRA is favored over denitrification in NO_3^- reduction (Dalsgaard & Bak 1994). The inverse correlation between the O_2 concentration and DNRA (III) implies that in the long term low O_2 conditions, the sediment N cycling is altered and instead of being a site for N removal, the sediment becomes a site for N storage.

One of the consequences of low bottom water O_2 conditions are alterations in the benthic animal community composition, or even the complete loss of benthic animals (Diaz 2001). The areas sampled in this study had very high abundances of *Marenzelleria* spp. (II, III). *Marenzelleria* spp. is an invasive polychaete that was first discovered in the Baltic Sea in the late 80s (Bick & Burchardt 1989). *Marenzelleria* spp. are known to be tolerant of low O_2 conditions (Schiedek 1997, Hahlbeck et al. 2000) and the increase in their abundance might be an indication of decreased O_2 availability in the sediment surfaces. The appearance of *Marenzelleria* spp. can also be a sign of recovery in areas that suffer from low O_2 conditions. For example, denitrification was measured at station XV-1 in June 1994, and then the O_2 concentration was 3.2 ml l^{-1} and the denitrification rate was 13 µmol N m^{-2} d^{-1} (unpublished data). In 2008, station XV-1 had the highest denitrification rate of all stations sampled and the O_2 concentration had increased from the values measured in mid 90s (Table 2). Interestingly, 2008 was the first year when *Marenzelleria* spp. was found at station XV-1 (J. Norkko 2011). It may be that the burrowing activity by *Marenzelleria* spp. increased the diffusion of O_2 into the sediment and restored the sediment N removal capacity.

The highest nitrification potential in the water column was always found in low O_2 concentrations (0.5–0 ml l^{-1}), which indicated that water column nitrification is also stimulated in low O_2 availabilities (IV). Interestingly, nitrification was found to be active even when H_2S was present (IV), which suggests that the nitrifiers in the Baltic Sea water column are AOA, which are known to be tolerant to H_2S (Coolen et al. 2007). The minimum O_2 concentration required for nitrification could not be determined from these samples because nitrification was measured in all samples. However, the results from the OMZs suggest that only nanomolar concentrations of O_2, which are far below the detection limits of the analysis methods used in the study (IV), is required for nitrification (Lam et al. 2007). It has also been proposed that manganese (MnO_x; Mn(III)/(IV)) may potentially serve as electron acceptors for anaerobic nitrification (Luther et al. 1997, Hulth et al. 1999) and no O_2 is necessarily required for nitrification. Therefore, nitrification may reach much deeper anoxic layers than previously thought.

The observed negative correlation between DIN and the volume of hypoxic water in the Baltic Sea (Vahtera et al. 2007, Savchuck 2010) cannot be explained by more intense denitrification in the sediments (II, III). The significance of water column denitrification is also controversial. In the Gulf of Finland, N removal from the water column cannot be a significant sink for N, because the volume of hypoxic water is very small. However, N concentration in the Gulf of Finland has not increased between 1995-2005 despite decreased denitrification rates and an unchanged N load (HELCOM 2009), hence in hypoxic conditions N must be either transported away from the Gulf of Finland or stored in the sediments. The latter option is supported by the results of the publication III and the study published by Kuparinen & Tuominen (2001). Kuparinen & Tuominen (2001) showed that massive NO_3^- releases occurred in the western Gulf of Finland sediments when an inflow that provided O_2 to bottom water entered the area. The occurrence of the NO_3^- release parallel with the entrance of O_2 was explained by the oxidation of NH_4^+ that had been stored into the sediment during the anoxic period.

4.5 Implications of the results for modeling phytoplankton blooms and N budgets in the Baltic Sea

Modeling is a powerful and cost effective tool for examining N cycling rates and an aid for management strategies. In order to create reliable predictors for phytoplankton blooms, the fluctuations of the N load should be linked to seasonal changes in the N removal rates. The lowest N removal rates occurred in early spring, when the N load from the drainage basin was at its highest due to snow melt (II). Therefore, the N that originates from the drainage basin is almost directly available for the spring bloom, with only minor N reductions by denitrification. The N load in spring is critical for the development of hypoxia because spring blooms are often N limited and the sedimentation of intense spring blooms are the main cause of seasonal hypoxia (Conley et al. 2009a). Hypoxia causes P release from the sediments, which stimulates phytoplankton growth in late summer. The N removal rates were maximal in late summer (Table 2) when the N load from the drainage basin is typically at its lowest. Consequently, the availability of P increases and availability of N for primary production decreases, which allows the N fixing cyanobacteria to dominate the phytoplankton species composition. The N fixation by diazotrophic cyanobacteria can contribute substantially to the total N load at this time (Figure 3). The maximal N removal rates decrease within a few months after primary production ceases (Table 2). At the same time, the N load of the drainage basin increases due to higher precipitation rates that increase the flow of N discharging from the drainage basin. However, phytoplankton cannot use this N until availability of light increases in spring.

One of the most used predictors for the N and P cycling rates in the Baltic Sea is the Simple As Necessary Baltic Long-Term Large-Scale model (SANBALTS; Savchuck & Wulff 2007). This model has also been the basis for nutrient management strategies for the Gulf of Finland (Pitkänen et al. 2007) and for the entire Baltic Sea (Wulff et al. 2007). The SANBALTS model calculates for conditions that prevailed between 1991-2002 in the Gulf of Finland an average denitrification rate of 430 µmol N m^{-2} d^{-1} (Savchuck & Wulff 2007), and the modeled rate matches the actual rates measured in the early 90s (Tuominen et al. 1998). The 1991-2002 period covers rather different hydrographic and biogeochemical conditions (Savchuck & Wulff 2007) but it is clear that the estimated 430 µmol N m^{-2} d^{-1} for an average denitrification rate is too high for conditions that were prevailing in the Gulf of Finland in 2008-2009 (Table 2).

A clear weakness of the SANBALTS and some other models (Vahtera et al. 2007, Savchuk 2010) is that they do not differentiate coastal sediment N dynamics from those of open sea sediments, although the response to varying O_2 levels is very different between these areas. In the open sea, hypoxia is more long term due to a stronger halocline and deeper water depths, whereas in the coastal areas hypoxia is a late summer phenomenon with normal O_2 conditions returning when the water column is mixed in the autumn. Short term anoxia in the coastal sediments may even enhance sedimentary denitrification (Hietanen & Lukkari 2007). In contrast, the long term hypoxia present in the open Gulf of Finland clearly decreased denitrification rates, which caused N storage into the sediment (III). Only in the deep basins located in the Baltic Proper, was the volume of hypoxic water so high that conditions were favorable for substantial water column N removal to take place (IV). Another weakness of the SANBALTS model and other N budget calculations (Granéli & Granéli 2008) is that they do not consider storage of NH_4^+ into the sediments, which occurred in the Gulf of Finland in 2008-2009 (III). The results of this thesis show that when the bottom water was hypoxic, N was removed from the water column but not from the sediments (III). This may seem like N removal in budget calculations that are based on N concentrations in the water column. However, the NH_4^+ stored into the sediment can be easily released (Kuparinen & Tuominen 2001) and should not be considered as lost from the system.

Predicting the effect of climate change on Baltic Sea N cycling is challenging. The HELCOM (2007) climate change scenarios predict increasing rains and

Table 2. The physicochemical characteristics and mean nitrification, denitrification, anammox, and DNRA rates of the sediment sampling stations. n/a =data not available. GoF = Gulf of Finland. BS = Baltic Sea. SE = standard error. *denitrification rate estimated from $^{15}NH_4^+$ oxidation samples because denitrification was not NO_3^- limited.

Station	Location	Sampling time	O_2 ml l^{-1}	NH_4^+ µM	NO_3^- µM	LOI %	Temp °C	Denitrification µmol N m^{-2} d^{-1} (SE)	DNRA µmol N m^{-2} d^{-1} (SE)	Anammox µmol N m^{-2} d^{-1} (SE)	Nitrification µmol N m^{-2} d^{-1} (SE)	Nitrification NH_4^+ limited	Salinity
Storfjärden	Coastal GoF	Sep 07	3.7	4.5	2.8	22	8.9	301 (42)	29 (3)	0	291 (34)	no	6.3
		Apr 08	9.2	2.5	2.6	17.7	2.4	161 (37)	10 (2)	0	156 (40)	yes	5.4
		Aug 08	4.6	6.2	1	21	16.2	293 (18)	12 (2)	0	278 (17)	no	5.9
		Nov 08	9.5	0.3	5.3	10.8	6.4	44 (17)	11 (2)	11 (3)	43 (14)	no	6.5
		Apr 09	8.8	0.1	6.9	13.2	0.8	47 (16)*	14(4)	0	689 (80)	yes	5.3
		Aug 09	4.0	1.8	3.5	31.6	5.7	193 (17)	42 (11)	0	530 (83)	no	6.6
		Dec 09	6.5	1.4	4	8.6	6.3	14 (4)	n/a	5 (1)	13 (4)	no	6.3
Muncken	Coastal GoF	Apr 08	9.2	0	1.1	7.5	2.7	24 (11)*	25 (4)	0	47 (251)	yes	5.3
		Aug 08	5.0	4.9	0.7	9.3	16.6	248 (13)	5 (1)	0	242 (14)	no	5.9
		Nov 08	8.1	0.2	5.4	7.6	6.3	38 (12)	1 (0)	13 (3)	379 (223)	yes	6.5
		Apr 09	9.5	0.2	4	5.2	1.7	32 (9)*	n/a	0	252 (70)	yes	5.2
		Aug 09	6.0	1.4	2.2	7.8	14.3	130 (5)	5 (0)	0	288 (32)	yes	5.8
		Dec 09	8.1	0.1	4.3	8.6	4.6	41 (17)	n/a	0	31 (14)	no	4.5
LL9	Open GoF	May 08	2.2	0	10.7	4.9	4.9	46 (7)	125 (25)	0	n/a	n/a	9.3
GF 1		May 08	0.9	1.3	1.5	0	5.4	49 (16)	604 (92)	0	n/a	n/a	10.1
GF 2		May 08	0.7	1.5	5.1	2.7	5.2	89 (12)	1060 (148)	0	n/a	n/a	9.8
XV-1		May 08	5.4	6.7	8.7	0.9	3.5	1619 (413)	329 (76)	0	n/a	n/a	6.9
St1	Open GoF	Jun 08	1	6.1	8.2	12.6	4.9	90 (33)	592 (119)	0	n/a	n/a	9.3
St2		Jun 08	2.5	6.5	2	10.9	4.5	316 (138)	327 (120)	0	n/a	n/a	8.7
St5		Jun 08	4.1	4.2	1.2	9.9	3.7	387 (174)	487 (66)	0	n/a	n/a	7.5
HYPERGoF		Jun 09	2.7	1.2	8.4	n/a	4.4	93 (67)	33 (6)	0	n/a	n/a	7.9
HBP 137	Southern BS	Jun 09	1.1	0.2	8.5	n/a	4	121 (8)	33 (8)	0	n/a	n/a	n/a
LF1		Jun 09	5.4	0.1	2	n/a	4.3	61 (14)	13 (2)	0	n/a	n/a	7.9
HAS		Jun 09	3.9	0.2	3.3	n/a	8.3	38 (10)	23 (8)	0	n/a	n/a	8.2

shorter periods of ice cover for the northern parts of the Baltic Sea. The increasing rains will increase nutrient loads discharging from the drainage basin as more water with nutrients from land will be transported to the sea. Nitrification and denitrification rates were higher in the Gulf of Finland when there was no ice cover (II) hence increased N removal rates may mitigate the effects of increasing N loads. The effects of ocean acidification, caused by elevated CO_2 concentration in the atmosphere, are largely unknown. Nitrification is a pH sensitive process and decreasing pH has been shown to decrease nitrification rates in the open sea (Beman et al. 2011). This may lead to decreased N removal rates in the water column because the N removal processes are often highly coupled to nitrification. The effects of ocean acidification on sedimentary N processes are unknown but the effects are likely to be smaller when compared to the water column because sediments are relatively well buffered (Drake et al. 1987).

Although the computational power has increased substantially in the past years, which has allowed the development of more complex models, the models still can give only rough estimates for N cycling. This is because N cycling rates are spatially highly variable (II, III IV), N dynamics are rather poorly understood, and N cycling is highly linked to other biogeochemical cycles. In addition to the complexity of N cycling, the environmental change scenarios are also highly uncertain, and therefore predicting for the future N cycling with models will remain a challenging task.

5. Conclusions

- For low nitrification rates and/or when denitrification is not NO_3^- limited, the $^{15}NH_4^+$ oxidation technique is the only option for nitrification measurements

- The most convenient way to analyze the $^{15}NO_3^-$ in water samples is the SPINMAS method. However, in low ^{15}N concentrations the denitrifier method is more suitable.

- Nitrification controls N removal rates only when the sediment organic content is high during late summer, and denitrification is not limited by the availability of organic carbon. In other seasons N removal is limited by the low availability of organic matter.

- The NO_3^- reduction in the Gulf of Finland sediments is dominated by denitrification and DNRA, with anammox occurring only periodically when the total N_2 production rates are low.

- In hypoxic conditions NO_3^- is reduced by DNRA rather than by denitrification or by anammox that remove N from the water ecosystem. Therefore, in hypoxic conditions N is stored in rather than removed from the sediments.

- There is high N removal potential in the Baltic Sea water column, but the denitrification process was spatially separated by at least a 10 m from the substrate producing nitrification process and consequently the *in situ* denitrification rates in the water column rarely reach their maximum potential.

- The negative relationship between the volume of hypoxic water and the amount of DIN in the Baltic Sea could also be explained by N storage in the sediments, since high sediment DNRA rates were found under hypoxic conditions.

- Models that predict phytoplankton blooms and N cycling should include seasonal variation of N cycling and treat shallow coastal areas differently to those of deeper open sea areas.

6. Acknowledgements

The funding for this thesis was provided by the Onni Talas Foundation, Maa- ja vesitekniikan tuki (MVTT), BONUS EEIG projects AMBER and HYPER, and the graduate school for Environmental Science and Technology (EnSTe). I am very grateful for the financial support that I have received over the years. I would like to acknowledge my thesis reviewers, Kirsten Jørgensen and Anne Ojala for the valuable comments that helped to improve this thesis. I was pleased to have our newest professor Alf Norkko as the custodian. When it came to picking the opponent, I do not know a better expert on Baltic Sea nitrogen cycling than Maren Voss. You have been a great support over the years and an inspiring example of successful female scientist.

Over the years I have had the pleasure to work in various laboratories. A substantial part of my thesis was done in Tvärminne zoological station, and I would like to acknowledge the staff that enabled excellent working conditions. I also visited several laboratories abroad and I would like to acknowledge the working groups of Daniel Sigman and Bess Ward of Princeton University, Florian Stange's group in UFZ, and Christo Buizert in the Ice and Climate Center of the University of Copenhagen. I was for-

tunate to learn from all of you. Most of all, I would like to acknowledge all my AMBER/HYPER project members in the IOW. Joachim, Barbara, Iris, Frederike, and Claudia, thank you for all the good times during cruises and for the times in Warnemünde!

I cannot thank enough all the people in the division of Aquatic Sciences. This thesis would have not been possible without my supervisor Susanna Hietanen. I am very grateful for all the time and effort you have put into guiding my work and seeking cruise opportunities and funding for my thesis. We have been the lone "Baltic Sea nitrogen duo" in Helsinki, but things have worked out despite the limited group size. I would also like to recognize my second supervisor Elina Leskinen. You have been a great help during the writing process and also good mental support throughout the PhD. Professors Jorma Kuparinen and Jukka Horppila were always willing to help when I needed a senior scientist's opinion. If I ever needed help in the lab, I could always get it from our lab technicians Raija Mastonen and Jouko Saren. I would also like to acknowledge Eeva Nikkola, at first a lab technician trainee and now a lab professional, who processed hundreds and hundreds of samples. Post docs Juha, Sanna, and Zeynep gave valuable advice on how to survive the PhD. You all were also excellent company during lunch breaks. Last but not least I would like to thank all my fellow PhD students. Hanna, you have been a great support over the years. You decided to take a new position a year ago and leave us but you stayed as a good friend despite the thousands of kilometers distance between us. Thank you for all the good times, I know you will do great in life. Heidi, your kind comments have been an invaluable help in the frustrations and difficulties of carrying out a PhD. Thank you for all the laughs and great pastries. Susi, Adrien, Tiina, Jolla, Saulis, Petti, Inari, and Sanna, you guys are the best. Grabbing coffee (and occasionally couple beers) with you guys just made my days a whole lot more fun.

My family and friends have been an endless support over the years. My parents, Lea and Kalevi, you have always encouraged me to pursue an academic career. Kuopio and Talluskylä visits have been a nice break from the hectic rat race and a reminder of the important things in life. Finally, I would like to thank my husband Petri. You have believed in me even in times when I have been in doubt. My life would be meaningless without you.

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