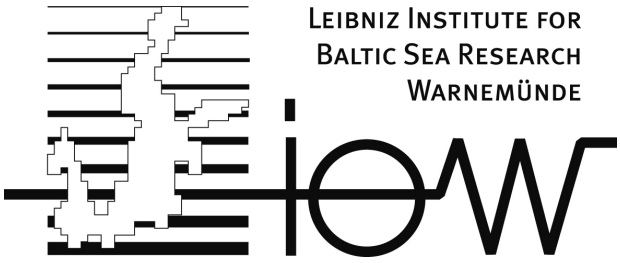

Measurement of Denitrification Rates in Estuarine and Offshore Sediments of the Baltic Sea with the Isotope Pairing Technique

Diplomarbeit

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Monika Wilhelm

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Erstgutachter:	PD Dr. Maren Voss
Zweitgutachter:	PD Dr. Stefan Forster

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Abstract

Denitrification rates in estuarine, coastal and central basin sediments of the Baltic Sea were measured with the Isotope Pairing Technique and related to prevailing environmental conditions. Rates ranged from mean 13 to 690µmol N_2 m^{-2} d^{-1}, with highest rates found at the coastal and intermediate mud stations (493 and 690 µmol N_2 m^{-2} d^{-1}) and lowest rates found at the sandy coastal and estuarine stations and the anoxic station in the Gotland basin (13-60µmol N_2 m^{-2} d^{-1}). The former rates lie within the range of highest denitrification rates measured in the Baltic Sea and the latter represent typical rates found in sandy environments with low organic carbon content. At the coastal mud stations, denitrification was dominated by D_n, which was also the case at the estuarine sandy station. At two of these stations this was apparent in the nitrate profiles, which showed high nitrate concentrations within the sediment, presumably due to nitrification. At the central stations, the share of D_n was lower but a distinct coupling of nitrification and denitrification was still present. With a maximum share of 35%, denitrification played an important role in remineralisation of organic C at most study sites. A multiple stepwise regression of total denitrification rates in relation to environmental conditions yielded a significant correlation only to organic C content ($R^2 = 0.83$). Glucose addition, however, did not have a stimulatory effect on denitrification rates. This suggests that the bacteria present were not acutely carbon limited and that the correlation of *in-situ* rates with organic C content was owed to a larger denitrifying community rather than a lack of electron donors of the existing denitrifiers.

Keywords: Denitrification, Isotope Pairing Technique, Environmental conditions, Sediments, Baltic Sea, Glucose fertilisation.

Kurzfassung

Es wurden Denitrifizierungsraten in Küsten- und zentralen Sedimenten der Ostsee mit der Isotope Pairing Technique gemessen und in Zusammenhang zu den vorherrschenden Umweltbedingungen gebracht. Die gemessenen Raten lagen zwischen 13 und 690 µmol N_2 m^{-2} d^{-1}, wobei die höchsten Raten an den küstennahen und intermediär gelegenen Schlickstationen (493 bzw. 690 µmol N_2 m^{-2} d^{-1}) und die niedrigsten Raten an den küstennahen und ästuarinen Sandstationen sowie der anoxischen Station im Gotlandbecken gemessen wurden. Erstere liegen im typischen Bereich hoher in der Ostsee gemessener Denitrifizierungsraten und letztere repräsentieren typische Raten für sandige Sedimente mit geringem organischem C-Gehalt. An den küstennahen Schlickstationen sowie der ästuarinen Sandstation wurde die Denitrifizierung von D_n dominiert. Dies wurde bei zwei dieser Stationen auch in den Nitratprofilen in Form von hohen Konzentrationen innerhalb des Sediments sichtbar, die Nitrifizierung nahe legen. An den Stationen der zentralen Becken war der Anteil von D_n deutlich geringer, aber eine Kopplung von Nitrifizierung und Denitrifizierung war trotzdem vorhanden. Denitrifizierung spielte an den meisten Standorten eine wichtige Rolle bei der Remineralisierung von organischem C, mit einem maximalen Anteil von 35%.Eine multiple stufenweise Regression der Denitrifizierung im Bezug auf verschiedene Umweltparameter ergab nur einen signifikanten Zusammenhang zum organischen C-Gehalt ($R^2 = 0,83$). Durch die Zugabe von Glucose konnte die Denitrifizierung jedoch nicht erhöht werden. Daraus lässt sich schließen, dass die vorhandenen Bakterien nicht akut kohlenstofflimitiert waren und dass die Korrelation der *in-situ* Raten mit dem organischen C-Gehalt durch das Vorhandensein größerer Denitrifizierergemeinschaften und nicht durch einen Mangel an Elektronendonatoren der anwesenden Mikroorganismen begründet ist.

Schlagwörter: Denitrifizierung, Isotope Pairing Technique, Umweltbedingungen, Sedimente, Ostsee, Glucosedüngung.

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Abbreviations

Anammox: Anaerobic Ammonium Oxidation

C: Carbon

C_{org}: Organic Carbon

C rem.: Carbon remineralisation

DNRA: Dissimilatory nitrate reduction to ammonia

DN: Denitrification rate

DW: Dry Weight

FW: Fresh Weight

GW 1: Gollwitz 1

GW 2: Gollwitz 2

IPT: Isotope Pairing Technique

KS: Kreidesegler

N: Nitrogen

^{15}N-N_2: Isotope labelled dinitrogen

NO_x^-: Sum of NO_3^- and NO_2^-

N_{org}: Organic Nitrogen

N rem.: Nitrogen remineralisation

P: Phosphate

Std.: Standard Deviation

TN: Total nitrogen

TP: Total phosphate

1 Introduction

1.1 The Aquatic Nitrogen Cycle

Nitrogen is a key constituent of many important biomolecules, such as amino acids, nucleic acids, chlorophylls and amino sugars, and it is essential to all living organisms. Between its most oxidized and reduced compounds, there is an eight-electron difference, which is why the redox cycling between nitrogen compounds forms the basis for numerous microbial metabolisms. Many of these microbial processes, in turn, control the availability of nitrogen in the environment and therefore are significant in regulating the activities of primary producing eukaryotes, which require a ready source of nitrogen for growth (Canfield et al. 2005b).

Most nitrogen input into the marine environment occurs via riverine runoff, precipitation and biological nitrogen fixation (Larsson et al. 1985; Rönner 1985; GESAMP 1990; Rabalais 2002; HELCOM 2003), although the importance of each of these sources differs greatly between areas. In the central basins of the Baltic Sea, for instance, most nitrogen is derived from biological nitrogen fixation and atmospheric deposition, while the coastal areas are dominated by riverine nitrogen input (Voss et al. 2005). Through the process of nitrogen fixation, atmospheric dinitrogen (N_2) is converted into a bioavailable form, namely ammonium, which can be incorporated into biomolecules (Sprent and Sprent 1990). Most particulate organic nitrogen (PON) is recycled into inorganic form by a process known as ammonification in which nitrogen-containing biomolecules are degraded by microorganisms or digested by animals. In addition to PON, some of the fixed N is released in the form of dissolved organic nitrogen (DON), which can also be assimilated by phytoplankton and bacteria. The so formed PON is recycled into inorganic form as well. The released ammonium (NH_4^+) can either be re-assimilated by microbes or plants and transformed into new biomolecules, or it can be oxidised by an assemblage of largely chemolithoautotrophic prokaryotes in a process known as nitrification (Frankland and Frankland 1889). The resulting oxidised nitrogen forms, nitrite (NO_2^-) and nitrate (NO_3^-) (together named as NO_x^-), can either be assimilated by microorganisms and plants or be denitrified to N_2 by a number of heterotrophic prokaryotes using oxidised nitrogen as electron acceptor and

organic carbon, for example, as electron donor. The produced N_2 is then returned to the large atmospheric pool and thus, on short term, is lost for further biological transformations. Most bio-available nitrogen is recycled several times between autotrophic and heterotrophic organisms, because the rates of nitrogen input to the biosphere by N fixation, and output by denitrification, are at least an order of magnitude slower than the internal cycling rates. This conventional view of the nitrogen cycle has recently been amended with the anammox process, in which ammonium oxidation is coupled to nitrite reduction, leading to the production of N_2 (Mulder et al. 1995). This process is performed by anaerobic, autotrophic bacteria and thus is restricted mainly to areas of low oxygen concentrations. According to Thamdrup and Dalsgaard (2002), anammox can play an important role in removing nitrogen from the sea, especially in sediments of moderate organic carbon content. Being a sink of nitrogen in the ecosystem, denitrification and anammox are of particular importance, especially in the context of additional N input into the ecosystem through anthropogenic activities.

1.2 The Process of Denitrification

As first reviewed by Payne (1973), denitrification is defined as the microbial process, by which nitrate and nitrite are sequentially reduced to nitrogen gas and nitrous oxide. Denitrifying bacteria are mostly facultative anaerobic and heterotrophic bacteria, using organic carbon compounds as electron donors and nitrate as electron acceptor, with some also being able to use H_2S as alternative electron donor (Brettar and Rheinheimer 1991). The process of denitrification consists of a number of respiratory reduction steps:

$$NO_3^- \xrightarrow{Nitratereductase} NO_2^- \xrightarrow{Nitritereductase} NO \xrightarrow{NO-reductase} N_2O \xrightarrow{N_2O-reductase} N_2$$

Although complete denitrification of NO_3^- and NO_2^- to N_2 is most common, a number of denitrifiers lack one or more enzymes or environmental conditions favour the accumulation of intermediate products like N_2O. When pH decreases in the environment, for example, the end product of denitrification gradually shifts from N_2 to N_2O due to the increasing inhibition of N_2O-reductase under acidic conditions (e.g. Knowles 1982). Denitrification in aquatic sediments can be subdivided into two processes of varying importance, depending on the prevailing environmental conditions: (1) Denitrification of nitrate from the overlying water and (2) coupled nitrification-denitrification in the sediment. D_w denotes the denitrification of nitrate from the overlying water, while D_n constitutes the coupled nitrification-denitrification driven by nitrate production from nitrifica-

tion in the oxic-anoxic interface of the sediment (Jenkins and Kemp 1984). In most river, lake and coastal marine sediments underlying an aerobic water column, D_n driven by nitrate produced in the sediments is the prevailing process (Seitzinger 1988).

The most important factors controlling denitrification are generally believed to be low oxygen concentration (< 0.2ml L^{-1}), availability of organic carbon (as electron donor), and the nitrate concentration (as electron acceptor) (Knowles 1982; Hattori 1983). Limitation of denitrification by organic carbon supply is particularly important in sandy sediments, which mainly consist of coarser mineral particles and lack a sufficient supply of organic material. Concerning the share of D_w and D_n in total denitrification, it seems that high nitrate concentrations in the overlying water, e.g. in rivers and estuaries, promote a high contribution of D_w due to the steep concentration gradient towards the sediment and thus efficient penetration of nitrate into the denitrification zone (Silvennoinen et al. 2007). In addition, the oxygen concentration is an influential factor in the way that low oxygen concentrations reduce the oxygen penetration depth of the sediment, which in terms shortens the distance that nitrate needs to diffuse to reach denitrifying sediment horizons (Silvennoinen et al. 2007). Furthermore, the presence of H_2S may inhibit coupled nitrification-denitrification (Joye and Hollibaugh 1995), but in some cases can also have a positive effect on denitrification as an alternative electron donor when organic carbon is scarce (Brettar and Rheinheimer 1991).

Apart from physico-chemical conditions, biological activity of bioturbating macrofauna have been found to be beneficial, either by enhancing the nitrate flux from the overlying water into the sediment (Karlson et al. 2005) or by enlarging the surface area of the sediment and producing tightly coupled oxic-anoxic microhabitats (Tuominen et al. 1999; Kuparinen and Tuominen 2001; Gilbert et al. 2003).

1.3 N_2 production in the Baltic Sea and its capacity to reduce the nitrogen load in the context of eutrophication

It has long been recognized that denitrification affects ecosystems and biogeochemical cycles at local, regional, and global scales (Codispoti and Richards 1976; Nixon et al. 1976; Seitzinger 1988; Revsbech and Sørensen 1990). Denitrification removes fixed N that would otherwise be available for primary production or microbial assimilation. Especially in rivers and lakes, as well as estuaries, denitrification appears to remove a major portion of the mineralised nitrogen and the loss of nitrogen via denitrification may

exceed the input via N_2 fixation (Seitzinger 1988). In low-N systems, denitrification therefore contributes to N limitation by further decreasing N concentrations and by reducing the N:P ratio of recycled nutrients. In systems highly enriched with N from anthropogenic sources, removal of fixed N by denitrification reduces the export of N and, in the case of rivers and estuaries, thus potentially reduces eutrophication of downstream ecosystems.

Due to its intra-continental geographic position with its large and densely populated southern drainage area and the prevailing hydrographical conditions, the Baltic Sea is particularly sensitive to nutrient input: As a result of the salinity stratification, the oxygen supply to the central basins is limited and nutrients tend to accumulate in the bottom water. The irregular inflow from the North Sea introduces relatively nutrient-rich bottom water and drainage water carries additional nutrients into the surface layer. A significant increase in total nitrogen (TN) and phosphorus (TP), as well as inorganic nitrate and phosphate, have been recorded for most parts of the Baltic Sea between 1970 and 1990 (Sandén and Rahm 1993). The continuous increasing trend in the 1990s (Conley 2000) caused progressive eutrophication, thus altering nutrient cycles with unpredictable consequences for the ecosystem (HELCOM 2003; ICES 2003). As opposed to nitrogen input, nitrogen loss through denitrification seems to be comparatively high in shallow areas of the Baltic Sea like the Danish coastal waters or the Gulf of Finland (Tuominen et al. 1998), where high nitrate concentrations in the water sustain high denitrification rates. This is confirmed by isotope ratio ($^{15}N/^{14}N$; $\delta^{15}N$) and nutrient data, which suggest that despite pronounced eutrophication in the coastal rim of the Baltic Sea, coastal sediments appear very efficient in removing river borne nitrogen by denitrification (Voss et al. 2005). However, in fast flowing rivers and estuarine areas with short residence times, the capacity to reduce the nitrogen load to the Baltic Sea seems to be limited, even if absolute denitrification rates are high (Silvennoinen et al. 2007). Denitrification rates measured recently at a coastal deposition area in the northern Baltic Sea ranged from 90 to 400µmol N m^{-2} d^{-1} (Hietanen and Kuparinen 2008) and were slightly lower than rates measured in the central Gulf of Finland (100–650µmol N m^{-2} d^{-1}, Tuominen et al. 1998). In the deep basins, denitrification rates are lower compared to regions of similar depth in other oceans, presumably due to the prevailing anoxic conditions (Tuominen et al. 1998). In anoxic accumulation bottoms denitrification may be replaced by dissimilatory nitrate reduction to ammonium (DNRA), which accumulates in the sediments. When anoxic deep water is mixed into oxic water, ammonium is

oxidised to nitrate (nitrification) and thus "bursts" of high NO_3^- levels appear in deep waters. One such NO_3^- "burst" was recorded after the inflow event in 1976 and two after the strong inflow in 1993 (Kuparinen and Tuominen 2001). After the second burst in 1996 - 1997, NO_3^- levels below the halocline were quickly reduced, which suggests an efficient "selfpurification" by denitrification after mixing with oxygenised water. The efficiency of NO_3^- removal by denitrification may also explain some of the decline in long-term NO_3^- concentration recorded for the northern Baltic Proper between 1973 and 1999 (Kuparinen and Tuominen 2001). In contrast to the situation before the strong saltwater inflow in 1976 when moderate and strong inflows had occurred almost annually , long stagnation periods have marked the situation in recent years (Matthäus and Schinke 1999) causing anoxia to spread over extensive areas. This has resulted in a weakening of the denitrification and thus self-purification capacity in wide areas of the Baltic Proper (Kuparinen and Tuominen 2001).

The second major process removing N_2 from the water and sediment, anammox, has only recently been studied extensively, which is why reliable data on its particular importance in the Baltic Sea is still scarce. It has been found in the Skagerrak, where it accounted for 24 to 67% of total N_2 production (Thamdrup and Dalsgaard 2002). In sediments of the Gulf of Finland, anammox made up less than 20% and could not be detected in a shallow estuary (Hietanen 2007). Different results have been obtained for estuarine sediments within the Randersfjord (Denmark), where anammox rates were comparable to those found in Skagerrak sediments, even though the contribution to total N_2 production was low (Risgaard-Petersen et al. 2004a).This low share of anammox in N_2 production compared to Skagerrak was due to ten to fifteen times higher denitrification rates, which most probably can be attributed to the high availability of easily accessible carbon in sediments of the Randersfjord.

All in all, the available data are not yet sufficient to draw reliable conclusions on the nitrogen sinks of the Baltic Sea and the application of a wide range of methods with their individual drawbacks has caused additional confusion. Furthermore, only limited data is available on denitrification in sandy sediments with limited supplies of organic carbon (C_{org}) and highly oxygenated pore water, as often found along the coast of the Baltic Sea and in some areas of the central Baltic. Despite the fact that they make up approximately 70% of the global continental shelf area, these sandy environments have often been overlooked due to unsolved sampling challenges and presumably unfavourable conditions for denitrification. Particularly in the Baltic Sea, they can be assumed to

be of major importance for the N removal and in order to counteract eutrophication, a substantiated knowledge on the dynamics of N removing processes is essential. This is why denitrification rates and anammox were measured with the Isotope Pairing Technique (Nielsen 1992; Risgaard-Petersen et al. 2003) in representative habitats (estuarine and coastal areas and the central basins) and sediment types (sand, bolder clay and mud) of the Baltic Sea and related to the prevailing environmental conditions. In glucose fertilisation experiments, the role of organic carbon supply was further investigated, which should be particularly important in sandy sediments. Against the background of previous findings, the following hypotheses should be proven:

1. At coastal stations with high organic carbon contents and nitrate concentrations (mud stations), high denitrification rates are promoted. With the water-body above the sediment being well-oxygenised, coupled nitrification-denitrification should be important, even though denitrification of NO_3^- from the overlying water should also increase with increasing nitrate levels in the water. The anammox process might be present but does not contribute greatly to the total nitrogen loss due to more favourable and competitively advantageous conditions for denitrifying bacteria.
2. Denitrification rates will be distinctly lower in the sediments of the central Baltic Sea due to limited substrate availability (in particular organic carbon) and inhibition of coupled nitrification-denitrification where oxygen concentrations are too low for microbial ammonium oxidation to nitrate to take place. The anammox process can be expected to be present due to competitively more advantageous conditions, namely less oxygenised sediments and moderate to low organic carbon contents.
3. At estuarine and coastal sandy stations, denitrification rates are low due to limited organic carbon supply, which cannot support an extensive denitrifying community.
4. Denitrification rates are significantly higher in glucose treatments compared to treatments without glucose, particularly at study sites with low organic carbon content in the sediment. In non-glucose treatments, *in-situ* denitrification should increase linearly with increasing C_{org} content in the study sites' sediments, as higher organic carbon levels will sustain a larger denitrifying microbial community.

2 Materials and Methods

2.1 Study Sites

Isotope Pairing experiments were performed at 8 study sites representing estuarine, coastal and deep basin habitats of the Baltic Sea. Figure 1 and Figure 2 show maps of the Baltic Sea with the position and sediment type of the study sites. These are further characterised in Table 1. As can be seen, the coastal and estuarine stations comprise three sandy stations (Breitling, Gollwitz 1 and Gollwitz 2) and one mud station (Kreidesegler). The Breitling forms the most seaward part of the lower Warnow estuary with an area of approximately 3.75 km^2 and is characterised by strongly fluctuating salinities and high nutrient loads due to former input from the fertilizer factory in Poppendorf via the Peezer stream (Bachor 2005). Gollwitz is situated in the north-east of the island Poel in the outer Wismar Bight, close to the island of Langenwerder. Compared to the Breitling, salinity is more stable, although fluctuations can occur after inflows from the Mecklenburg Bight. In the area of Gollwitz close to Langenwerder, sediments of different grain sizes occur at close proximity (as at stations Gollwitz 1 and 2), depending on the degree of shelter from the island of Langenwerder. The Wismar Bight is generally considered as strongly eutrophied and inputs of inorganic nitrogen compounds through agriculture are high, although less pronounced in the outer part of the bight (von Weber and Gosselck 1997). The station Kreidesegler is situated in the Mecklenburg Bight, off the coast of Rostock towards Wismar. Like most coastal environments of the Baltic Sea, the Mecklenburg Bight also suffers from eutrophication and related environmental changes (e.g. increased nutrient concentrations) have been detected (LUNG 2007), although a decreasing trend of chlorophyll a concentrations in spring has also been found from 1979 to 2006 (Wasmund et al. 2007). The station is located within a depositional area and represents a typical coastal mud environment with fine sediment particles and a high share of organic compounds as opposed to the above described coastal sand stations. The stations within the central basins represent two stations characterised by mixed sediments consisting of mud and boulder clay material (NS6 and NS7), as well as two true mud stations (NS1 and Arkona), of which one was covered by anoxic bottom water (NS1) at the time of sampling. As shown in the map, NS1, NS6 and NS7 are central stations inside the Gotland basin, while the Arkona station is located further south-

west and less central (Arkona basin). With the exception of NS1 (anoxic conditions), all stations lie within the depth range where oxygen and nitrate are present in the bottom water. A more detailed description of environmental conditions at the time of sampling is given in the results section.

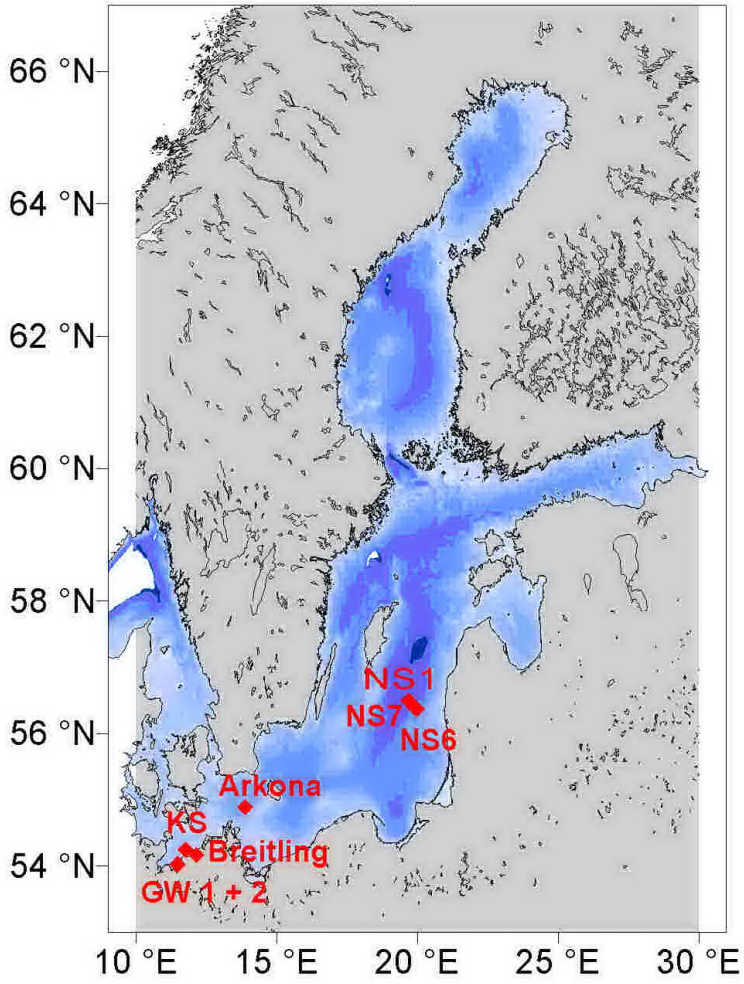

Figure 1: Map of the Baltic Sea with position of study sites. KS = Kreidesegler; GW = Gollwitz. The map was created with the program Surfer 8.

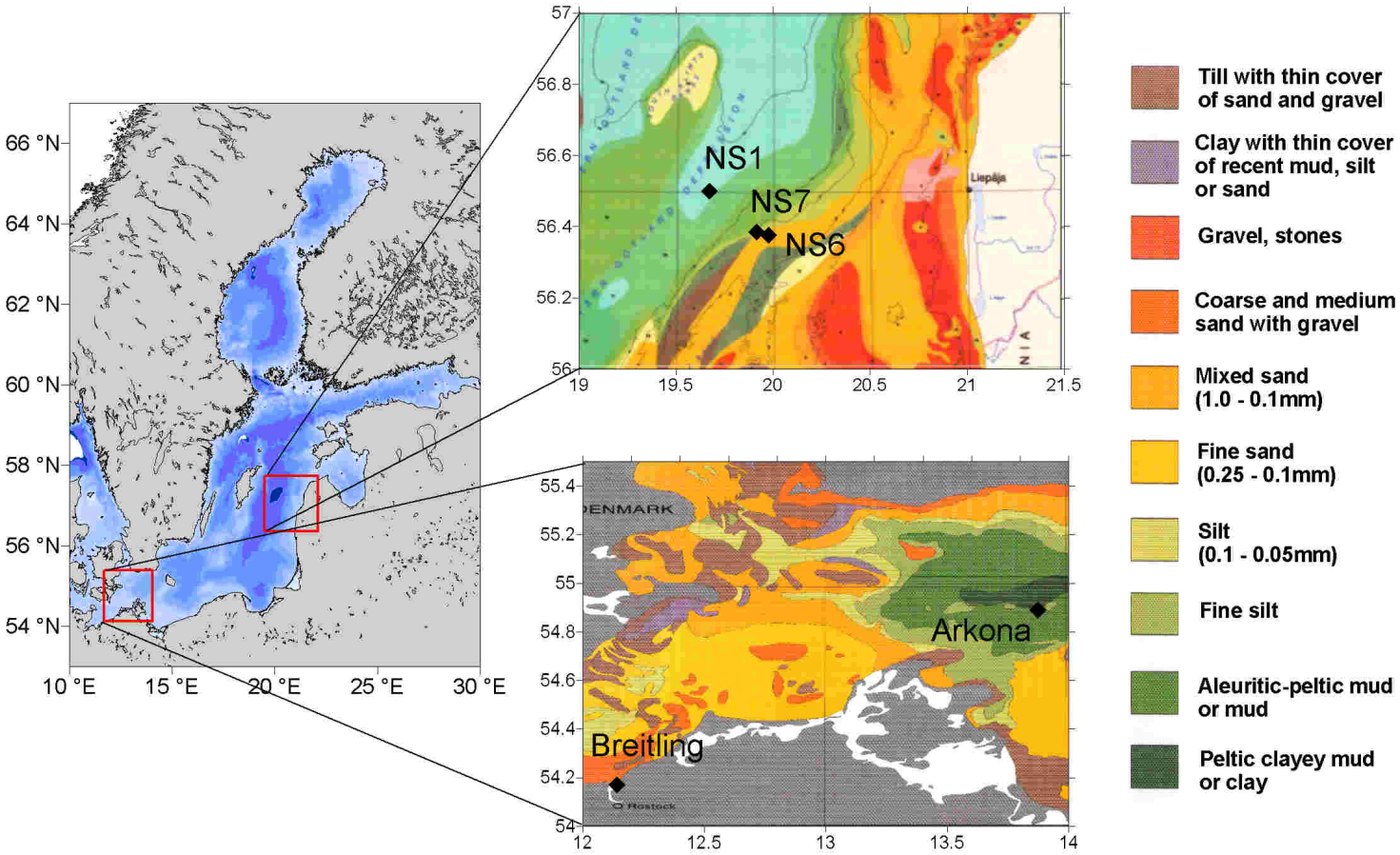

Figure 2: Map of the Baltic Sea (left) with sediment details of study areas (right). For the coastal stations Kreidesegler, Gollwitz 1 and 2, no sediment maps were available. Maps were created with the program Surfer 8.

Table 1: Geophysical characterisation of study sites

Station	**Geographic Position (dec)**	**Proximity to coast**	**Sediment type**	**Depth [m]**	**Bottom temperature [°C]**	**Salinity [PSU]**	**O_2 in overlying water [µmol/L]**
Kreidesegler	54.25°N 11.77°O	Coastal	Silt with high C_{org} (Mud)	25	5.3	14.9	236.2
Breitling	54.17°N 12.14°O	Estuarine	Fine Sand	0.2	22.6	13.8	311.5
Gollwitz 1	54.02°N 11.48°O	Coastal	Fine Sand	0.2	15.3	22.0	354.1
Gollwitz 2	54.02°N 11.49°O	Coastal	Very Fine Sand with higher C_{org}	0.2	15.3	22.0	354.1
NS1	56.50°N 19.67°O	Central basin	Silt with very high C_{org} (Mud)	160	6.27	12.0	0
NS6	56.38°N 19.98°O	Central basin	Silt with moderate C_{org} and coarser partikels at depth (Boulder clay)	75	5.2	9.5	99.1
NS7	56.38°N 19.92°O	Central basin	Silt with relatively high C_{org}/ Boulder clay	80	5.5	9.9	80.8
Arkona	54.89°N 13.87°O	Intermediate proximity to coast	Silt with high C_{org} (Mud)	46	14.6	14.2	85.7

2.2 Sampling and Sample Processing

Sediment cores were taken with a multicorer (deep basins), a Ruhmor corer (coastal station Kreidesegler) and by hand (coastal and estuarine stations Breitling, Gollwitz 1 and Gollwitz 2).

3 cores (10 cm diameter) per station were sliced into pieces of one centimetre (upper 6 centimetres) and 2 centimetres (from 6 to 10 centimetres depth) thickness, respectively, to be subsequently analysed for organic C/N content, porosity, grain size distribution and nutrient concentrations in the pore water. The bottom water overlying the sediment was sampled for oxygen content, as well as nitrate and nitrite (NO_x^-), ammonium and phosphate concentration. In the case of the central sampling stations, water for a depth profile of the latter parameters in the water column was also taken and temperature and salinity profiles were recorded.

To determine denitrification and anammox rates, whole sediment cores (25cm height, 3.6cm diameter) were incubated with $^{15}NO_3^-$ and processed as described under 2.5. During Isotope Pairing incubation experiments, the cores were maintained at *in-situ* temperatures in the dark.

2.3 Physico-chemical characterisation of the water column

For the stations NS1, NS6, NS7 and Arkona, temperature and salinity profiles of the water column were recorded and the water from different depths was sampled with the help of a CTD and analysed for oxygen content, NO_x^-, NH_4^+ and PO_4^{3-} as described under 2.4. From the profile progression of salinity and temperature, the depth horizons of the halocline and thermocline were estimated.

2.4 Sediment characterisation

2.4.1 Grain size analysis

Apart from the Breitling sediment, grain sizes were analysed with the help of a laser sizer (CILAS 1180). For this, a small amount of sediment (approximately 0.5g for muddy sediments and 1g for sandy sediments) was suspended in 20ml of distilled water and amended with 10ml of H_2O_2 to oxidise organic compounds. Afterwards, approximately 10ml of sodium polyphosphate were added to the sample to keep particles in

suspension. This suspension was left to react overnight and measured straight away the next morning. In the case of the Breitling sediment, which was too coarse for laser analysis, grain sizes were analysed by wet sieving approximately 100g through a series of sieves with mesh sizes of 1000µm, 500µm, 250µm, 125µm and 63µm. After sieving, the sieves were dried at 60°C overnight and the sediment weight in each size fraction corresponding to the mesh size of the sieves were determined. The fraction of < 63µm was calculated from the weight difference to the original weight. For each sediment, the first ten centimetres were analysed in slices of one centimetre (1-6 cm depth) and 2 centimetres (6-10cm depth), respectively. The percentage of each size fraction was calculated, as well as the median grain size and trask sorting coefficient (Trask 1932). From these parameters, the sediments were assigned to sediment types according to a modified Folk triangle (Folk 1954) and the Udden-Wentworth scale of sediment classification (Wentworth 1922) (Figure 3). Where values remained relatively constant throughout the whole depth horizon (all stations except NS6), values were averaged over the entire depth. In the case of station NS6, where the material was coarser below 5cm depth, two separate means were taken for the first 5 centimetres and below this depth.

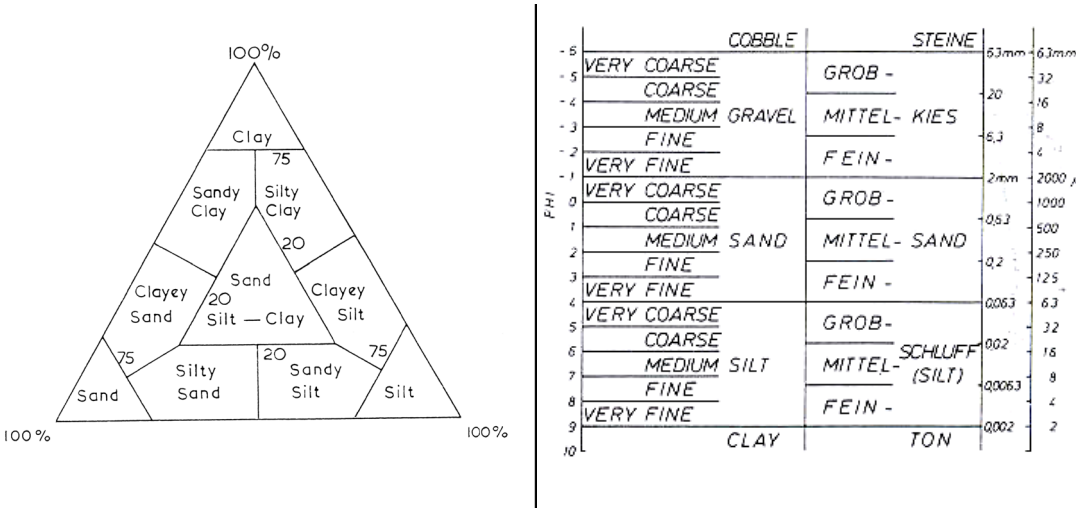

Figure 3: left: Modified Folk triangle of sediment type distinction; right: modified Udden-Wentworth scale of sediment classification.

2.4.2 Determination of oxygen concentration in the bottom water and oxygen penetration into the sediment

The oxygen concentration was determined by Winkler titration (Winkler 1888). For this, glass bottles filled bubble-free with a known volume of bottom water were amended with 0.5 $MnCl_2$ and 0.5 alkaline iodide solutions and shaken vigorously. The

resulting brownish precipitate ($Mn(OH)_3$) was left to settle for at least 30min but no longer than 10 hours before 1ml of sulphuric acid was added to the sample, which was shaken well, again. The iodine formed by oxidation of iodide under acidic conditions was then titrated by adding 0.02N thiosulphate solution with the help of a Titrino (716 DMS Titrino). The oxygen concentration was automatically calculated from the amount of thiosulphate added to reach the endpoint of titration.

Where oxygen was present in the bottom water, the oxygen penetration into the sediment was investigated by recording O_2 profiles of the sediment with the help of an oxygen microelectrode (Unisense standard OX25). The microelectrode was introduced into the sediment in steps of 200µm with a micromanipulator. From the resulting profiles, the oxygen penetration depth was read. Due to logistic constraints (bad weather conditions during research cruise, lack of availability of micromanipulator) it was only possible to record profiles at the coastal station Kreidesegler and at the central station NS6. As NS7 and NS6 were very similar in terms of sediment characteristics, they were assumed to have similar oxygen penetration depths. In the case of the Breitling and Gollwitz stations, oxygen penetration was estimated from the colour of the sediment.

2.4.3 Pore water extraction and determination of nutrient concentrations

For nutrient concentration determination, the pore water of the sediment was extracted for each of eight depth slices in two ways depending on the coarseness of the sediment. In the case of fine, muddy sediment with high water content, approximately 50ml of wet sediment was simply centrifuged at 3000-5000g for ten minutes and the extracted pore water was transferred into clean test tubes. For sandy sediments, it was necessary to use the pore water sampler according to Saager et al. (1990). Here, approximately 50ml of wet sediment was filled into special centrifuge tube inserts, which were equipped with GFF filters and placed into centrifuge tubes. The sediment was then centrifuged as described above to extrude the pore water into the centrifuge tubes underneath the insert.

The resulting pore water was analysed for NO_x^-, NH_4^+ and PO_4^{3-} concentration. For this, 1ml of pore water for each analysis was transferred to 2ml Eppendorf tubes and the nutrient concentration was determined calorimetrically.

2.4.3.1 Determination of phosphate concentration

The phosphate concentration was determined slightly modified to Grasshoff et al. (1983). For this, 0.02ml of a mixture reagent consisting of watery molybdate solution and antimony dissolved in 50% sulphuric acid were added to 1ml of the filtered sample and mixed well before 0.01ml of ascorbic acid solution was added. This mixture was left to react in the dark for at least 20min but no longer than 2 hours and the extinction was measured at 885nm. Phosphate concentration was calculated from a calibration curve obtained from standard solutions of potassium phosphate. According to Grasshoff et al. (1983) the detection limit of the method is 0.01µmol/L and the precision is 0.02 - 0.06µmol/L depending on the concentration of the sample. Due to low sample volumes and the resulting use of a 0.5cm micro-cuvette even at low concentrations for pore water samples, the detection limit is assumed to be higher, in the range of 0.05 - 0.1µmol/L.

2.4.3.2 Determination of ammonium concentration

The ammonium concentration was determined slightly modified to Grasshoff et al. (1983). For this, 1ml of the filtered sample was amended with 0.03ml of phenol reagent and shaken well before adding a mixture reagent consisting of sodium hypochlorite solution and alkaline citrate buffer solution. The sample was left to react at room temperature in the dark for a minimum of 6 hours but no longer than 18 hours. Afterwards, the extinction was measured at 630nm against MilliQ and the ammonium concentration was determined either from a calibration curve (all stations except Kreidesegler) or by multiplying the extinction by a reagent factor obtained from the extinction of a 2µmolar ammonium chloride standard solution (station Kreidesegler). The calibration curve was obtained from ammonium chloride standard solutions. According to Grasshoff et al. (1983) the detection limit of the method is 0.1µmol/L and the precision is 0.1µmol/L. Due to low sample volumes and the resulting use of a 0.5cm micro-cuvette even at low concentrations for pore water samples, the detection limit is assumed to be in the range of 0.5µmol/L.

2.4.3.3 Determination of NO_x^- concentration

NO_x^- concentration was determined with the Spongy Cadmium method (Jones 1984). The spongy cadmium used in this method was produced by placing zinc metal sticks into 20% cadmium sulphate solution for 6-8 hours and breaking the resulting cadmium into small particles in 6N HCL solution with the help of various pipette tips and spatula.

Afterwards, the cadmium was washed with MilliQ to obtain a pH above 5 and kept in MilliQ until use. 200µL ammonium chloride buffer (pH = 8.5) was added to the filtered pore water sample before it was amended with one spoon of wet spongy cadmium (approximately 20mg) and shaken for 90 minutes in the dark. After this, the solution was transferred cadmium-free into a clean test tube and 50µL of Colour Reagent B (Sulfanilamide N-(1 naphtyl) ethylenediamide dihydrochloride solution) was added. The mixture was left to react for 20 minutes in the dark before the extinction of the solution was measured photometrically at 540nm. To calculate the concentration from the measured extinction, a calibration curve was created by plotting the concentration of 5 nitrate standards (1µmol/L, 2µmol/L, 5µmol/L, 10µmol/L and 15µmol/L KNO_3) against their extinction at 540nm. Samples with nitrate concentrations above 15µmol/L were diluted accordingly to make sure that they fall within the linear section of the calibration curve. Above this concentration, a complete reduction of nitrate by the added cadmium can no longer be ensured, which leads to a possible underestimation of nitrate concentrations. The detection limit of this method is given by Jones (1984) as 0.033µmol NO_3^- for 50ml sample. Due to the use of a 0.5cm micro-cuvette even at low concentrations and higher reagent blank values, the detection limit is assumed to be higher, in the range of 0.5 - 1µmol/L.

2.4.4 Calculation of NO_x^- and NH_4^+ fluxes and carbon (C) remineralisation in the sediment

NO_x^- and NH_4^+ fluxes (J) were calculated with the first Fick's law in the following way:

$$J = \phi \times Ds \times \frac{dC}{dz}$$

where φ is the sediment porosity, Ds is the sediment's diffusion coefficient, and $\frac{dC}{dz}$ is the concentration gradient across the sediment-water interface (flux up) or from the top centimetre towards deeper parts of the sediment (flux town). Ds in turn, was estimated from the diffusion coefficients of nitrate and ammonium (Do, taken from Boudreau 1997) as follows:

$$Ds = \frac{Do}{(1 - \ln \phi^2)}$$

For nitrate, it was discriminated between an upward and downward flux where NO_x^- maxima occurred within the top sediment layers (Kreidesegler, Breitling, Gollwitz 1 and Gollwitz 2). Here, the upward flux was calculated from the concentration gradient between the top centimetre of the sediment and the overlying water and the downward flux was calculated from the gradient of the top centimetre towards deeper sediment layers. For the remaining stations, only the downward flux was calculated from the gradient of the bottom water towards the sediment. From the downward nitrate fluxes, denitrification rates were calculated by dividing the flux by 2, in order to account for the molar ratio of nitrate reduced to nitrogen gas produced (two NO_x^- ions are consumed per N_2 molecule). These were compared to rates obtained by the IPT (2.5).

Ammonium fluxes were converted to carbon fluxes with the help of the Redfield ratio (C:N = 106:16). The same was done for denitrification rates (DN, from IP experiments), assuming a stoichometric equivalent of metabolised C of 1.25 ($C:N_2$ = 2.5) from the equation of denitrification:

$$\tfrac{4}{5} NO_3^- + \tfrac{3}{5} H^+ + \tfrac{1}{2} C_2H_3O_2^- \rightarrow \tfrac{2}{5} N_2 + HCO_3^- + \tfrac{1}{5} H_2O$$

The sum of carbon fluxes from ammonium and denitrification were taken as a proxy of total C remineralisation (Crem.):

$$Crem. = J(NH_4^+) \times \frac{106}{16} + DN \times 2.5$$

This was related to denitrification rates (converted to carbon units) to estimate the importance of denitrification in carbon remineralisation.

The value of Crem. (in mmol) was converted to carbon mass (mg) by multiplying with the molar weight of carbon.

2.4.5 Determination of sediment porosity

To determine the porosity, exactly 5ml of wet sediment of each depth were placed in prepared aluminium cups with the help of a syringe and weighed before dried in a drier at 60°C overnight. After drying, the sediment was weighed again and from the loss of weight the porosity φ was calculated in the following way (Pettijohn et al. 1973):

$$\phi = \frac{\frac{FW - DW}{1}}{\frac{FW - DW}{1} + \frac{DW}{2.65}}$$

where FW and DW stand for the fresh weight and dry weight of the sediment and the constants 1 and 2.65 represent standard densities of water and quartz sediment. The density of water was not corrected for salinity and temperature as it was found that the values of φ only changed negligibly and it was more convenient in that way for calculations. Despite the fact that sediment density can be assumed to vary between sediment types, the constant of 2.65 determined for sandy sediments was applied for all stations as an exact sediment density determination was too time-consuming within the time limits of this work. Apart from that, denitrification rates only changed marginally when assuming a ten percent higher or lower porosity. For calculations of denitrification rates, mean porosities of the depth horizon homogenised in tracer experiments were used.

2.4.6 Determination of organic carbon (C_{org}), organic nitrogen (N_{org}) and C/N ratio

Particulate organic carbon and nitrogen, as well as C/N ratios were determined with a C/N elemental analyser (EA 1110 CHN, CE Instruments).

Depending on the sediment type and expected C_{org} and N_{org} content, 5-10mg and 30-35mg of freeze-dried sediment were weighed in silver cups for muddy and sandy sediments, respectively. The sediment was acidified with 6N HCL and left to react for several hours to remove inorganic carbonate before placed in a dryer at 60°C overnight. Afterwards, silver cups were folded and pressed prior to analysis in the elemental analyser. To determine the C_{org} and N_{org} content from the area below the elements' peaks as given by the elemental analyser, a calibration curve was recorded from 5 acetanilide standards of different weights with known C_{org} and N_{org} content (71.09 and 10.36%).

2.5 Isotope pairing experiments

Denitrification was measured with the revised Isotope Pairing Technique (r-IPT) according to Risgaard-Petersen et al. (2003), which relies on stable isotope tracer ($^{15}NO_3^-$) experiments and subsequent determination of the isotopic composition of N_2 gas produced by microbial activity. The original IPT was developed by Nielsen (1992) and was adjusted by Risgaard-Petersen et al. (2003) to conditions where denitrification is not the only N_2 producing process. The principle of the method is that the $^{15}NO_3^-$ tracer added to the overlying water of sediment cores mixes with the ambient $^{14}NO_3^-$ in the sediment to be denitrified to a mixture of $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ gas. From the isotopic composition of the produced N_2 gas as measured by isotope ratio mass spectrometry, the rates of genuine N_2 production at *in-situ* NO_3^- levels (without tracer addition, p_{14}) can be derived as described under 2.5.4. In contrast to alternative methods, the r-IPT allows an exact determination of total N_2 production rates and denitrification (including coupled nitrification-denitrification) in intact sediment cores where denitrification and anammox coexist. Under such conditions, essential assumptions of the classical IPT that the genuine N_2 production rate (p_{14}) is independent of the added tracer concentration and that the produced $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ are binomially distributed (Nielsen 1992), are violated. This is the case, because anammox will also produce $^{28}N_2$ and $^{29}N_2$ via the reaction of $^{14}NH_4^+$ with $^{14}NO_3^-$ and $^{15}NO_3^-$, leading to an apparent increase of p_{14} with tracer concentration (Figure 4). The independence of p_{14} from the added tracer concentration, however, forms the basis for the calculation of p_{14} from the production of ^{15}N-N_2 species ($^{29}N_2$ and $^{30}N_2$) as suggested by Nielsen (1992) (see 2.5.4). This results in an overestimation of true N_2 production under conditions where p_{14} increases with tracer addition. Thus, the calculation of true N_2 production needs to be altered by including the ratio of $^{14}NO_x^-$ and $^{15}NO_x^-$ in the NO_x^- reduction zone (r_{14}) to counterbalance this error (Risgaard-Petersen et al. 2003). At the same time, an increase of p_{14} as calculated by the classical IPT when adding rising concentrations of $^{15}NO_3^-$ also serves as an indirect prove of the anammox process.

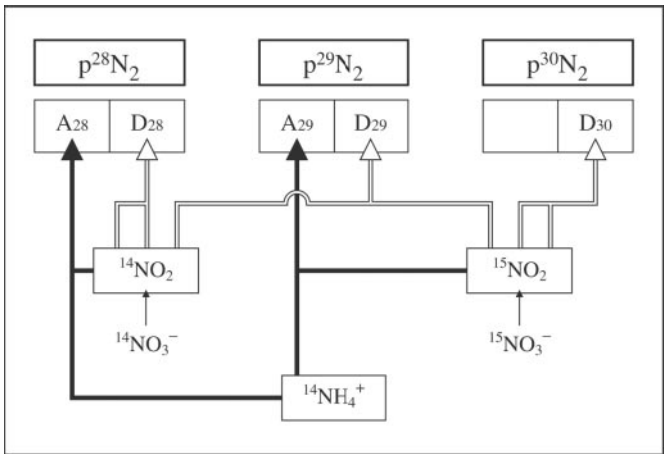

Figure 4: Distribution of N_2 isotopes in ^{15}N labeling experiments where anammox and denitrification co-occur. A_{28} and A_{29} are the produced pools of $^{28}N_2$ and $^{29}N_2$ originating from anammox, whereas D_{28}, D_{29}, and D_{30} denote the pools of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ produced via denitrification. $p^{28}N_2$, $p^{29}N_2$, and $p^{30}N_2$ are the integrated pools of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$, respectively. Dark arrows represent the anammox process and light arrows denitrification. Thin arrows represent NO_3^- reduction. (after Risgaard-Petersen et al. 2003).

2.5.1 Concentration series and time series experiments

To determine the rates of denitrification and anammox, whole core incubation experiments were carried out according to Risgaard-Petersen et al. (2003). To check whether the assumptions underlying the classical Isotope Pairing Technique are valid, it is necessary to perform tracer concentration series experiments. For this, sediment cores were incubated at *in-situ* temperatures in a box equipped with two rotating magnets, after having been spiked with different concentrations of $^{15}NO_3^-$ (25, 50, 100 and 150 µmol/L). The desired tracer concentration was obtained by adding a certain volume of a 10mmolar $^{15}NO_3^-$ solution, which was calculated from the water volume overlying the cores. The water volume, in turn, was estimated from the radius of the core (1.8 cm) squared, multiplied by π and the sediment height subtracted from the total height of the core. A table of tracer addition according to desired concentration is included in the appendix. After tracer addition, samples of the overlying water were taken to verify nitrate concentrations in the cores (see appendix).

Three parallel cores per tracer concentration were prepared to ensure statistic validity. In addition, 2 background cores were directly homogenised and sampled for N_2 analysis and 2 to 3 reference cores were incubated without tracer addition. As described above, testing different tracer concentrations is essential to check for tracer concentration dependency of genuine N_2 production and thus to determine the contribution of anammox

to total N_2 production. If anammox is absent and therefore denitrification is the only N_2 producing process, the true N_2 production (p_{14}) is assumed to be independent of the added tracer concentration. If anammox and denitrification coexist, however, this independence of tracer concentration is no longer valid, which makes it necessary to correct for the contribution of anammox. Cores of the concentration series were incubated for 24 hours in total in the case of the Kreidesegler and sediments of the Gotland basin, 20 hours for the Arkona sediment and 18 hours in the case of the Breitling and Gollwitz sediments. Reducing the incubation time for the Arkona, Breitling and Langenwerder sediment was necessary to prevent too high oxygen consumption of the sediment due to high *in-situ* temperatures (15, 23 and 15°C, respectively).

In order to get a temporal resolution of denitrification, a time series experiment was performed for the stations Kreidesegler, Breitling and NS1 to complement the concentration series data. Here, cores were amended with $^{15}NO_3^-$ at a concentration corresponding to at least 20% of the oxygen content of the overlying water and ensuring a relative nitrate mass enrichment of at least 30% (Dalsgaard et al. 2000). After a pre-incubation period of 24 hours (Kreidesegler), 18 hours (Breitling) and 12 hours (NS1), respectively, 2 cores were sacrificed to determine a starting point for N_2 production (t_0). Afterwards, cores were sampled every hour for a period of 6 hours and every 2 hours for another 6 hours to reach a total incubation time of 12 hours (plus 12 hours pre-incubation). In the case of the remaining sampling stations, time series experiments were not carried out due to time constraints and low success at previous stations (see results section).

When cores were sacrificed for N_2 analysis, the sediment of each core was homogenised with the help of a homogenising device connected to a drill and samples of the sediment slurry were taken with a 50ml syringe and filled in 12ml gas-tight vials (exetainers). 100µl of zinc chloride was added to the samples of N_2 analysis to stop bacterial activity. Finally, the exetainers were capped bubble-free and stored dry at room temperature prior to analysis.

2.5.2 Organic Carbon fertilisation experiments with Glucose

In addition to the experiments described above, tracer concentration experiments with glucose addition were carried out for a selection of stations to investigate whether denitrification is carbon limited and thus can be stimulated by giving an external carbon source in the form of glucose. The stations chosen for these experiments were NS7,

Arkona and the Gollwitz stations to compare the effect on denitrification at clay-like, muddy and sandy sediments, which differ in their ambient organic carbon content. Glucose was chosen as an organic carbon source as it has been successfully used in previous investigations (Obenhuber and Lowrance 1991; Bradley et al. 1995; Davidsson and Stahl 2000) and is readily available and easy to handle.

The experiments were carried out according to the concentration series experiments described above with the alteration that glucose was added besides $^{15}NO_3^-$ to reach a concentration of 1mM in the water overlying the cores. The same concentration of Glucose was used by Bradley et al. (1995) and insures an excess of carbon compared to the available nitrate. As for nitrate addition, a certain amount of stock solution of Glucose (100mM), which was calculated as described above, was added to the water on top of the cores to obtain the desired concentration. Cores were incubated for the same period of time as in the concentration series experiments without glucose addition to insure comparability and the sediments were homogenised and sampled for N_2 analysis as described above.

2.5.3 ^{15}N-N_2 gas analysis

^{15}N-N_2 analyses were performed at the Environmental Research Institute in Silkkeborg (Denmark).

For N_2 analysis, a headspace (2ml analytical grade He) was introduced into the gas-tight vials using a 2-way valve and a syringe. The vials were then shaken vigorously, inverted, and stored upright at 22°C to allow N_2 to equilibrate between the water phase and the headspace. Subsequently, a 50µl sample was taken from the headspace and introduced into an elemental analyser. From the mass ratios of $^{29}N_2$ and $^{30}N_2$ obtained from the mass spectrometric signal, the concentration of each nitrogen isotope was calculated by multiplying the ratios with the total amount of N_2 in the exetainer. The values were corrected for background values of the isotopes to yield the excess $^{29}N_2$ and $^{30}N_2$ concentrations, which were used for downstream calculations (see below).

2.5.4 Calculation of denitrification (N_2 production) rates

As anammox was not detected in the experiments, calculations were carried out according to the classical isotope pairing technique (Nielsen 1992), which assumes denitrification to be the only N_2 producing process. Here, the genuine N_2 production (p_{14}) is de-

scribed as the production rate of $^{28}N_2$ times 2 (two ^{14}N atoms) plus the production rate of $^{29}N_2$ (one ^{14}N atom) after addition of $^{15}NO_3^-$ to the system. As a production of $^{28}N_2$ cannot be detected directly by mass ratio spectrometry due to high background values, p_{14} is estimated from the production rates of $^{29}N_2$ and $^{30}N_2$ ($p^{29}N_2$ and $p^{30}N_2$). These, in turn, are calculated from the excess $^{29}N_2$ and $^{30}N_2$ concentrations in the following way (here shown for $p^{29}N_2$):

$$p^{29}N_2 = \frac{[^{29}N_2]}{A \times t} \times (V_W + \phi V_S)$$

where $[^{29}N_2]$ represents the excess $^{29}N_2$ concentration in µmol obtained from the mass spectrometric signal, A is the surface area of the incubated sediment in m^2, t is the incubation time in days, V_w is the incubated water volume, φ is the sediment porosity and V_s is the incubated sediment volume.

With the help of $p^{29}N_2$ and $p^{30}N_2$, the production rate of ^{15}N-N_2 (p_{15}), which forms the basis for the calculation of p_{14}, can be calculated as shown below:

$$p_{15} = p^{29}N_2 + 2 \times p^{30}N_2$$

This equation is derived from the fact that in $^{29}N_2$ only one atom of ^{15}N contributes to form the N_2 molecule, while in $^{30}N_2$ both the two atoms are labelled ^{15}N. Finally, the genuine N_2 production rate (p_{14}) is calculated as

$$p_{14} = p_{15} \times \frac{p^{29}N_2}{2 \times p^{30}N_2}$$

The share of D_w and D_n can be estimated in the following way (Steingruber et al. 2001):

$$D_w[\%] = \frac{p_{15} \times [NO_X^-]_W}{[NO_X^-]_a} \times \frac{100}{p_{14}}$$

and

$$D_n[\%] = 100 - D_W$$

where $[NO_x^-]_w$ is the ambient NO_x^- concentration in the overlying water and $[NO_x^-]_a$ is the concentration of added $^{15}NO_3^-$ tracer. By multiplying the p_{14} denitrification rates with the percentage of D_w and D_n, absolute values for both processes can be obtained.

2.6 Statistical Data analysis

Experiments were conducted with at least 3 parallels. The data is shown as mean values of triplicates with standard deviations. Denitrification rates of different sampling sites were compared by oneway ANOVA. The Tukey test was applied as *Post Hoc* test to create homogeneous subgroups of mean values which were significantly different. Data analysis was performed with the help of the computer programs Excel, Sigma Plot 10.0 and SPSS 14.0.

2.6.1 Testing the assumptions underlying the IPT

Summarising the information on the applicability of the IPT as given in earlier sections of this work, there are four assumptions underlying the IPT (Nielsen 1992; Steingruber et al. 2001):

1. The added $^{15}NO_3^-$ does not interfere with denitrification of *in-situ* NO_3^-. This assumption is not valid if anammox is present, in which case an increase of N_2 production with tracer concentration is observed and calculations would have to be altered according to the r-IPT (see above).
2. Total denitrification of NO_3^- from the water column should increase linearly with the NO_3^- concentration in the overlying water (first order kinetics of denitrification).
3. Labelling of *in-situ* NO_3^- with $^{15}NO_3^-$ in the water column and in the sediment must be homogenous.
4. A stable NO_3^- concentration gradient across the sediment water interface must be established shortly after $^{15}NO_3^-$ addition.

As explained above, all four conditions are met when genuine N_2 production (p_{14}) is independent of tracer concentration and the production of ^{15}N-N_2 (p_{15}) increases linearly with tracer concentration.

To test whether genuine N_2 production was independent of the added concentration of $^{15}NO_3^-$, mean N_2 production rates at different tracer concentrations were statistically compared by analysis of variance (oneway ANOVA). With p-values above 0.05, means were not considered significantly different and an independence of tracer concentration and thus absence of anammox was assumed.

To test whether p_{15} increased linearly with tracer concentration, a linear regression of mean p_{15} versus $^{15}NO_3^-$ addition was performed. The coefficient of determination, R^2, was used as an indicator of statistic significance.

2.6.2 Comparison of denitrification rates at different study sites and relating observed differences to environmental conditions

Mean denitrification rates were compared by analysis of variance (oneway ANOVA) as described above. To relate the observed differences in denitrification to environmental conditions, a multiple stepwise regression of mean denitrification rates and the measured environmental parameters (C_{org}, N_{org}, C/N, nutrient concentrations, oxygen, salinity, temperature and median grain size) was performed. In this regression model, the variable with the highest correlation enters the equation first if the probability associated with the test of significance (p) is less than or equal to the default 0.05. In subsequent steps, variables are entered according to their partial correlation if they meet the entry criterion ($p<0.05$). After the entry of each variable, variables are examined backwards for removal (removal criterion of $p>0.1$) and excluded from the model where necessary. This yields a prediction model which combines all variables that show a significant correlation to denitrification. The multiple stepwise regression was done in two different ways, one including all stations and the second excluding the station NS1 due to its particular property of anoxic bottom water.

2.6.3 Share of D_w and D_n

The share of D_w and D_n as calculated from the equations shown under 2.5.4 was compared between sites and correlated to NO_x^- concentration in the overlying water and the oxygen content. NO_x^- in the overlying water is important as the definition of D_w already implies that D_w directly depends on the amount of NO_x^- available in the water overlying the sediment. The oxygen content is particularly important for D_n, because the coupling of denitrification to nitrification depends on the presence of an oxic-anoxic interface within the sediment due to the aerobic metabolism of nitrifying bacteria.

2.6.4 Organic carbon fertilisation experiments with Glucose

Mean denitrification rates with and without glucose addition were compared by t-test after having been tested for normal distribution. Means were considered significantly different at p-values equal to or below 0.05.

3 Results

3.1 Physico-chemical characterisation of the water column

3.1.1 Temperature and salinity profiles

Water column profiles of temperature and salinity for the stations in the deep basins are given in Figure 5. It can be seen that at the time of sampling, a thermocline was present at all stations at a depth of approximately 30m. Here temperature dropped from near surface values of 17°C down to temperatures below 10°C. At NS1, NS6 and NS7, temperature decreased to 5°C and remained low underneath the thermocline. At Arkona, however, temperature went down to 9°C and increased again below 30m to reach a value of 14.2°C at the bottom. Salinity remained more or less constant for the top 70m at stations NS1, NS6 and NS7 and showed a steep increase in the depth horizon of the halocline between 60 and 80m. At stations NS6 and NS7, the halocline reached down to the sea floor. At the deeper station NS1, salinity increased marginally below the halocline down to the sediment surface. At the Arkona station, the halocline lay at the depth of the thermocline around 30m and showed a salinity gradient from 8 to 14 PSU. As at stations NS6 and NS7, it reached down to the sea floor.

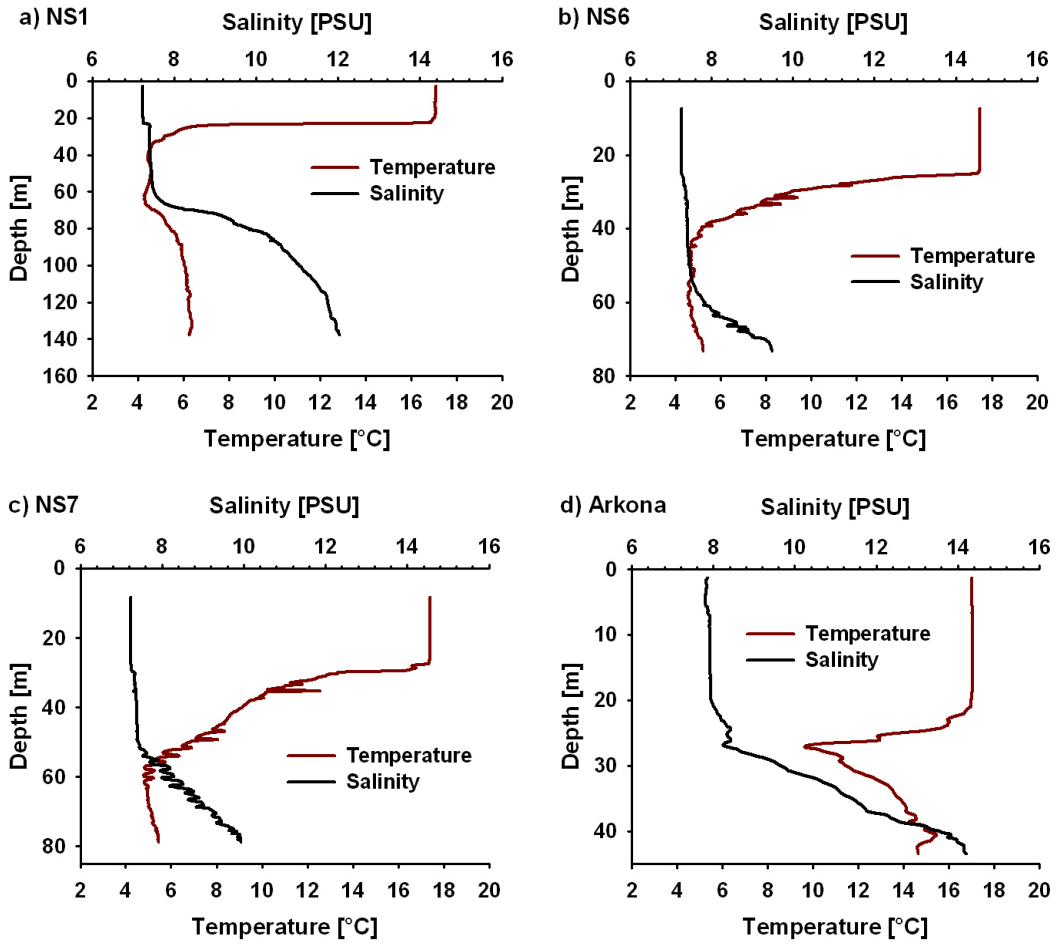

Figure 5: Water column profiles of temperature and salinity for the stations inside the central basins.

3.1.2 Oxygen profiles

Oxygen profiles of the water columns overlying the stations in the central basins are displayed in Figure 6. Near the surface, conditions were close to oxygen saturation with oxygen concentrations ranging from 250 to 350µmol/L. There are some fluctuations in oxygen concentrations apparent between 20 and 60m depth at station NS6 and NS7. Within the depth horizon of the halocline (60-80m for NS1, NS6 and NS7; 30-40m for Arkona), there is a prominent drop in oxygen down to values below 100µmol/L. In the case of NS6, NS7 and Arkona, these values correspond to bottom values of oxygen, while at NS1 oxygen continues to decrease and is completely depleted near the sediment surface at a depth of 140m.

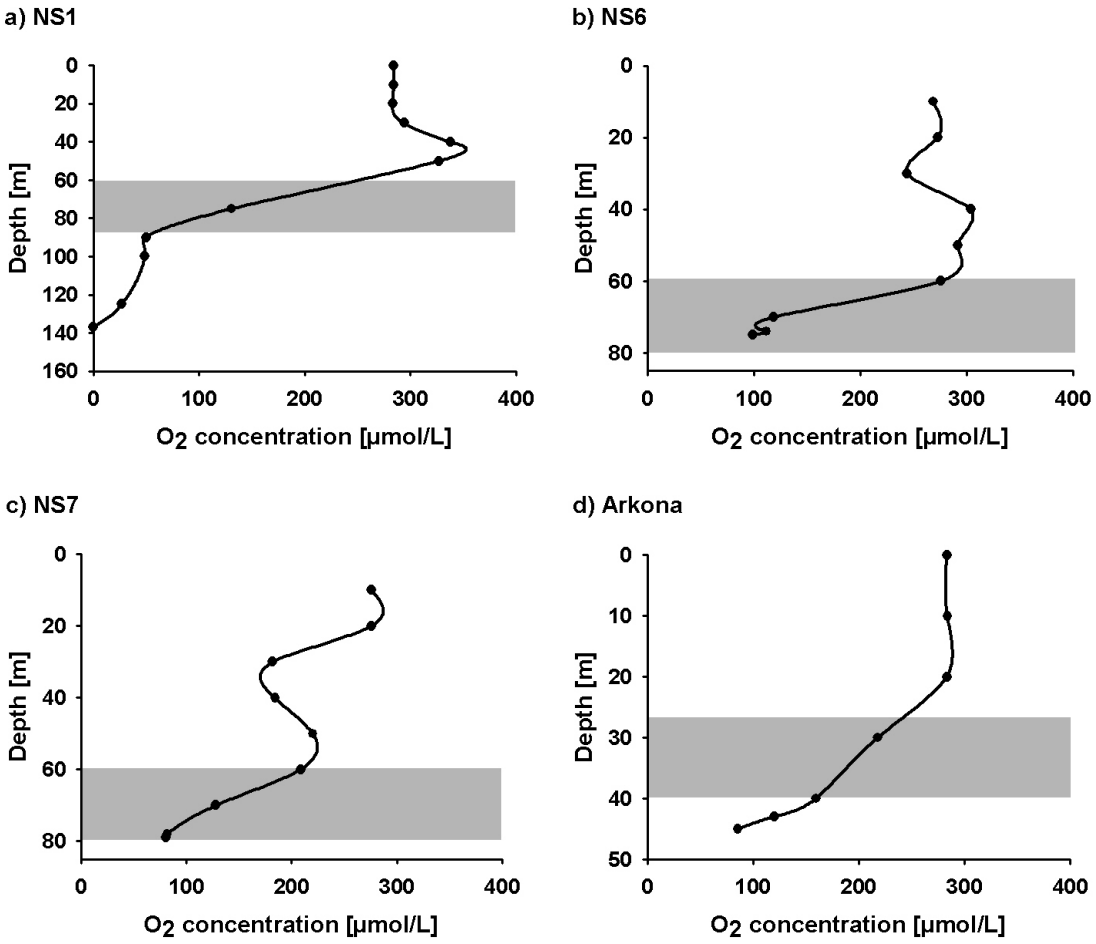

Figure 6: Oxygen profiles of the water columns of NS1, NS6, NS7 and Arkona. Grey bars mark depth horizon of the halocline.

3.1.3 Nutrient profiles

Nutrient profiles of the water columns of stations NS1, NS6, NS7 and Arkona are shown in Figure 7. It becomes clear that NO_x^- was depleted near the surface and down to a depth of about 20m (NS7 and Arkona) and 40m (NS1 and NS6), respectively. At stations NS7 and Arkona, there is an increase in NO_x^- between 20 and 40m, which corresponds to the depth horizon of the thermocline. At Arkona station, this increase is steeper than at station NS7 and continues down to the bottom. At station NS7 there is a small NO_x^- peak at 40m followed by a slight dip an a steep increase within the halocline to a maximum value of 7.4µmol/L. A steep increase within the depth horizon of the halocline is also obvious at stations NS6 and NS1 (6.9 and 7.1µmol/L). At station NS1, this is followed by a slight dip at 100m and a prominent NO_x^- peak at 120m (11.2µmol/L). Parallel to the depletion of oxygen below this depth, NO_x^- as well decreases to a value close to zero. Concerning the profile of NH_4^+, concentrations are generally low (0-2µmol/L), with a slight minimum within the halocline at stations NS1,

NS6 and NS7. At stations NS6, NS7 and Arkona, there is a small increase between 30 and 40m, which corresponds to the depth of the sea floor in the case of Arkona. At NS1 the NH_4^+ minimum within the halocline is followed by a small peak at 90m. Below, ammonium steeply increases up to 11µmol/L where the water turns anoxic. Like the NO_x^- profile, the first 20 metres of the phosphate profile are marked by a nearly depleted phosphate pool at all stations. Below this depth, phosphate rises steadily with depth to reach maximum values between 2 and 5µmol/L. At station NS1, the phosphate profile follows that of ammonium, but the increase is not as pronounced.

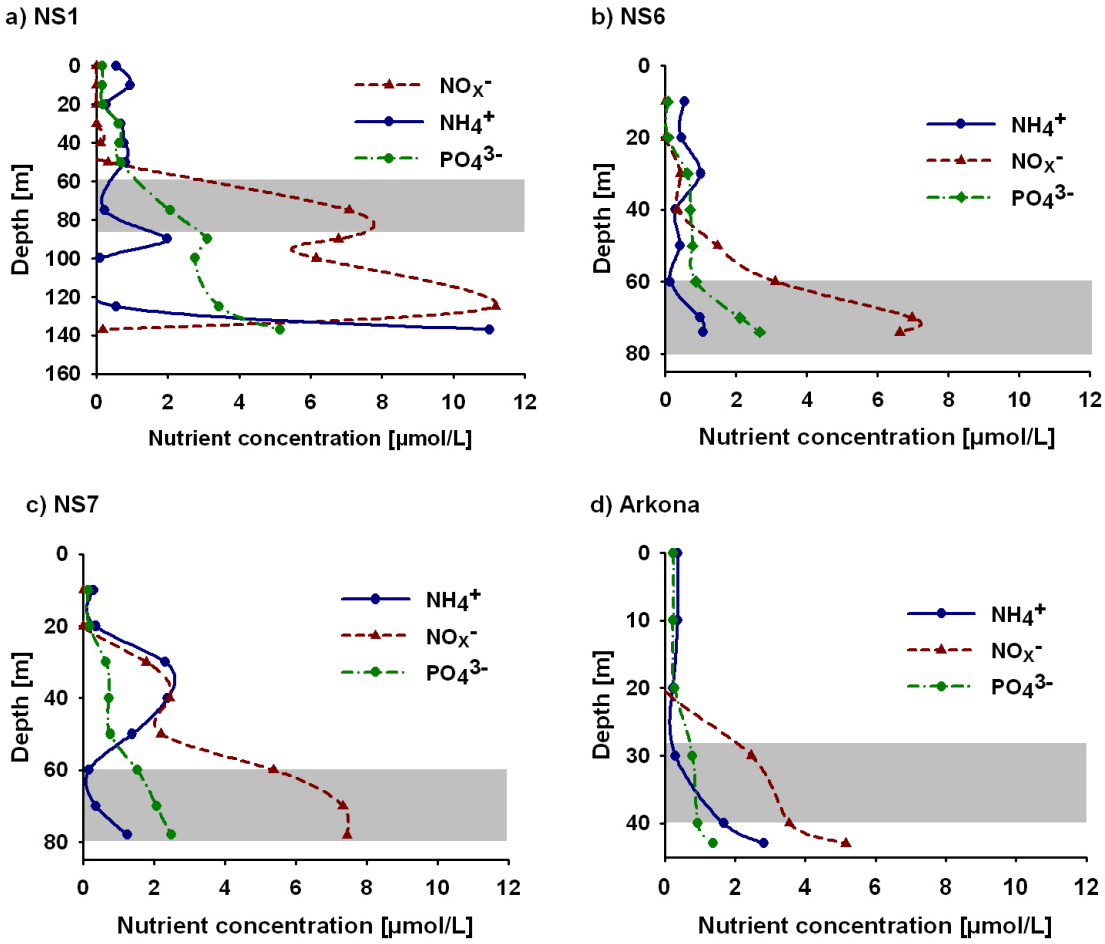

Figure 7: Nutrient profiles of the water columns of NS1, NS6, NS7 and Arkona. Grey bars mark depth horizon of the halocline.

3.2 Sediment characterisation

3.2.1 Grain size analysis

Results of the grain size analysis are summarised in Figure 8 and Table 2. It becomes clear that the true mud stations (Kreidesegler, NS1 and Arkona) have the lowest grain size median (9.5, 11.0 and 10.8µm, respectively) and therefore the highest percentage of particles in the silt and clay fraction (<63µm).

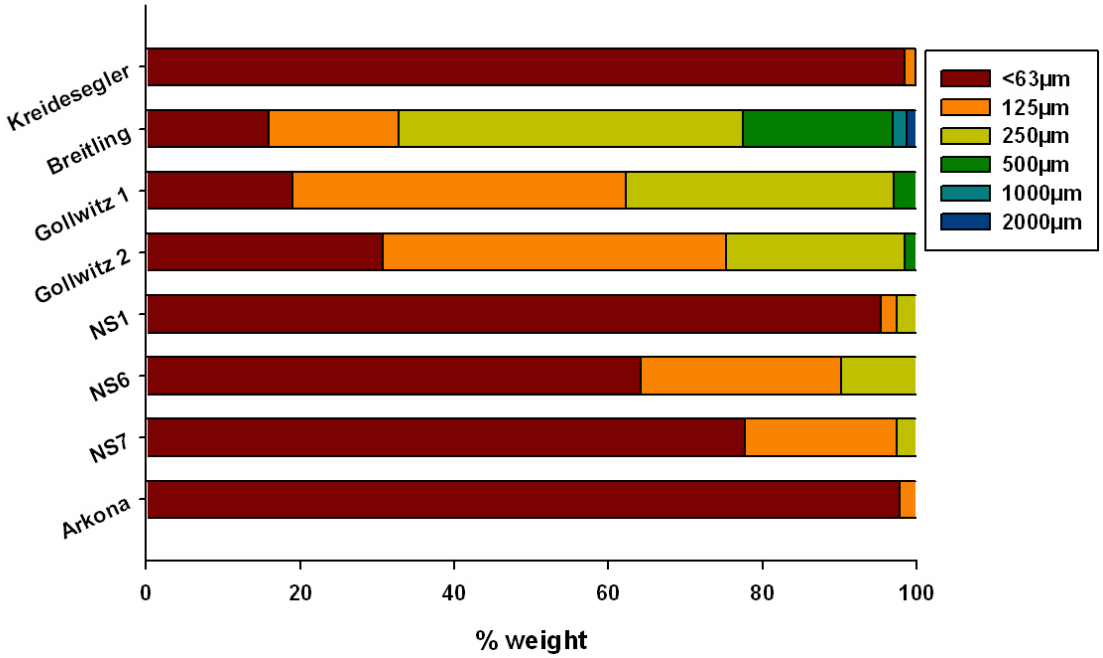

Figure 8: Grain size distribution at study sites. Colours represent grain size fractions as indicated in the legend on the right.

At station NS6, there is layer of fine material on top (median values in brackets, Table 2) and a layer with a higher fraction of coarser particles at a depth below 5cm, which is in line with the station's position within an area of prevailing glacial drift material (boulder clay). Despite the geographic proximity to NS6, NS7 does not have a layer of coarser particles at depth (data not shown).

The trask sorting coefficients of the sediments range from 0.5 to 1.6. Here, the sandy stations Gollwitz 1, Breitling and Gollwitz 2 show the best sorting (lowest sorting coefficients), while NS7, Arkona and NS6 have the highest sorting coefficients and are therefore less well sorted.

Table 2: Summary of grain size analysis. Values in brackets (NS6) represent means of the first 5 centimeters excluding the coarser particles at depth.

Station	Sediment type and material	Description	Median grain size	Trask sorting coefficient
Kreidesegler	Alluvial mud	Silt with high C_{org}	9.5	1.2
Breitling	Sand	Fine Sand	165.0	0.7
Gollwitz 1	Sand	Very Fine Sand	107.5	0.5
Gollwitz 2	Muddy Sand	Silty sand with higher C_{org}	87.5	0.6
NS1	Alluvial mud	Silt with very high C_{org}	11.0	1.2
NS6	Glacial drift (boulder clay)	Silt with moderate C_{org} and coarser partikels at depth	42.4 (14.6)	1.3 (1.6)
NS7	Glacial drift, alluvial mud	Silt with relatively high C_{org}	24.5	1.5
Arkona	Alluvial mud	Mud (Silt with high C_{org})	10.8	1.3

3.2.2 Oxygen concentration and oxygen penetration depth

Oxygen concentrations in the bottom water can be seen from Table 1 (materials and methods section) and the bottom values shown in the oxygen profiles of the water columns (Figure 6). It can be seen that the water overlying the shallow coastal stations Breitling, Gollwitz 1 and Gollwitz 2 was oxygen saturated (311.5 and 354.12µmol/L, respectively) at the time of sampling and the oxygen penetration depth as estimated from the colour of the sediment was around 2cm in the case of Breitling and Gollwitz 1 and slightly less in the case of Gollwitz 2 (data not shown). At the coastal mud station Kreidesegler, the oxygen concentration was still high in the bottom water (236.16µmol/L, water depth 25m) but oxygen penetration into the sediment was only 3mm. At all central stations except NS1, oxygen was present in the overlying water (99, 80 and 85µmol/L at NS6, NS7 and Arkona), although at low concentrations compared to the more shallow coastal stations. As estimated from the oxygen profile of NS6, oxygen penetration into the sediment was accordingly low (<1mm).

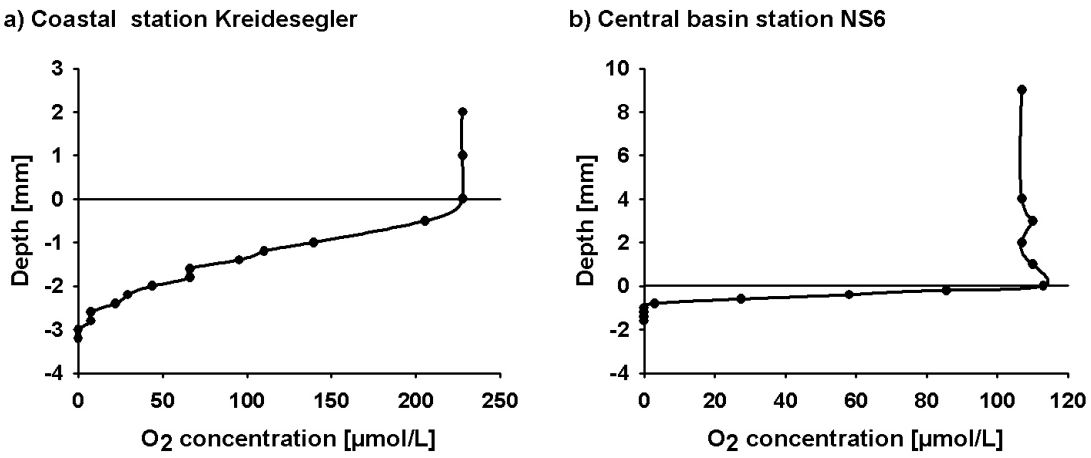

Figure 9: Oxygen profiles of the coastal mud station Kreidesegler and the central basin station NS6.

3.2.3 Pore water profiles of nutrient concentrations

Depth profiles of NO_x^-, NH_4^+ and PO_4^{3-} are shown in Figure 10 and Figure 11. At all stations except NS1 (anoxic bottom water), NO_x^- was present at the sediment surface. The highest nitrate level in the bottom water was apparent at station NS7 with 7µmol/L, followed by NS7 (6.3µmol/L) and Arkona (5.2µmol/L). In the case of the mud stations Kreidesegler, NS6 and NS7, nitrate was depleted below the top sediment centimetre to values close to zero where the sediment became anoxic. A remarkable particularity of the nitrate profile of the Kreidesegler is the nitrate peak of 78µmol/L in the surface layer of the sediment. Compared to the mud stations, the sandy stations Breitling and Gollwitz 1 show different nitrate profiles in the way that there is an increase in the top centimetre (NO_x^- peaks of 8µmol/L and 5µmol/l, respectively) followed by a decrease (close to 0µmol/L) and a distinct re-increase to 40µmol/L and 13µmol/l, respectively. At the very fine sand station Gollwitz 2, NO_x^- declines to zero in the top centimetre, followed by a nitrate maximum of 3.5µmol/L. Below, NO_x^- decreases once again, but the decreasing trend is intermitted by some fluctuation to slightly higher values at a depth of 6cm.

The ammonium profiles of all stations are characterised by NH_4^+ depletion in the bottom water and top centimetre of the sediment, followed by a sharp increase to concentrations clearly above 100µmol/L at depth. In the case of Breitling and Gollwitz 1, this general increase is intercepted by small dips. Highest ammonium levels are reached at the coastal and estuarine stations Gollwitz 2 and Breitling (637 and 618µmol/L, respectively), followed by the central stations NS7 (576µmol/L), Arkona (506µmol/L) and NS1 (430µmol/L).

The phosphate profiles show high variation between sites, but in general the concentration increases with depth. In the bottom water and top centimetre of the sediment, phosphate concentrations are close to detection limit and go up to 100µmol/L at 4-5cm sediment depth in the case of Gollwitz 2. At the Breitling station, there is a pronounced peak at 3cm depth. The phosphate profile of Gollwitz 1 is marked by a series of peaks and dips in the sediment horizon between 1 and 5cm depth.

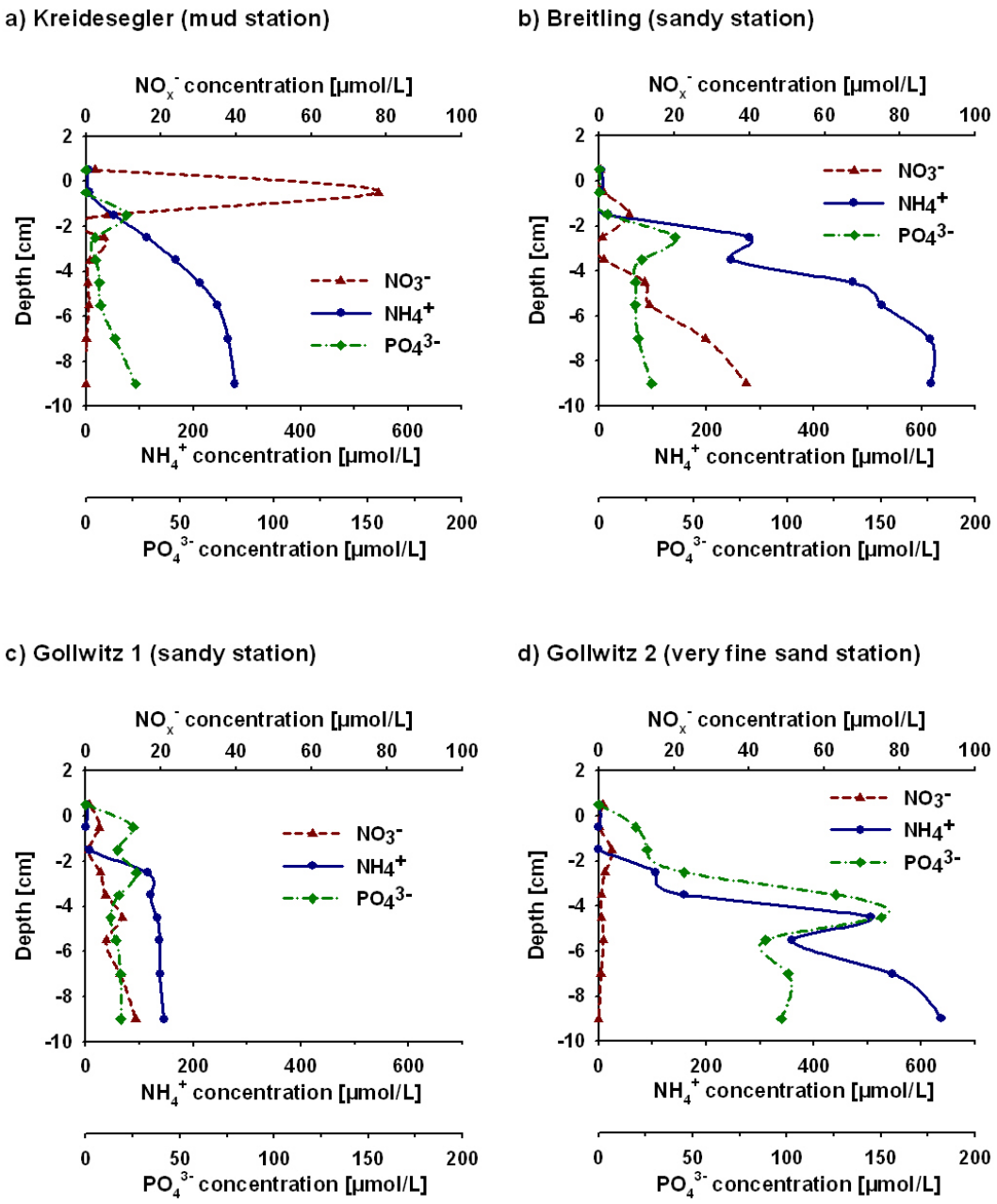

Figure 10: Nutrient pore water depth profiles of the coastal and estuarine stations.

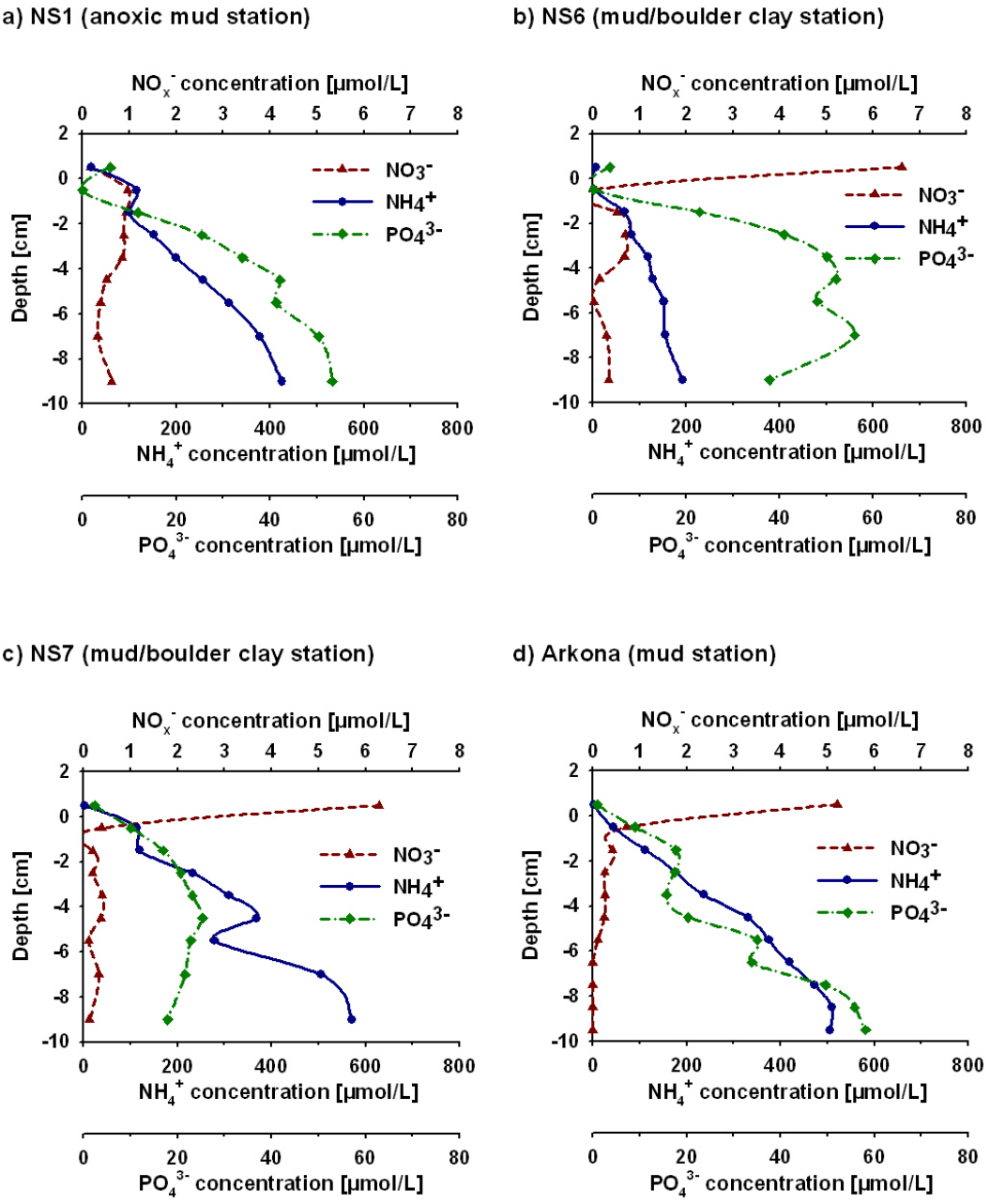

Figure 11: Nutrient pore water depth profiles of the stations inside the central basins.

3.2.4 NO_x^- and NH_4^+ fluxes as estimates of denitrification and C remineralisation

NO_x^- and NH_4^+ fluxes and derived denitrification rates are displayed in Table 3. The NH_4^+ flux is directed out of the sediment (negative values) at all study sites. Highest fluxes were found at Arkona (-553µmol m^{-2} d^{-1}), followed by NS7 (-378µmol m^{-2} d^{-1}), Kreidesegler (-359µmol m^{-2} d^{-1}) and NS1 (-359µmol m^{-2} d^{-1}). The lowest NH_4^+ flux was found at the sandy station Gollwitz 1 (-80µmol m^{-2} d^{-1}). The other sandy stations Breitling and Gollwitz 2 had distinctly higher NH_4^+ fluxes (-198 and -222µmol m^{-2} d^{-1}). For nitrate, there was both an outward flux towards the overlying water (negative up-

ward flux) and an inward flux from the top towards deeper layers of the sediment (positive downward flux) at the stations where a nitrate peak appeared within the sediment (Kreidesegler, Breitling Gollwitz 1 and Gollwitz 2). For the remaining stations, only a positive flux into the sediment was present. Denitrification rates calculated from the downward flux of nitrate into the sediment ranged from 2 to 260µmol N_2 m^{-2} d^{-1} with highest rates found at the Kreidesegler station. Compared to denitrification rates measured with the IPT (see 3.3), rates are distinctly lower at all study sites.

Table 3: Nutrient fluxes across the sediment-water interface and towards deeper sediment layers. An upward flux of NO_x^- was calculated only for stations where a nitrate peak occurred in the top centimetre of the sediment. Negative values indicate a flux out of the sediment. DN = denitrification rate.

Station	**NH_4^+ flux up** [µmol m^{-2} d^{-1}]	**NO_x^- flux up** [µmol m^{-2} d^{-1}]	**NO_x^- flux down** [µmol m^{-2} d^{-1}]	**DN from flux** [µmol N_2 m^{-2} d^{-1}]	**DN (IPT)** [µmol N_2 m^{-2} d^{-1}]
KS	-359	-544	519	259.5	493.5
Breitling	-198	-16	17	8.5	59.4
GW 1	-80	-5	6	3	13.2
GW 2	-222	-7	3	1.5	26.9
NS1	-359				29.1
NS6	-113		36	18	162
NS7	-378		38	19	145.8
Arkona	-553		45	22.5	689.2

The calculation of total C remineralisation revealed highest remineralisation rates at Arkona and Kreidesegler (65 and 43mg C m^{-2} d^{-1}, respectively), followed by NS7 (34mg C m^{-2} d^{-1}) and NS1 (29mg C m^{-2} d^{-1}). C remineralisations of the Breitling and Gollwitz 2 were similar (17.5 and 18.5mg C m^{-2} d^{-1}) and lowest values were present at NS6 and Gollwitz 1 (14 and 7mg C m^{-2} d^{-1}). The share of denitrification in C remineralisation ranged from 3 to 35%. The highest share was present at NS6 (35%). High contributions were also found at the stations with highest denitrification rates, Kreidesegler and Arkona (34 and 32%, respectively). The sandy stations Breitling, Gollwitz 1 and Gollwitz 2 showed distinctly lower contributions (10, 6 and 4%). The lowest value was present at NS1 (3%).

Table 4: Estimates of total C remineralisation (C rem.) and the share of denitrification (DN) in these processes.

Station	**total C rem.** [mg C m^{-2} d^{-1}]	**% C by DN**
Kreidesegler	43.38	34.1
Breitling	17.51	10.2
Gollwitz 1	6.77	5.9
Gollwitz 2	18.49	4.4
NS1	29.41	3.0
NS6	13.81	35.2
NS7	34.42	12.7
Arkona	64.61	32.0

3.2.5 C_{org} and N_{org} content and C/N ratio

Mean organic carbon and nitrogen contents and C/N ratios in the denitrification zone (top three centimetres of the sediment) are shown in Table 5. It can be seen that NS1 has the highest C_{org} content, followed by Arkona and Kreidesegler. For N_{org}, Arkona shows the highest value, followed by NS1 and Kreidesegler. These high contents of organic carbon and nitrogen in muddy sediments are opposed to very low values at sandy stations, demonstrated by the low C_{org} and N_{org} contents of the coastal stations Breitling, Gollwitz 1 and Gollwitz 2.

Table 5: Results of the C/N analysis. Carbon and nitrogen contents are given as % dry weight. C/N is given as elemental ratio.

Station	**C_{org} [%]**	**N_{org} [%]**	**C/N ratio**
Kreidesegler	4.53	0.52	10.31
Breitling	0.26	0.05	6.31
Gollwitz 1	0.23	0.04	7.06
Gollwitz 2	0.46	0.07	7.62
NS1	6.06	0.60	11.90
NS6	1.15	0.15	9.02
NS7	3.76	0.52	8.59
Arkona	5.54	0.79	8.21

3.3 Results of denitrification measurements with the IPT

3.3.1 Testing the assumptions underlying the IPT

3.3.1.1 Tracer concentration dependency of genuine N_2 production and presence of anammox

There was no tracer concentration dependency of genuine N_2 production detected at any of the study sites ($p>>0.05$). Thus, the first assumption of the IPT could be validated and provides a negative proof of anammox for all study sites. Representative for all stations, this is shown for the coastal station Kreidesegler and the central station NS7 (Figure 12).

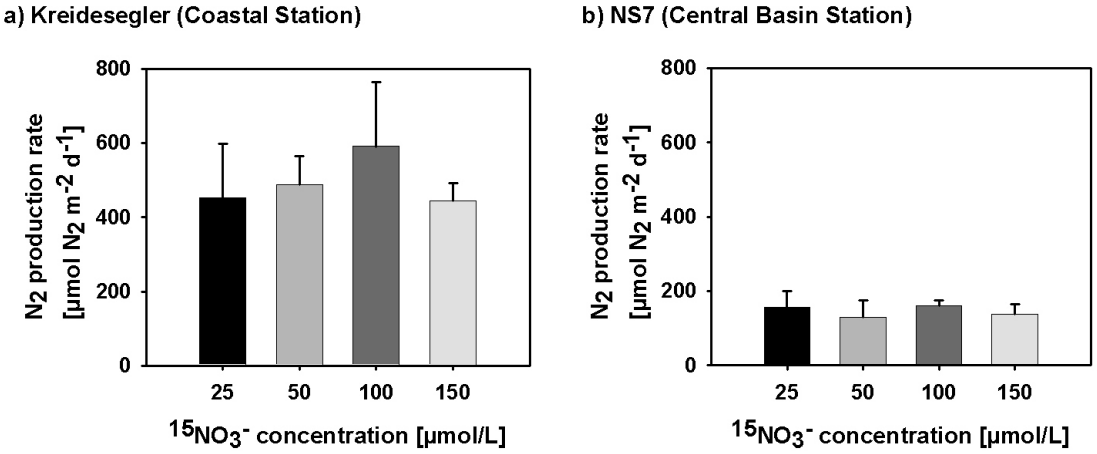

Figure 12: Bar charts of genuine N_2 production rates at different $^{15}NO_3^-$ concentrations. Bars represent mean N_2 production rates and errors indicate standard deviations.

3.3.1.2 Linear correlation between tracer addition and production of ^{15}N-N_2

The linear regression of p_{15} versus tracer concentration yielded a significant linear increase of p_{15} with tracer concentration for all stations ($R^2 > 0.88$). Against this background, the assumptions underlying the IPT as described under 2.6.1 could be assumed to be valid and thus calculations derived from these assumptions as described by Nielsen (1992) could be applied without additional corrections. Representative for all stations, results of the linear regression are displayed for the coastal station Kreidesegler and the central station NS7 (Figure 13).

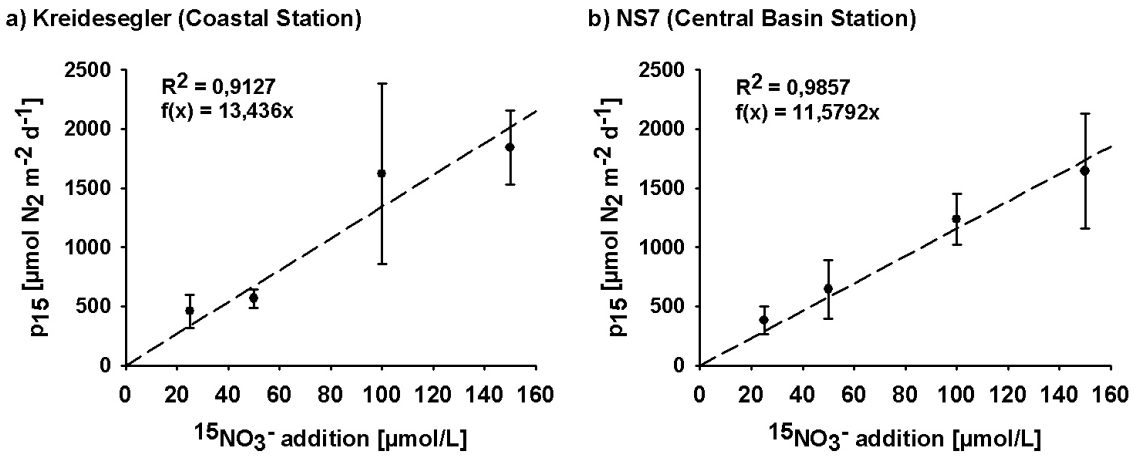

Figure 13: Results of the linear regression of p_{15} versus $^{15}NO_3^-$ concentration at the coastal station Kreidesegler (left) and the central basin station NS7 (right).

3.3.2 Temporal variability of denitrification rates

Representative for all sites, the results of the time series experiments of the stations Kreidesegler and Breitling are displayed in Figure 14. It can be seen that denitrification rates were not constant with time and that the variation between parallels (Breitling station) was as high as the temporal variation in general. Furthermore, the production of ^{15}N-N_2 per surface area did not increase linearly with time (data not shown).

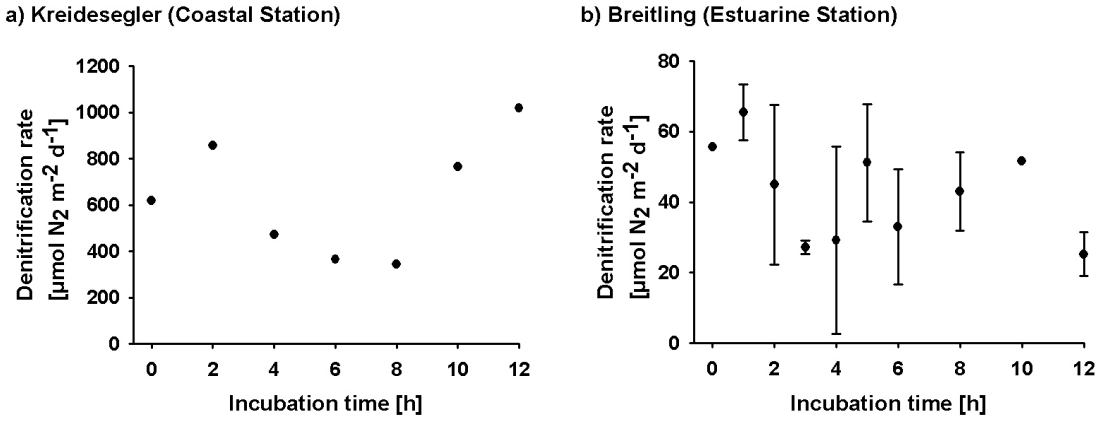

Figure 14: Scatter plot of the temporal variation of denitrification rates. For the Kreidesegler, data points represent single measurements, while at the Breitling station parallel measurements were performed. Error bars (right) represent standard deviations.

3.3.3 Comparison of denitrification rates at different study sites and relating observed differences to environmental conditions

Denitrification rates of the study sites are displayed in Figure 15. As can be seen, the oneway ANOVA has yielded four groups of sites with statistically the same denitrification rates ($p>0.05$). The mud station Arkona shows the highest mean denitrification rate ($689 \pm 129 \mu mol\ N_2\ m^{-2}\ d^{-1}$), which is significantly higher than the rates at all other stations. The second highest rate ($494 \pm 120 \mu mol\ N_2\ m^{-2}\ d^{-1}$) was measured at the coastal mud station Kreidesegler (KS), which again is significantly different from all remaining sites. The central stations NS6 and NS7 form a group with mid range denitrification rates (162 ± 50 and $146 \pm 33 \mu mol\ N_2\ m^{-2}\ d^{-1}$), which do not differ significantly from each other but from the rest of the sites. Finally the anoxic station NS1 and the coastal sandy stations Breitling, Gollwitz 1 (GW 1) and Gollwitz 2 (GW 2) comprise a group with low mean denitrification rates (29 ± 15, 59 ± 20, 13 ± 8 and $27 \pm 19 \mu mol\ N_2\ m^{-2}\ d^{-1}$). From the size of the boxes and whiskers, it becomes clear that values of denitrification show a high scatter, particularly in the case of the stations with the highest denitrification rates, Arkona and Kreidesegler. Median values generally lie central within the data range and are therefore close to mean values, which is in line with the normal distribution of the data as confirmed by Kolmogonov-Smirnov test (data not shown).

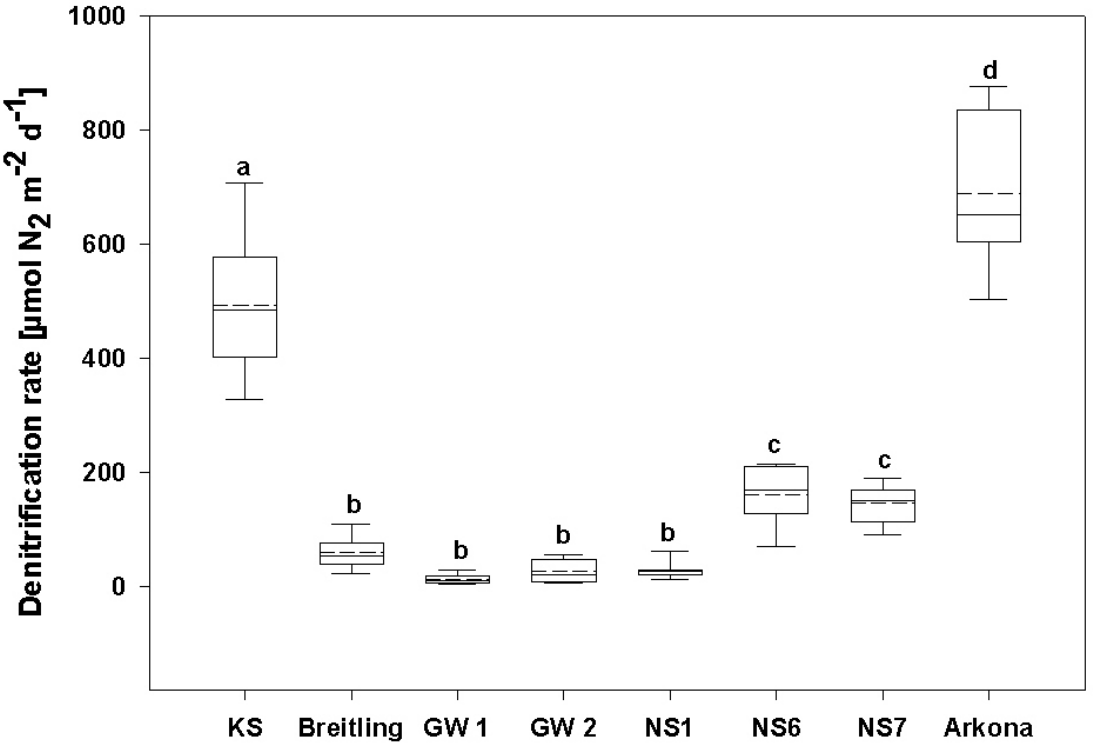

Figure 15: Box-whisker plot on denitrification rates at different study sites. Boxes include 25-75% of overall data. Solid lines are median and dashed lines mean values of denitrification. Whiskers comprise the whole range of data. Letters above plots refer to the ANOVA results with identical letters indicating no significant difference between denitrification means at corresponding sites.

In order to assign the pattern of denitrification rates to the prevailing environmental conditions, a multiple stepwise regression was performed as described under 2.6.2. When all stations were included into the model, there was no significant correlation of denitrification to any environmental parameters. However, when excluding station NS1 due to anoxia, the regression showed a different result in the way that it yielded a significant correlation of denitrification to C_{org} ($R^2 = 0.813$, $p<0.05$; Figure 16). The remaining parameters still did not meet the model's entry criteria ($p<0.05$) and were therefore excluded from the regression model (Table 6).

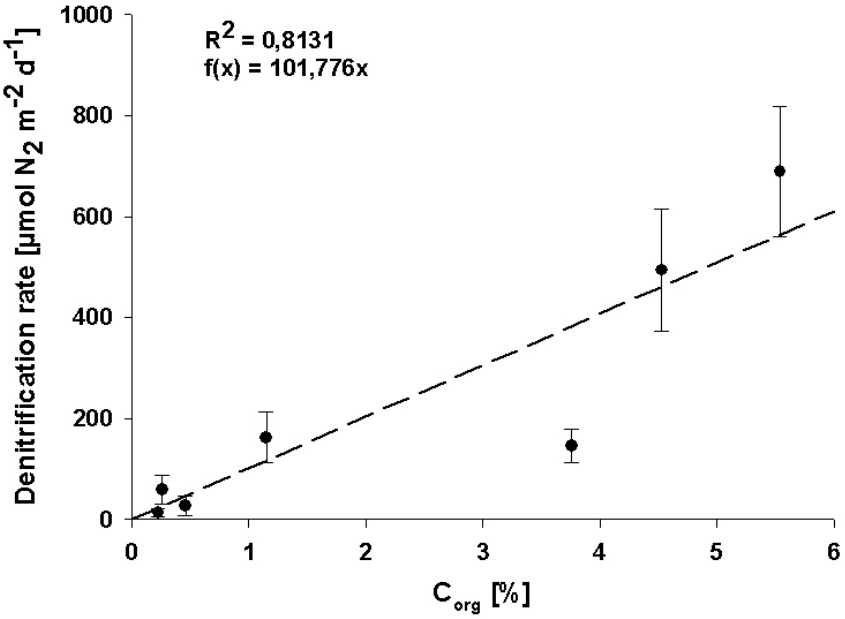

Figure 16: Correlation between denitrification rates and C_{org} in the denitrification zone. Error bars represent standard deviations. NS1 was excluded from the correlation and is not shown in the figure.

Table 6: Significance level (p) and partial correlation of variables excluded from the multiple stepwise regression model of denitrification. The entry criterion for variables was a p-value <0.05. At p-values >0.1 variables were excluded from the regression model.

Excluded Variables	p	Partial Correlation
NO_x^- (water)	0.595	0.277
NO_x^- (denitrification zone)	0.797	0.136
NH_4^+	0.744	0.173
PO_4^{3-}	0.617	0.261
N_{org}	0.977	0.015
C/N	0.883	0.078
Temperature	0.405	0.422
Salinity	0.683	0.214
O_2 water	0.698	0.204
Median Grain Size	0.731	0.181

Parameters like the nitrate concentration in the overlying water and within the denitrification zone (Figure 17), as well as temperature (Figure 18) did not contribute significantly to explain the denitrification pattern.

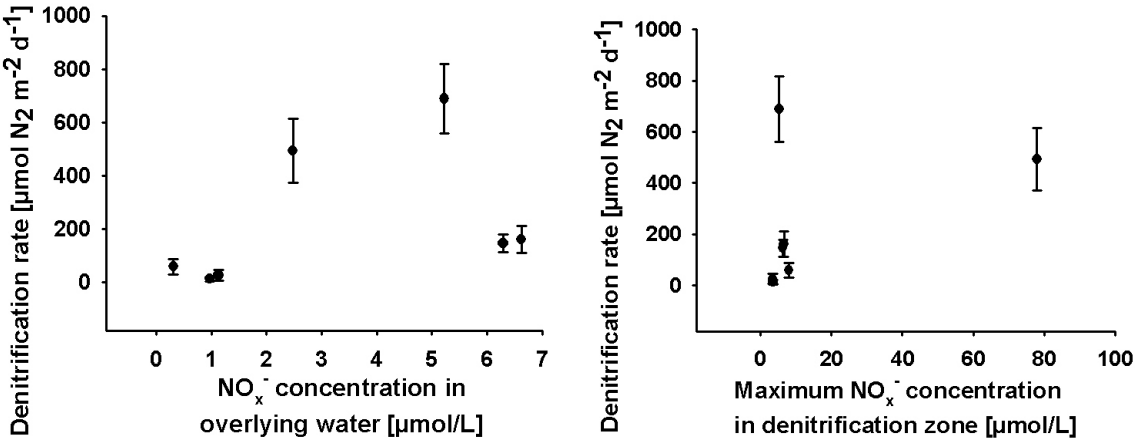

Figure 17: Scatter plots of the relation between denitrification rates and NO_x^- concentrations. On the left, denitrification is plotted against the NO_x^- concentration in the overlying water and on the right against the maximum NO_x^- concentration within the denitrification zone. Error bars represent standard deviations.

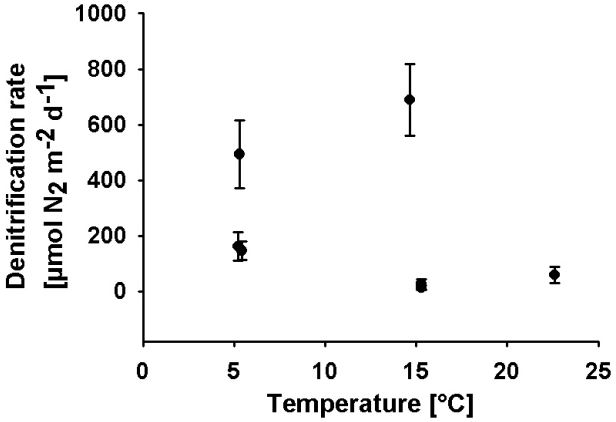

Figure 18: Scatter plot of the relation between denitrification rates and temperature. Error bars represent standard deviations.

3.3.4 Share of D_w and D_n

The share of D_w and D_n in total denitrification is presented in Figure 19 for all study sites. It can be seen that the highest share of D_w was found at Gollwitz 1 (72.15 ± 23.38%), followed by NS6 (61.92 ± 10.46%), Gollwitz 2 (58.58 ± 23.21%), and NS7 (55.34 ± 7.77%). In contrast to these stations where denitrification is dominated by D_w, the remaining stations show a high share of D_n in total denitrification (>70%). Here, the highest relative D_n was calculated for the station Kreidesegler (90.53 ± 4.2%), followed by NS1 (88.61 ± 5.93%), Arkona (81.97 ± 3.06%) and Breitling (73.42 ± 11.89%).

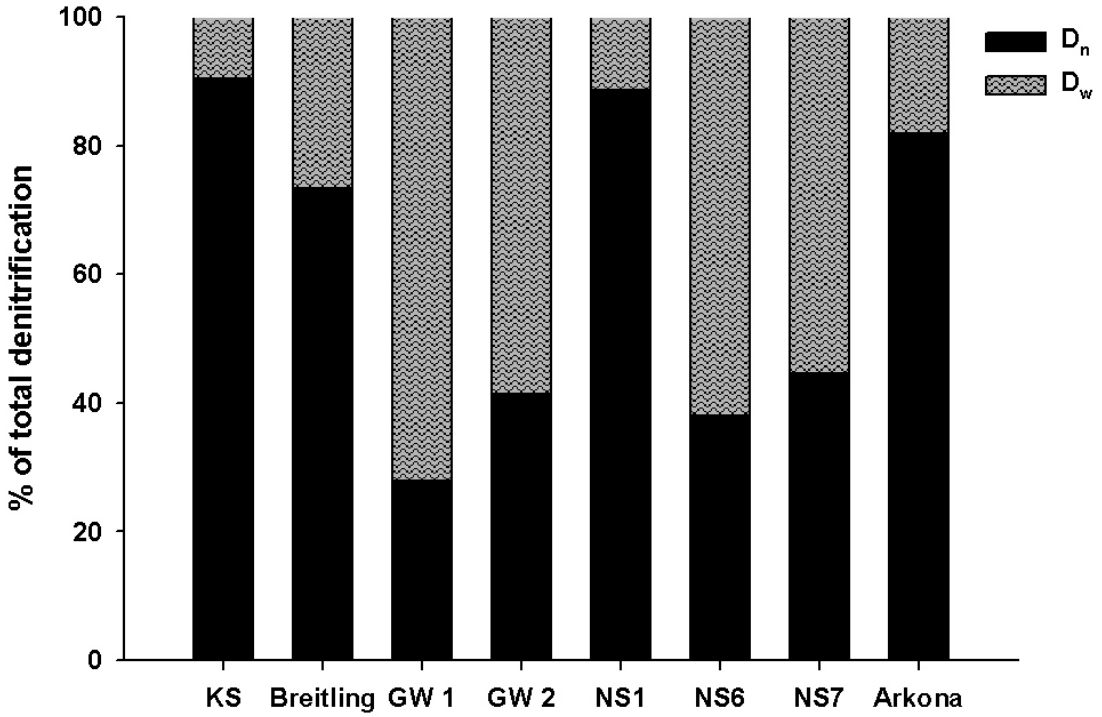

Figure 19: Stacked bar chart of the share of D_w and D_n in total denitrification.

The absolute D_w rates showed a significant linear correlation with NO_x^- in the overlying water ($R^2 = 0.85$, Figure 20) Absolute D_n rates did not show a significant correlation to any of the measured environmental parameters (data not shown). The same holds for the relative share of D_w and D_n in total denitrification, which yielded no significant correlation to the concentration of NO_x^- in the overlying water and the oxygen concentration in the bottom water, respectively (Figure 21).

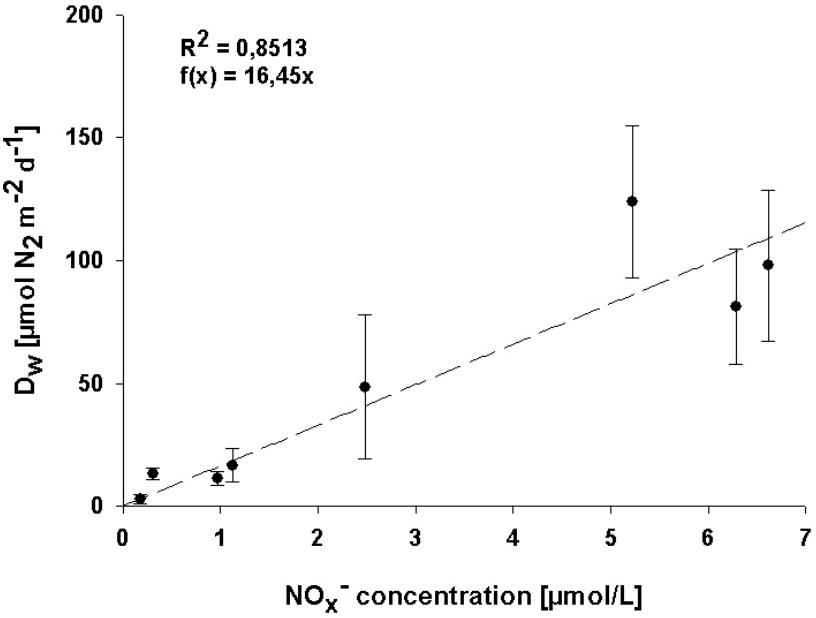

Figure 20: Correlation between NO_x^- concentration in the overlying water and absolute D_w rates. Error bars represent standard deviations.

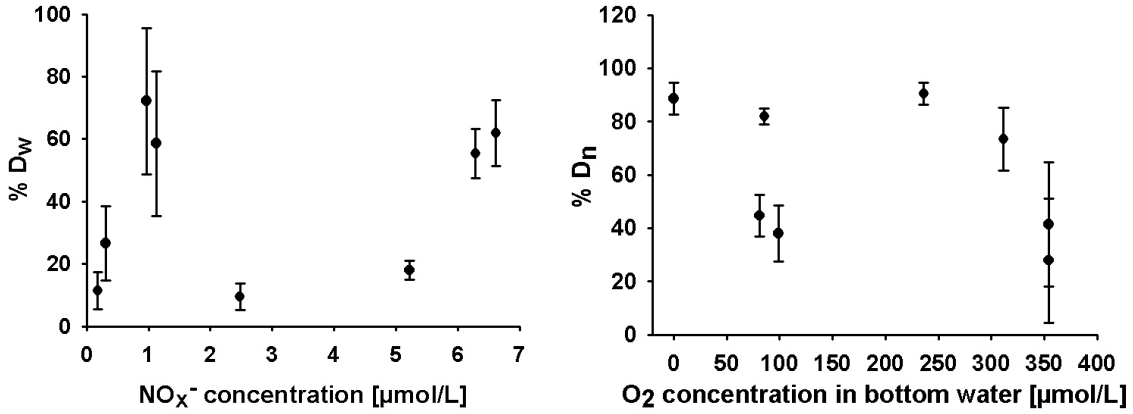

Figure 21: Scatter plot of the relation of relative D_w to the concentration of NO_x^- in the overlying water (left) and relative D_n to the oxygen concentration in the bottom water (right). Error bars represent standard deviations.

3.3.5 Organic carbon fertilisation experiments with Glucose

Results of the organic carbon fertilisation experiments are shown in Figure 22. The t-test yielded no significant differences between denitrification rates with and without glucose fertilisation ($p>0.05$) for all stations.

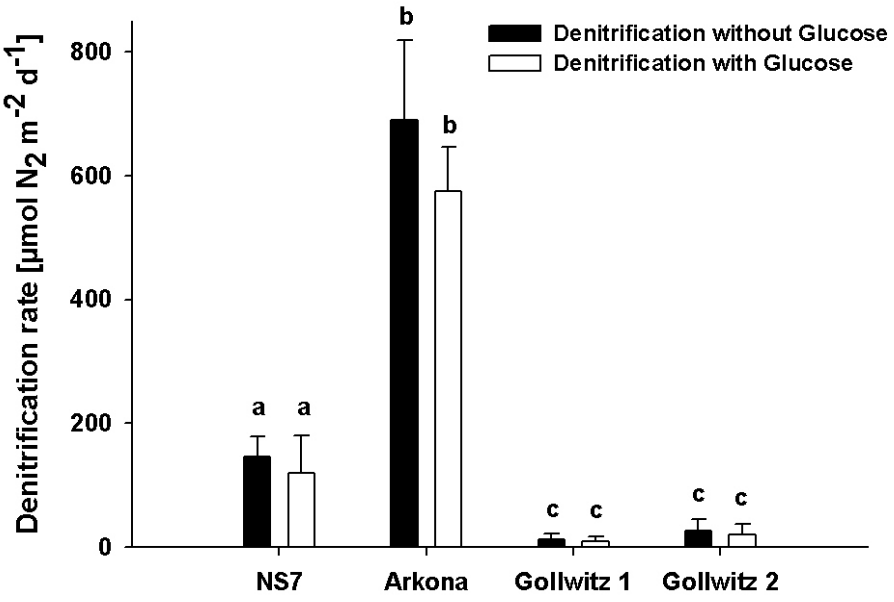

Figure 22: Bar chart of the results of organic carbon fertilisation experiments. Error bars represent standard deviations. Letters above plots refer to the t-test and ANOVA results with identical letters indicating no significant difference between denitrification means with and without glucose addition.

4 Discussion

4.1 Applicability of the Isotope Paring Technique (IPT) in the current study

The IPT is a well-established and widely used method for measuring denitrification rates in the water column and sediment of different ecosystems (Risgaard-Petersen et al. 2003 and references therein). In comparison to other methods, it has the advantage that denitrification of both NO_x^- diffusing from the overlaying water and NO_x^- from nitrification within the sediment can be quantified. The IPT has been reviewed by Steingruber et al. (2001) and has been adapted to conditions when denitrification and anammox coexist (Risgaard-Petersen et al. 2003; Risgaard-Petersen et al. 2004b; Trimmer et al. 2006). Despite its easy handling, the assumptions underlying this technique (see 2.6.1) are complex and have to be tested in the course of experiments to ensure the correct applicability of the method. The methodological tests performed in this study comprised the verification that genuine N_2 production (p_{14}) was independent of tracer concentration and that denitrification followed first-order kinetics as reflected in a linear increase of ^{15}N-N_2 production (p_{15}) with tracer concentration. Thus it can be insured that the $^{15}NO_3^-$ tracer is uniformly distributed in the sediment and that denitrification is the only N_2 producing process present in the sediment. If uniform mixing is not insured, denitrification rates will be underestimated since immeasurable $^{14}N^{14}N$ pairs will be formed. On the other hand, genuine N_2 production will be overestimated if anammox is present besides denitrification due to an additional formation of $^{29}N_2$ through this process (Risgaard-Petersen et al. 2003). In this study, the concentration series experiment yielded an independence of genuine N_2 production of tracer concentration (Figure 12), so that mistakes derived from the latter problems can be assumed to be negligible. The assumption of first-order kinetics of denitrification forms the base for the calculations of the IPT and is therefore an essential prerequisite of the method. This could be also verified in the concentration series experiments, as a significant linear increase of p_{15} with tracer concentration was detected for all study sites (Figure 13). Concerning the denitrification of NO_x^- from the overlying water, the basis of calculation is the linear increase of D_w (absolute values) with NO_x^- concentrations in the overlying water, which was also

found in this study (Figure 20). The standard deviations of p_{15}, p_{14} and D_w, however, were high, which is a general problem of quasi *in-situ* measurements due to the naturally occuring high spatial variability of denitrification activity (e.g. Folorunso and Rolster 1984). The station with lowest denitrification rates, Gollwitz 1, sometimes showed negative denitrification rates, which were excluded from downstream calculations. It is most probable that at this station, the $^{15}N\text{-}N_2$ production was at the detection limit with the present incubation time and set-up. Thus, minor variations in the $^{15}N\text{-}N_2$ ratio had tremendous effects on the calculated D_w and D_n, which resulted in values above 100% for D_w in some cases (again these were excluded from calculations).

A problem which has become apparent in the time series experiments is the temporal variability of denitrification. Here the results implied a high temporal fluctuation of denitrification over the incubation period, which is against the assumption of more or less constant rates over time and a linear increase of $^{15}N\text{-}N_2$ production with time. This, however, should be again assigned to a high variability between sediment cores, because N_2 production for each time point was calculated from a different sediment core. To improve the time series experiment, parallel cores were used for each time point in the case of the Breitling station. The results have revealed that the variability among parallels is as high as between time points, which makes it difficult to draw reliable conclusions. Despite some variability between parallels in the concentration series experiments, it was not as high in the latter experiments compared to time series experiments, although the sampling and processing procedure was the same for both types of experiments. Thus, it should be concluded that something went generally wrong in the time series experiments, potentially due to lower tracer additions and the resulting low production of $^{15}N\text{-}N_2$ species. This, however, does not impair the data quality obtained from concentration series experiments where higher levels of $^{15}NO_3^-$ were used.

In summary it can be said that the assumptions underlying the IPT could be validated in this study and that despite the naturally occurring variability between sediment cores, some distinct patterns in relation to environmental conditions (see below) could be discerned between sites.

4.2 Current denitrification rates in the literature context of the Baltic Sea and aquatic ecosystems around the world

Denitrification rates have been shown to differ significantly between ecosystems in the aquatic environment, with highest rates generally found in rivers and lakes, followed by estuaries, coastal ecosystems and the ocean (Seitzinger 1988; Piña-Ochoa and Álvarez-Cobelas 2006; Seitzinger et al. 2006). There might be a discrepancy to this general ecosystem trend of denitrification on the regional scale due to site-specific variability (Piña-Ochoa and Álvarez-Cobelas 2006). In this work, it was attempted to cover different ecosystems (estuarine/coastal and deep basins) and sediment types (sandy and muddy) of the Baltic Sea and relate patterns of denitrification rates to the prevailing environmental conditions. The results have shown that possible differences between estuarine and coastal habitats and the deep basins as such were superimposed by differences due to the specific environmental conditions of the sediments.

As described in the results section, denitrification rates measured in this work ranged from mean 13 to 690µmol N_2 m^{-2} d^{-1}. The Post Hoc test following the oneway ANOVA has yielded four groups of significantly different denitrification rates (Figure 15). These groups are mainly consistent with the prevailing environmental conditions with the exception of NS1, which is treated separately due to anoxia. A summary of group characteristics is given in Table 7. Here the stations Kreidesegler and Arkona have been assigned to one group despite significant differences in their denitrification rates, because environmental conditions were similar and rates still lay in the same range. In the following, denitrification rates will be discussed by group. A summary of cited denitrification rates of the Baltic Sea and around the world is given in at the end of this section.

Table 7: Characterisation of station groups by environmental conditions and denitrification.

Group	Included stations	Characteristics
1	Kreidesegler, Arkona	Fine mud with high C_{org} (4.5-5.5%), low oxygen penetration, high denitrification rates
2	NS6, NS7	Mixed sediment with moderate to high C_{org} (1-3.5%), low oxygen penetration and mid range denitrification rates
3	Breitling, Gollwitz 1, Gollwitz 2	Sandy sediment with low C_{org} (0.2-0.4%) and high O_2 penetration, low denitrification rates
4	NS1	Fine mud with highest C_{org} (6%), anoxic bottom water, no NO_x^- in overlying water

Highest rates were present at the Arkona station (689 ± 129µmol N_2 m^{-2} d^{-1}), followed by the coastal station Kreidesegler (493 ± 120µmol N_2 m^{-2} d^{-1}.). These are around the highest denitrification rates of the Baltic Sea compared to the Gulf of Finland (Tuominen et al. 1998; Silvennoinen et al. 2007; Hietanen and Kuparinen 2008) and the Bothnian Bay (Silvennoinen et al. 2007). Extremes of denitrification rates have been found in Denmark in the Norsminde Fjord (1600µmol N_2 m^{-2} d^{-1}, Nielsen et al. 1995) and Randersfjord (up to 8000µmol N_2 m^{-2} d^{-1}, Nielsen et al. 2001), which should, however, not be considered representative of the Baltic Sea due to the extremely eutrophied nature of these fjords (~700µmol/L NO_3^-). Interestingly, ambient nitrate levels at the present study sites (2-5µmol/L) were somewhat lower than at the study sites with comparable rates in the Gulf of Finland and Bothnian Bay (~10µmol and up to 24µmol/L, respectively), suggesting that denitrification might have been controlled by environmental factors other than the ambient nitrate concentration in the overlying water (see 4.3). Comparing the denitrification rates of Arkona and Kreidesegler to values of ecosystems around the world, they fall within the lower range of estuarine and coastal denitrification rates (Piña-Ochoa and Álvarez-Cobelas 2006). In the Nueces River estuary (USA), for example, denitrification rates around 650µmol N_2 m^{-2} d^{-1} were found (Bernot et al. 2003), which is very close to the rates determined at the Arkona station. At another estuarine site on Rhode Island (USA), denitrification rates of 480µmol N_2 m^{-2} d^{-1} were measured (Nowicki 1994), which in turn is similar to the rate of the Kreidesegler. Compared to these estuarine sites (NO_3^- concentration of 67 and 21µmol/L), nitrate concentrations were low.

The central stations NS6 and NS7 comprised a group of medium denitrification rates, as also found in previous studies in central parts of the Baltic Sea. In the Northern Baltic Proper rates ranged from 15 to 300µmol N_2 m^{-2} d^{-1} (Tuominen et al. 1998) and were only slightly lower than the rates recently measured at a coastal station in the Northern Gulf of Finland (90-400µmol N_2 m^{-2} d^{-1}, Hietanen and Kuparinen 2008). In a global context, the rates measured at NS6 and NS7 fall well within the range of denitrification rates in the ocean (Piña-Ochoa and Álvarez-Cobelas 2006). For example, similar denitrification rates have been measured at some stations in the Artic Sea in Svalbard (Norway) (100-135µmol N_2 m^{-2} d^{-1}, Blackburn et al. 1996) and off Greenland (33-265µmol N_2 m^{-2} d^{-1}, Rysgaard et al. 2004), as well as in the Pacific Ocean off the coast of Mexico (~ 130µmol N_2 m^{-2} d^{-1}, Christensen et al. 1987). It is interesting to note that all of these study sites lay at a much higher depth than NS6 and NS7 and that the site of comparable

depth in the current study, NS1, showed very low denitrification rates compared to the latter mentioned sites in other oceans. This again supports the finding by Tuominen et al. (1998) that denitrification rates in the central Baltic Sea are generally lower in comparison to areas of the same depth in other oceans, presumably due to prevailing anoxic conditions.

The sandy estuarine and coastal stations made up a group of low denitrification activity. These rates are close to the lowest denitrification rates measured in the Northern Baltic Proper (~15µmol N_2 m^{-2} d^{-1}, Tuominen et al. 1998), as well as to rates measured in sandy littoral areas of the Baltic Sea (10-85µmol N_2 m^{-2} d^{-1}, Sundbäck et al. 2006) and sandy sediments in the North East Kattegat (36-40µmol N_2 m^{-2} d^{-1}, Sundbäck et al. 2000). For the littoral areas Sundbäck et al. (2006) only found higher rates at silty sediments compared to sandy environments in September, while rates were similar in April. This was assigned to the fact that high photosynthetic oxygen production combined with low remineralisation rates kept denitrification rates at a low level at the silty site in April. In the open North Sea, denitrification rates at different coastal and offshore stations with sandy sediments also lay in the same range as the current rates (0-45µmol N_2 m^{-2} d^{-1}; Lohse et al. 1993) In the Wadden Sea, mean annual denitrification rates in sand and fine sand environments were slightly higher (100 and 72µmol N_2 m_{-2} d^{-1} ± std., respectively; Jensen et al. 1996). In sandy sediments of the Georgia continental shelf (Atlantic Ocean, USA) denitrification rates ranged from 23 to 34µmol N_2 m^{-2} d^{-1} (Vance-Harris 2005). This confirms that the rates measured at the three sandy stations Breitling, Gollwitz 1 and Gollwitz 2 seem well representative of denitrification in sandy sediments in general. However, Kähler (1990) has shown for the Kiel Bight that denitrification rates can be temporarily very high (up to 1000µmol N_2 m^{-2} d^{-1}) in sandy coastal sediments after food input from spring blooms. This would have important consequences for the N-removal along sandy coastal areas of the Baltic Sea.

As a result of anoxia, there was no nitrate available for denitrification at NS1 either from the overlying water (the small measured nitrate concentration lies within the error of the method), or from coupled nitrification-denitrification. Thus, the measured rate (29 ± 15µmol N_2 m^{-2} d^{-1}) should be viewed as a denitrification potential rather than a true denitrification rate, as it is most probable that in the course of core handling some oxygen was introduced into the water and denitrification was also stimulated due to $^{15}NO_3^-$ addition. This denitrification potential against the background of environmental conditions will be discussed in more detail in the following section.

Table 8: Summary of cited denitrification rates (DN) in the Baltic Sea.

Region	Conditions	DN [µmol N_2 m^{-2} d^{-1}]	Method	Reference
Central Gulf of Finland	depositional area, ~ 10µmol/L NO_3^-	150 - 650	Classical IPT	Tuominen et al. 1998
Entrance to Gulf of Finland (80m)	depositional area, ~ 10µmol/L NO_3^-	100 - 400	Classical IPT	Tuominen et al. 1998
Northern Baltic Proper	depositional area, ~ 10µM NO_3^-	15 - 300	Classical IPT	Tuominen et al. 1998
Bothnian Sea	depositional area, ~ 5µmol/L NO_3^-	250 - 300	Classical IPT	Tuominen et al. 1998
Northern Gulf of Finland	Coastal depositional station	90 - 400	Revised IPT	Hietanen and Kuparinen 2008
Archipelago Sea (southern Gulf of Finland)	Estuary bays, 2-24 µM NO_3^-	90 - 910	Direct N_2 measurement	Silvennoinen et al. 2007
Gulf of Finland	Estuary bays, 3-10 µmol/L NO_3^-	230 - 320	Direct N_2 measurement	Silvennoinen et al. 2007
Bothnian Bay	River mouths, high C_{org}, 20-80 µmol/L NO_3^-	330 - 905	Direct N_2 measurement	Silvennoinen et al. 2007
Norsminde Fjord	Eutrophied fjord, high C_{org}, up to 300µmol/L NO_3^-	100 - 1600	Classical IPT	Nielsen et al. 1995
Randersfjord	Eutrophied fjord, very high C_{org}, 150-750µmol/L NO_3^-	4000	Classical IPT	Nielsen et al. 2001
Kalmar Sound	Littoral sandy sediments, low C_{org}, 0.2-10µmol/L NO_3^-	10 - 85	Classical IPT	Sundbäck et al. 2006
North East Kattegat	Shallow brackish bay, low C_{org}, ~2µmol/L NO_3^-	36 - 40	Classical IPT	Sundbäck et al. 2000

Table 9: Summary of cited denitrification rates (DN) in aquatic ecosystems around the world.

Region	Conditions	DN [µmol N_2 m^{-2} d^{-1}]	Method	Reference
Nueces River Estuary (USA)	Sediment with high C_{org}, 67µmol/L NO_3^-	650	Acetylene Block, Membrane Inlet Mass Spectrometry	Bernot et al. 2003
Estuary Rhode Island (USA)	Sediment with high C_{org}, 21µmol/L NO_3^-	480	N_2 Gas Flux	Nowicki 1994
Svalbard (Norway)	Deep water stations, moderate C_{org}, maximum 5µmol/L NO_3^-	100 - 135	Classical IPT	Blackburn et al. 1996
East and West Greenland	Oceanic sediments, moderate to low C_{org}, 2-15µmol/L NO_3^-	33-265	Revised IPT	Rysgaard et al. 2004
Mexico and Gulf of Maine	Oceanic, oxygen deficient zones	130	NO_3^- flux	Christensen et al. 1987
South East North Sea	Open North Sea sandy sediments, low C_{org}, 1-40µmol/L NO_3^-	0 - 45	Acetylene Block	Lohse et al. 1993
Sylt, Wadden Sea	Sand and fine sand with low to moderate C_{org}, 0-75µmol/L NO_3^-	72 - 100	IPT, Isotope Dilution	Jensen et al. 1996
Georgia Continental Shelf (USA)	Sandy sedments with low C_{org}, NO_3^- concentration not given	23 - 31	Modified IPT	Vance-Harris 2005

4.3 Controlling factors of denitrification

Factors influencing denitrification in aquatic ecosystems include the supply of nitrate, concentration of oxygen, dissolved organic carbon and phosphorus, temperature, light, water residence time and the presence of plants (Golterman 2004). In the present study, the multiple regression model of denitrification only yielded a significant correlation to the C_{org} content of the sediment, while other important factors like the NO_x^- concentration in the overlying water did not show a consistent relation to total denitrification rates. D_w, in contrast, was significantly related to nitrate in the overlying water, suggesting that the pattern of total denitrification was mainly determined by D_n.

The reducing power of organic carbon compounds is a very important factor controlling the activity of the majority of denitrifying bacteria. The importance of this substrate was

clearly reflected in the denitrification activity of the study sites, as it was the only environmental factor which showed a significant correlation with denitrification rates (Figure 16). These findings have a strong support within the literature. The carbon supply stimulates denitrification activity directly by supplying the necessary substrate for growth and indirectly as the oxygen consumption is increased by the supply of carbon, thereby decreasing the thickness of the oxic zone (Chalamet 1985; Goering 1985; Seitzinger 1988). Piña-Ochoa and Álvarez-Cobelas (2006) found a linear correlation across all aquatic ecosystems between denitrification rates and organic carbon content in the sediment. In Iowa subsoils (USA), denitrification was stimulated by the addition of aqueous extracts of surface soils and the degree of stimulation increased with increased organic carbon content of the extracts (McCarty and Bremner 1992). Mitchell and Baldwin (1999) demonstrated a very strong increase of denitrification rates when organic carbon was added to the sediments of Lake Hume (Australia). Furthermore, Duff et al. (1984) suggested a supply of nitrate to a pristine stream did not stimulate denitrification due to the low organic carbon content. In the Baltic Sea and Kattegat, denitrification rates in silty sediments with high organic carbon were found to be higher compared to sediments with lower C_{org} in shallow water sites (Sundbäck et al. 2000) and non-tidal littoral habitats (Sundbäck et al. 2006). For the water column of the Baltic Proper, Brettar and Rheinheimer (1992) showed that denitrification was not nitrate limited but that a lack of available organic carbon seemed to limit denitrification rates and growth of denitrifiers. In contrast to this, no coherent relation could be proved by Tuominen et al. (1998) for sediments in the Northern Baltic Proper. The same holds for river estuaries of the Northern Baltic Sea (Silvennoinen et al. 2007), where no correlation to any environmental factors was found due to the high variability of denitrification rates. For the present study it has to be kept in mind that the general pattern of increased denitrification rates with increased organic carbon was only significant when excluding the anoxic station NS1, where due to anoxia denitrification was very low despite the highest content of organic carbon (~6%). In order to study the effect of organic carbon in more detail, glucose fertilisation experiments were performed at four of the study sites (Figure 22). The results have shown no significant difference between denitrification rates with and without glucose addition, which is in seeming contradiction to the positive correlation between C_{org} and *in-situ* denitrification. Different results have been obtained in most previous carbon fertilisation experiments, although results are not consistent. Microcosms constructed from coarse sandy material obtained from the Claiborne aquifer

(south central Georgia, USA) had enhanced denitrification rates and nitrate disappearance when nitrate laden water was infused with glucose (Obenhuber and Lowrance 1991). Bradley et al. (1995) have also found a stimulatory effect of glucose addition to sandy sediments of the South Platte River (Colorado, USA) from 35km downstream of a water treatment plant (C_{org} content of sediment <0.05%). Closer to the treatment plant, denitrification did not appear to be carbon limited despite low organic carbon content (0.3%) and it could instead be enhanced by nitrate addition. This situation is comparable to that in the present study, where minimum organic carbon contents were 0.2% and a stimulatory effect of glucose addition was initially expected. In agricultural streams in Illinois and Michigan (USA), it was found that benthic organic carbon only stimulated denitrification when nitrate levels were above limiting levels in the range of half saturation constants (K_m) for nitrate uptake (Arango et al. 2007). In the present study, ambient nitrate levels were all clearly below the K_m values cited by Arango et al. (2007) (27-53µM), which would therefore seem to indicate that nitrate levels were too low for a stimulation of denitrification by organic carbon to become apparent. On the other hand, if this was the case, one should expect that ^{15}N-N_2 production values of denitrification should be higher at high tracer concentrations (>50µmol/L) with glucose addition compared to treatments without glucose, which, however, was not the case either, even at highest tracer concentrations (150µmol/L). Similar results as in the present study have been obtained in a study on wetland soils in southern Sweden, where despite a positive relationship between soil organic matter and NO_3^- consumption, glucose fertilisation did not result in an increased N removal in the soils (Davidsson and Stahl 2000). The authors infer from this that there was no short-term lack of electron donors, including the sandy soils. It is most probable that the situation was similar in the present study in the way that the existing denitrifying microbial community was not acutely carbon limited and might have been adapted to carbon sources other than glucose. Therefore it did not respond to the glucose addition on a short-term basis of maximum 24 hours. Experiments like those by Bradley et al. (1995) and Obenhuber and Lowrance (1991) all lasted for several days or weeks, therefore allowing for extensive microbial growth and a resulting enhancement of denitrification activity. The present duration of fertilisation experiments was probably too short for extensive microbial growth to take place, which would explain the lacking stimulation of denitrification. Doubling times recorded for *Pseudomonas denitrificans* when grown in media containing 10-20mM formiate and 10mM NO_3^- were about 10 hours (Strohm et al. 2007), but growth on glucose might be

slower as it is not the preferred carbon source used under natural conditions. In agricultural soils, for example, denitrification rates after 72 hours of incubation were significantly lower in glucose treatments compared to treatments with malate, citrate and acetate (Lescure et al. 1992). For a strain of *Desulfovibrio desulfuricans*, Dalsgaard and Bak (1994) determined a doubling time of 21h under optimal denitrifying growth conditions. It is most likely, however, that growth rates are much slower under *in-situ* conditions and are highly variable depending on the species composition of the denitrifying community. In the permanently ice-covered Antarctic Lake Bonney, for example, doubling times of three denitrifying bacterial strains under near *in-situ* culturing conditions were in the order of 50 hours (Ward and Priscu 1997). Considering the experimental conditions used in the present study (1mM Glucose, 150µmol/L maximum added NO_3^-), it is therefore likely that the growth of denitrifiers was also slow. This would support the hypothesis that the lacking stimulation of denitrification can be explained by the short experimental duration and the fact that the existing microbial community was not acutely carbon limited. Indirectly this also implies that the observed linear relationship between denitrification rates and sediment organic carbon content was due to the presence of larger denitrifying microbial communities with increased carbon content rather than a carbon limitation of the existing denitrifiers.

Nitrate availability at the physical place for denitrification may be driven by water column concentration, by sediment nitrification and through groundwaters (Jenkins and Kemp 1984; Seitzinger 1988; Nielsen et al. 1995; Cornwell et al. 1999). Nitrate produced in the sediment via nitrification of ammonium released from benthic oxidation of organic matter appears to be the major substrate source for denitrification in most aquatic sediments (Seitzinger 1988). However, Christensen et al. (1990) pointed out that for systems with significant nitrate concentrations in the overlying water, the denitrification rate is inversely proportional to the thickness of the oxic surface layer, as nitrate has to diffuse through this layer. At the same time it is proportional to the nitrate concentration in the overlying water. Such a proportional increase of denitrification rate with nitrate concentration has been found in many site-specific studies (Christensen et al. 1990; El-Harb and Golterman 1990; Pelegri et al. 1994; Nielsen et al. 1995; Rysgaard et al. 1995; Ogilvie et al. 1997; Kana et al. 1998; Royer et al. 2004; Inwood et al. 2007). The same has been found across different biomes for streams (Mulholland et al. 2008) and in a cross-system analysis including different types of aquatic ecosystems (Piña-Ochoa and Álvarez-Cobelas 2006). All of these studies were performed in nitrate-

rich environments with effective penetration of bottom water nitrate into the sediment, namely rivers and streams, estuaries and agriculturally contaminated aquatic environments. Thus, a correlation of denitrification with ambient nitrate can be expected, because denitrification is dominated by D_w. In the present study, nitrate levels in the overlying water were low (<7µmol/L) compared to the latter investigations and the share of D_w was below 50% for most study sites. An exception was Gollwitz 1 (D_w > 70%), but due to ^{15}N-N_2 production close to detection limit this value was viewed critically (see 4.1). Thus, the pattern of total denitrification was dominated to a higher extend by D_n, which supports the original hypothesis of Seizinger et al. (1988) concerning the importance of D_n in most aquatic ecosystems. This also explains the lack of a linear increase of total denitrification rate with ambient NO_x^-. Similar results have been obtained by Tuominen et al. (1998) for study sites in the Northern Baltic Proper, the Gulf of Finland and the Bothnian Sea, where only D_w and not total denitrification was correlated with nitrate concentrations in the bottom water. This is similar to recent results in the Archipelago Sea (Southern Gulf of Finland) (Silvennoinen et al. 2007) and the Northern Gulf of Finland (Hietanen and Kuparinen 2008). A significant linear increase of D_w with nitrate concentration could be also demonstrated in the present study. In contrast to this, neither total denitrification nor D_n revealed a coherent relation to the maximum nitrate concentrations within the sediment, which here served as a proxy of nitrate produced in the sediment by nitrification. Only in the case of the Kreidesegler and the Breitling, high nitrate levels within the sediment coincided with a high share of D_n in total denitrification and thus supported the assumption that high nitrate production via nitrification in the sediment promotes coupled nitrification-denitrification. However, tight coupling of denitrification to nitrification could also result in a more or less complete consumption of produced nitrate, so that no increased nitrate level in the sediment becomes apparent in spite of high nitrification activity. Despite a lacking general relationship between denitrification rates and the ambient nitrate concentration, nitrate must have been the limiting factor for denitrification at NS1 due to anoxia in the bottom water.

The importance of the oxygen concentration in the overlying water on denitrification has been examined both experimentally (Andersen 1985; Bonin and Raymond 1990; Rysgaard et al. 1994) and at ecosystem level (Kemp et al. 1990). Furthermore, a cross-system analysis has yielded that denitrification is enhanced at oxygen concentrations below 0.35ml/L (< 15µmol/L) both in freshwater and marine systems (Piña-Ochoa and Álvarez-Cobelas 2006). This is in line with earlier estimates that oxygen concentrations

need to be below 0.2 ml/L for denitrification to function effectively (Knowles 1982; Hattori 1983). Andersen (1977) showed that anaerobic water resulted in a high rate of denitrification in six Danish lakes compared to conditions with oxygenated water overlying the sediment. With oxygen present, the uppermost sediment will be maintained at a high redox level. As denitrification will occur below this oxidized zone, a longer diffusion pathway for nitrate will result in lower rates of D_w and potentially total denitrification rates if D_w dominates the process of denitrification (Silvennoinen et al. 2007). In the present study, oxygen concentrations in the bottom water were a lot higher than 15µmol/L at all stations except NS1 and denitrification rates were nevertheless high. Furthermore, the bottom water oxygen concentration did not contribute significantly to explain the pattern of denitrification in the multiple regression model. This might be due to the low oxygen penetration depths for most stations despite high concentrations in the bottom water, which is reflected in the oxygen profiles of the Kreidesegler and NS6 (O_2 penetration depths < 3mm) and was probably a result of low permeability and high metabolic activity. Thus, the diffusion distance for nitrate to reach the denitrifying horizon was still short and at the same time an effective coupling of nitrification and denitrification could take place at the oxic-anoxic interface within the sediment. Kuparinen and Tuominen (2001), too, have stated that the most efficient removal of nitrogen by denitrification takes place where the sediment is moderately well oxidised. This is further sustained by the very low denitrification rate at the anoxic station NS1, which, as already mentioned, should be viewed as a denitrification potential. The reduction or even elimination of sediment denitrification under anoxic conditions in the deep basins has important implications for the N-removal capacity of these areas in the Baltic Sea. This is particularly important due to the less frequent and intensive salt water inflows into the Baltic Sea after the late 1970s (Matthäus and Schinke 1999), leading to extensive areas with anoxia in the bottom water of the central Baltic. However, prolonged stagnation periods also lead to a subsidence of the halocline, so that the total area of oxygenated bottom water in the Baltic Sea increases (Conley et al. 2002), which in turn could promote denitrification. During the last 20 years, significant inflows occurred only 3 times, namely in 1983, 1993 and the winter 2003. The period after the last inflow in 2003 was characterized by low inflow activities with only a slight intensification during 2006. Except in the southern Baltic, the stagnation period lasting since 2004/2005 is strengthening further, leading to a deterioration of the oxygen situation and a continuous increase of hydrogen sulphide concentrations in the deep water

(Feistel et al. 2007). A weakening of the self-purification capacity of the central Baltic Sea by denitrification has already been recorded by the year of 1999 (Kuparinen and Tuominen 2001), a trend which is likely to continue in the course of the recent stagnation period and seems to be reflected in the low denitrification rate at NS1. This could have a devastating effect on the Baltic Sea ecosystem, particularly in the course of continuing eutrophication, and it reflects the necessity for future efforts to minimize the anthropogenic N and P load. On the other hand, the detection of a prevailing denitrification potential even under anoxic conditions, also coupled to nitrification, implies that upon the oxygenation of the bottom water and the resulting introduction of NO_3^-, an extensive denitrifying bacterial community can develop and reduce the N-load accumulated in the sediment through remineralisation. A completely different situation concerning oxygen is generally found in shallow coastal sands represented by the stations Breitling, Gollwitz 1 and Gollwitz 2. These are characterised by high pore water dissolved oxygen concentrations. A good oxygenation, however, promotes nitrification and thus coupled nitrification-denitrification. Furthermore, tightly coupled oxic-anoxic microsites can be formed in the walls of makrofauna burrows, which have been found to stimulate nitrification and denitrification (Sayama and Kurihara 1983; Kristensen and Blackburn 1987; Henriksen and Kemp 1988; Pelegri et al. 1994; Mayer et al. 1995; Rysgaard et al. 1995; Gilbert et al. 1998). At the sandy coastal stations the measured denitrification rates were indeed low, which should, however, be attributed to the low organic carbon content (see above) and not the oxygen situation. In line with a potential stimulation of nitrification, the share of D_n was very high at the Breitling station (~80%), where nitrate was still present at high concentrations at depth. Compared to this, the share of D_n was distinctly lower at the station Gollwitz 2 (~40%), but here nitrate might not have been produced at such high quantities in the sediment (Figure 10) or errors due to low ^{15}N-N_2 production have lead to an underestimation of D_n. In other studies in the Northern Baltic Sea, a positive correlation of D_n with oxygen could be detected (Silvennoinen et al. 2007). A relation of D_n to oxygen is not consistently found in the literature (e.g. Hietanen and Kuparinen 2008) and was absent in the present study.

In contrast to the present study, an indirect relation of phosphate concentrations to denitrification rates has been found in some studies, although results are not consistent. Piña-Ochoa and Álvarez-Cobelas (2006), for example, have found that low concentrations of total phosphorus enhanced denitrification at a yearly scale, but not during the

period of highest water temperatures. A low N:P ratio was found in eutrophic systems, i.e. sites with high TP, which might cause an imbalance of the N and P supply to bacterial denitrification metabolism (Knowles 1982). A low N:P ratio results in a lack of N compared to P, which may lead to a higher competition for nitrogen from i.e. benthic micro algae und thus lower denitrification rates. Opposed to this, it was found for P-limited wetlands that increased P loading in the course of eutrophication leads to enhanced denitrification rates (Reddy et al. 1999), presumably through the general stimulation of the microbial metabolism. In the present study, it is most probable that the effect of phosphorus on denitrification was minor compared to other environmental factors and therefore did not become apparent in the regression model of denitrification.

There are only a few studies on the effect of temperature on denitrification rates. Most data show increasing rates with increasing temperature (Cavari and Phelps 1977; Sørensen et al. 1979; Chalamet 1985; Seitzinger 1988; Nowicki 1994; van Luijn et al. 1999). However, an inverse relationship between temperature and denitrification has been found in Danish coastal sediments (Sørensen et al. 1979) and in the mesohaline region of Chesapeake Bay (Kemp et al. 1990). El-Harb and Golterman (1990) demonstrated that, if the sediment was relatively rich in organic matter (about 5%), the temperature had a strong influence on the denitrification rate, while if the organic matter was about 2%, this influence was feeble. Furthermore, Piña-Ochoa and Álvarez-Cobelas (2006) did not find any latitudinal effect on denitrification, which served as a proxy of the water temperature effect. The latter findings are consistent with the current result of a lacking relation between bottom water temperature and denitrification, confirming once again that other environmental factors determined denitrification activity. Here, the results of El Harb and Golterman (1990) might be of importance as organic matter content varied between 0.3 and 6% at the study sites. In addition, factors such as nitrification rate, oxygen content and external nutrient loading may also be changing with changing temperatures (Andersen 1977; Seitzinger and Nixon 1985; Seitzinger 1988; Nowicki 1994), which makes it difficult to isolate the effect of temperature on denitrification rates alone.

4.4 The absence of anammox

According to the modern view of the nitrogen cycle, anammox can play an important role in removing nitrogen from marine ecosystems and the relative importance of anammox seems to be negatively correlated to mineralization rate and water depth (Dalsgaard and Thamdrup 2002; Dalsgaard et al. 2005; Engström et al. 2005). This negative correlation, however, does not have to be present for absolute rates.

In the scope of this work, anammox could not be detected at any of the here investigated study sites, which is against the initial expectation that anammox should be at least present at the stations inside the central basins of the Baltic Sea. Considering the prevailing environmental conditions, however, the absence of anammox can be explained and is in line with previous findings on this process.

The anammox process is mainly performed by anaerobic, autotrophic bacteria with slow growth rates (doubling time was measured to be in the range of 9 days under optimal growth conditions in waste water treatments) and comparatively low substrate affinity for NH_4^+ and NO_2^- (<0.1 mg N L^{-1}(~2µmol/L) in waste water treatments) (Jetten et al. 1998; Strous et al. 1999). This indicates that anammox bacteria need very specific environmental conditions to be able to compete with other bacterial groups, like denitrifiers. In the Skagerrak, it was found that the ratio of anammox to denitrification did not vary as a function of NO_2^- concentration and it was concluded that the affinity to NO_2^- was similar (K_m<<3µM) in the two groups of bacteria. On the other hand, the NO_2^- utilised by the anammox bacteria most probably originates from the reduction of NO_3^- by denitrifiers and dissimilatory nitrate reducers, which should only release excess NO_2^- when not nitrate limited (Dalsgaard and Thamdrup 2002). Assuming nitrate limitation to take place at concentrations corresponding to 2-3 times the K_m and assuming a K_m for nitrate uptake of denitrifiers of 1µM (Dalsgaard and Bak 1994), nitrate reducers should become nitrate limited at concentrations below 2-3µM (Dalsgaard et al. 2005). Higher K_m values for nitrate uptake have been also reported, with values generally ranging from 27-53µM (Seitzinger 1988), but maximum values going up to 500µM NO_3^- (Joye et al. 1996; García-Ruiz et al. 1998). Comparing the range of K_m values to the prevailing nitrate concentrations in the water column and in sediments of the study sites, it becomes clear that nitrate reducers may have been nitrate limited (NO_x^- levels ranging from 0 to 8µmol/L in the overlying water and top centimetres of the sediment). Only at the coastal

station Kreidesegler and at greater depth at the Breitling and Gollwitz 1 nitrate levels were higher. Thus, it could be inferred that NO_2^- release was comparatively low (potentially with the exception of the latter stations), so that only little to no nitrite would have been available for the anammox bacteria. In addition, remineralisation rates as estimated from the upward flux of ammonium in the sediment were high for most stations, suggesting that other processes involved in remineralisation were able to out-compete the anammox process.

4.5 Additional aspects of the sediment environment of denitrification

As shown in the previous sections, environment factors played an important role in determining the denitrifying activity in the sediment and their impact on denitrification has been reviewed by various authors (e.g. Focht 1982, Hattori 1983, Knowles 1982, Cornwell et al. 1999, Golterman 2004). This is why the study of the sediment environment formed an important part of this work and shall be further discussed in the following.

As described in the results section, all stations except NS1 were covered by oxygen containing water bodies, with highest oxygen contents at the shallow coastal and estuarine stations (Breitling, Gollwitz 1 and Gollwitz 2). At the latter stations, oxygen penetration into the sediment was also highest, which is typical for sandy environments. At the muddy stations, the oxygen penetration depth into the sediment was shallow despite high water values, thus creating favourable conditions for denitrification (see 4.3). As a result of the oxygen situation, nitrate was present in the bottom water of all stations except NS1. At the estuarine and coastal stations Breitling, Gollwitz 1 and Gollwitz 2, the NO_x^- concentrations in the water were very low. This is a typical summer situation for surface water (water depth of Breitling station: 0.2m), where nitrate is depleted due to primary production. Phosphate concentrations in the water were zero at all coastal stations, while some nitrate and ammonium were still present. This might suggest a phosphate limitation of primary production during the time of sampling as has been previously found in northern parts of the Baltic Sea (Bothnian Bay and Gulf of Finland) (Granéli et al. 1990; Moisander et al. 2003) and as implied by nutrient ratios of external loading (Granéli et al. 1990), which is particularly important in eutrophied coastal areas with high inputs from agricultural runoff. Nitrate was depleted with depth in the case of all central stations and the coastal stations Kreidesegler and Gollwitz 2, due to the re-

duction of nitrate below the redoxcline through denitrification. The similar depths of nitrate and oxygen depletion confirms that denitrification generally takes place at the oxic-anoxic interface of the sediment (Jenkins and Kemp 1984; Canfield et al. 2005b). The nitrate profile of the coastal station Kreidesegler showed a prominent nitrate peak in the top layer of the sediment, which can be attributed to nitrification activity. This is further supported by the apparent ammonium depletion in this sediment horizon. It could be shown that this had important consequences for the coupling of nitrification and denitrification (4.3). Compared to the other stations, Breitling and Gollwitz 2 revealed distinct differences concerning their nitrate profiles. The combination of low NH_4^+ values and small NO_x^- peaks, followed by a decrease of NO_x^- speaks for nitrification activity and a coupled denitrification in the centimetre below. For the subsequent increase of NO_x^-, there might be two possible explanations: On the one hand the influence of nitrate rich groundwater might be responsible for increased nitrate levels at depth. However, against this hypothesis speaks the rather low permeability of the sediment (3-6 x 10^{-12} m^2) as determined by Betke (2002), which makes an effective permeation of groundwater from greater depths improbable. On the other hand, the increased and fluctuating nitrate concentrations at depth might be due to zones of enhanced nitrification as a result of irrigated macrofaunal burrows (bioturbation). Bioturbation of sedimentary deposits typically generates close juxtapositions of oxic and anoxic microenvironments around biogenic structures and strongly influences both nitrification and denitrification. There is often a stimulatory effect of macrobenthic activity, and particularly bioirrigation, on sedimentary nitrification and denitrification (Sayama and Kurihara 1983; Kristensen and Blackburn 1987; Henriksen and Kemp 1988; Pelegri et al. 1994; Mayer et al. 1995; Rysgaard et al. 1995; Gilbert et al. 1998). The spacing of burrows also decides on the stimulatory effect on both processes and the effectiveness of their coupling, with best coupling occurring at medium burrow densities and less denitrification occurring at high densities when the water becomes completely oxygenated (Gilbert et al. 2003). For Breitling and Gollwitz 1, it is conceivable that irrigated macrofauna burrows have stimulated nitrification particularly in some parts of the sediment, so that the measured concentrations are means of concentrations in stimulated and unstimulated zones, leading to the observed concentration patterns. This might also explain the fluctuations of nitrate concentrations with depth at the station Gollwitz 2. The high presumable production of nitrate at tube walls inside the sediment compared to

nitrate levels in the overlying water have also become apparent in the share of D_n in total denitrification at the Breitling.

At all stations, there was a steady increase of ammonium with depth, which is typical for sediments as a result of remineralisation of organic material in the process of ammonification. Fluctuations of ammonium with depth as seen at the Breitling, Gollwitz 2 and NS7 again speak for zones of differing nitrification and denitrification activity at the walls of macrofauna tubes. The high NH_4^+ concentrations at stations Gollwitz 2 and Breitling (>600µmol/L) indicate a very active remineralisation of organic matter (see 4.6). In this respect Gollwitz 2 differs from Gollwitz 1, where ammonium concentrations speak for less active ammonification. This is particularly interesting when considering that the organic carbon and nitrogen content of both stations is more or less the same, suggesting that at Gollwitz 2 there is a higher turnover of deposited organic material.

Similar to ammonium, phosphate also accumulated with depth as a result of remineralisation. Small phosphate peaks, as for instance at the Kreidesegler station at a depth of 2cm and at Gollwitz 2 at a depth of 4cm probably indicate zones of particularly intensive P remineralisation.

4.6 Rates and contribution of denitrification in carbon remineralisation estimated from NO_x^- and NH_4^+ fluxes

An alternative method for estimating denitrification rates in sediments is their calculation from NO_x^- pore water profiles (Mengis et al. 1996; Mengis et al. 1997). In this study, nitrate fluxes distinctly underestimate denitrification, which has been previously found (e.g. Mengis et al. 1996, Steingruber et al. 2001 and Friedrich et al. 2003) and seems a general problem of this approach. On the one hand this is because flux calculations do not consider coupled nitrification-denitrification. On the other hand, denitrification is underestimated due to insufficient vertical resolution of the profiles and as only diffusive and not turbulent transport is considered when the fluxes are calculated (Mengis et al. 1996; Friedrich et al. 2003).

The respiration of organic matter releases ammonium (e.g. Blackburn and Henriksen 1983; Hammond et al. 1985; Jensen et al. 1990; Dahllöf and Karle 2003), which under oxic conditions can be converted into nitrate through nitrification. Thus, NH_4^+ and NO_3^- concentrations and -fluxes can be used as a proxy for remineralisation activity. The high

ammonium concentrations in deeper sediment layers of most sites (4.5) therefore indicate high remineralisation activity in the sediments. Accordingly, there is a high NH_4^+ flux directed out of the sediment so that all investigated sediments act as a source of ammonium for the overlying water. Flux measurements in the Kattegat also showed that sediments mainly act as sources of NH_4^+ (100-1500µmol m^{-2} d^{-1}), although positive fluxes into the sediment can occur during periods of high benthic photosynthesis (Sundbäck et al. 2000). Similarly, ammonium was released from the sediment at high quantities (>2000µmol m^{-2} d^{-1}) in shallow littoral areas of the Baltic Sea in September, while it was taken up during spring (Sundbäck et al. 2006). For nitrate, there is both an outward flux from the top sediment centimetre towards the overlying water and an inward flux towards deeper sediment layers at stations where nitrate peaks appeared within the sediment (Kreidesegler, Breitling, Gollwitz 1 and Gollwitz 2). This is particularly distinct at the Kreidesegler station, which means that nitrate must have been produced by nitrification in the top layer to be then consumed by denitrification in deeper sediment horizons. Accordingly, the share of D_n in total denitrification was very high at this station (4.3). Like NH_4^+ fluxes, NO_x^- fluxes obtained from incubation experiments in studies like those of Sundbäck et al. (2000) and Sundbäck et al. (2006) were distinctly higher (100-630µmol/L) than in the present investigation. This illustrates the problem of underestimating fluxes in calculations from pore water profiles.

The sum of ammonium fluxes and denitrification converted to C units should give a rough estimate of total carbon remineralisation rate. Relating denitrification rates to this estimate gives an idea of the importance of denitrification in total remineralisation of organic matter (personal considerations). The share of denitrification in total C remineralisation calculated from these considerations revealed a contribution of 3 to 35% (Table 4). Denitrification shows high contributions to remineralisation of organic matter at the stations with highest denitrification rates, Kreidesegler and Arkona. Thus, conditions presumably were favourable for denitrifying bacteria at these stations, so that the process of denitrification could successfully compete for resources with other remineralisation processes, e.g. ammonification. Interestingly, denitrification had the highest share in remineralisation at the central station NS6, despite lower absolute denitrification rates. As also seen from the low NH_4^+ fluxes, this means that remineralisation in general was not as active at this station, so that denitrification was relatively more important than at other sites. It is astonishing that in spite of similar environmental conditions, denitrification contributed less to remineralisation at the neighbouring station

NS7. This indicates that variations in the importance of denitrification in sediment remineralisation can even occur on a small spatial scale, which makes it difficult to extrapolate to greater areas.

A typical situation for the anoxic basins of the Baltic Sea was found at station NS1. Due to anoxia there is no nitrate available for denitrification, so that denitrification is replaced by other anaerobic respiratory processes, in particular sulphate reduction to sulphide, and therefore does not contribute significantly to C remineralisation. The high total remineralisation activity found at this station can be attributed to the very high organic carbon content (6%) of the sediment.

The share of denitrification in remineralisation of organic matter was also low at the sandy coastal stations. Total remineralisation activity, however, was high at the stations Breitling and Gollwitz 2, as opposed to Gollwitz 1 where low NH_4^+ fluxes suggest low remineralisation activity. The low organic carbon content of these stations indicates a high turnover of organic material in the sediment and a tight coupling of assimilatory and dissimilatory processes in the water column and sediment. Furthermore, the low share of denitrification in total remineralisation confirms that the typical conditions in sandy sediments like low organic matter content and high pore water dissolved oxygen are indeed disadvantageous for denitrification and favour aerobic remineralisation processes with a higher energy yield per carbon. However, as Kähler (1990) has shown, the situation may be different during pulses of C_{org} input in the course of phytoplankton blooms.

As mentioned for the calculation of denitrification from NO_x^- fluxes, all fluxes and the inferred remineralisation rates are only estimates and represent minimum values since the gradients may be underestimated and the conversion factor of NH_4^+ to carbon units may vary due to the influence of alternative NH_4^+ producing processes. Furthermore, there are other oxidation pathways (chemo litho-autotrophic processes), which consume NH_4^+ and impair the estimate of total remineralisation. The relative share of NO_3^- respiration processes in total C-oxidation in different sedimentary environments usually lies around 5% or less, with exceptions found in deep sea environments (20%) and Norsmindefjord (23%), both likely due to the high availability of nitrate (Canfield et al. 2005a and references therein). Thus, the present calculations probably overestimate the contribution of denitrification as a result of the mentioned drawbacks.

4.7 Conclusions and Outlook

Denitrification rates measured in this diploma thesis ranged from mean 13 to 690µmol N_2 m^{-2} d^{-1}, influenced by the prevailing environmental conditions at the study sites. As formulated in the first hypothesis (1.3), the coastal and intermediate mud stations Kreidesegler and Arkona displayed highest denitrification rates and the share of D_n in total denitrification was high. Denitrification rates were distinctly lower in the sediments of the central basins (second hypothesis), presumably due to lower organic carbon content. The share of D_n in total denitrification was lower at these stations (NS6 and NS7) but oxygen concentrations in the bottom water were still high enough to sustain microbial nitrate production, so that a coupling of denitrification to nitrification could take place. It might have been the case that DNRA also played an important role at these stations, particularly at the station NS7 with a high ammonium flux. It would be interesting to analyse the water samples of these stations taken after the incubation experiments for $^{15}NH_4^+$ to prove the presence of this process. At the anoxic station NS1, denitrification was very low and the measured rate represents a denitrification potential rather than an actual rate, because *in-situ* nitrate was depleted. The existence of this potential, however, is an important finding in the context of inflow events into the central basins of the Baltic Sea, suggesting that the microbial community is able to react rapidly to the introduction of nitrate in the course of bottom water oxygenation. At the estuarine and coastal sandy stations, denitrification rates were low, which is in line with initial expectations (hypothesis 3) and previous findings in the literature (see 4.2 and 4.3). Despite these low rates, the presence of a denitrifying community even in well oxygenated and low organic carbon environments has important implications for the oceans' nitrogen budget as over 70% of the global continental shelf area is composed of sandy sediments. In coastal areas in particular, this denitrification potential can help reduce the impact of anthropogenic nutrient and organic carbon input, even when the input occurs irregularly in pulses rather than in the form of a continuous source.

The lack of consistent relationships of denitrification to environmental patterns other than organic carbon reflects the high natural variability of denitrification rates. Furthermore, environmental controls are often different for D_n and D_w (Cornwell et al. 1999), which makes it even more difficult to identify clear relationships. It could nevertheless be proven that the organic carbon supply plays an important role in determining the *in-situ* denitrification rate (hypothesis 4), although a short term stimulation of denitrification via glucose addition did not occur. Here, future experiments with longer incubation

times (days to weeks) could address the long-term impact of organic carbon on denitrification rates in the Baltic Sea and microbial community structure investigations could help to understand the observed patterns from an organismic and physiological point of view. Finally, by including additional rate measurements of representative sites in the Baltic Sea, the absolute nitrogen loss through denitrification could be accurately extrapolated and the nitrogen budget of the Baltic Sea adjusted. The total nitrogen loss of 83 tonnes N_2 per year for the Breitling estuary seems to be an underestimation as the extrapolation was only based on rates obtained in sandy parts of the Breitling, while silty areas with high C_{org} load and presumably high denitrification rates could not be included. Thus, it would be important to perform additional measurements in rivers and estuaries draining into the Baltic, as they seem particularly important in reducing the load of anthropogenic nitrogen (Seitzinger 1988; Seitzinger et al. 2006).

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Appendix

Appendix 1: Table of tracer additions for concentration series experiments.

Stock [µmol/ml]:	**10**								
NO_3^-: [µmol/l]		**25**		**50**		**100**		**150**	
Sediment height [cm]	Volume [ml]	NO_3^- needed [µmol]	NO_3^- addition [ml]	NO_3^- needed [µmol]	NO_3^- addition [ml]	NO_3^- needed [µmol]	NO_3^- addition [ml]	NO_3^- needed [µmol]	NO_3^- addition [ml]
5	184,24	4,61	0,461	9,21	0,921	18,42	1,842	27,64	2,764
5,5	179,15	4,48	0,448	8,96	0,896	17,91	1,791	26,87	2,687
6	174,06	4,35	0,435	8,70	0,870	17,41	1,741	26,11	2,611
6,5	168,97	4,22	0,422	8,45	0,845	16,90	1,690	25,35	2,535
7	163,88	4,10	0,410	8,19	0,819	16,39	1,639	24,58	2,458
7,5	158,79	3,97	0,397	7,94	0,794	15,88	1,588	23,82	2,382
8	153,70	3,84	0,384	7,68	0,768	15,37	1,537	23,05	2,305
8,5	148,61	3,72	0,372	7,43	0,743	14,86	1,486	22,29	2,229
9	143,52	3,59	0,359	7,18	0,718	14,35	1,435	21,53	2,153
9,5	138,43	3,46	0,346	6,92	0,692	13,84	1,384	20,76	2,076
10	133,34	3,33	0,333	6,67	0,667	13,33	1,333	20,00	2,000
10,5	128,25	3,21	0,321	6,41	0,641	12,83	1,283	19,24	1,924
11	123,16	3,08	0,308	6,16	0,616	12,32	1,232	18,47	1,847
11,5	118,07	2,95	0,295	5,90	0,590	11,81	1,181	17,71	1,771
12	112,98	2,82	0,282	5,65	0,565	11,30	1,130	16,95	1,695
12,5	107,89	2,70	0,270	5,39	0,539	10,79	1,079	16,18	1,618
13	102,81	2,57	0,257	5,14	0,514	10,28	1,028	15,42	1,542
13,5	97,72	2,44	0,244	4,89	0,489	9,77	0,977	14,66	1,466
14	92,63	2,32	0,232	4,63	0,463	9,26	0,926	13,89	1,389
14,5	87,54	2,19	0,219	4,38	0,438	8,75	0,875	13,13	1,313
15	82,45	2,06	0,206	4,12	0,412	8,24	0,824	12,37	1,237
15,5	77,36	1,93	0,193	3,87	0,387	7,74	0,774	11,60	1,160
16	72,27	1,81	0,181	3,61	0,361	7,23	0,723	10,84	1,084
16,5	67,18	1,68	0,168	3,36	0,336	6,72	0,672	10,08	1,008
17	62,09	1,55	0,155	3,10	0,310	6,21	0,621	9,31	0,931
17,5	57,00	1,43	0,143	2,85	0,285	5,70	0,570	8,55	0,855
18	51,91	1,30	0,130	2,60	0,260	5,19	0,519	7,79	0,779
18,5	46,82	1,17	0,117	2,34	0,234	4,68	0,468	7,02	0,702
19	41,73	1,04	0,104	2,09	0,209	4,17	0,417	6,26	0,626
19,5	36,64	0,92	0,092	1,83	0,183	3,66	0,366	5,50	0,550
20	31,55	0,79	0,079	1,58	0,158	3,16	0,316	4,73	0,473
20,5	26,46	0,66	0,066	1,32	0,132	2,65	0,265	3,97	0,397
21	21,38	0,53	0,053	1,07	0,107	2,14	0,214	3,21	0,321
21,5	16,29	0,41	0,041	0,81	0,081	1,63	0,163	2,44	0,244
22	11,20	0,28	0,028	0,56	0,056	1,12	0,112	1,68	0,168

Appendix 2: Table of glucose additions for fertilisation experiments

Stock [µmol/ml]:	**100**		
Glucose: [µmol/l]		**1000**	
Sediment height [cm]	Volume [ml]	NO_3^- needed [µmol]	NO_3^- added [ml]
5	179,24	179,24	1,792
5,5	178,74	178,74	1,787
6	178,24	178,24	1,782
6,5	177,74	177,74	1,777
7	177,24	177,24	1,772
7,5	176,74	176,74	1,767
8	176,24	176,24	1,762
8,5	175,74	175,74	1,757
9	175,24	175,24	1,752
9,5	174,74	174,74	1,747
10	174,24	174,24	1,742
10,5	173,74	173,74	1,737
11	173,24	173,24	1,732
11,5	172,74	172,74	1,727
12	172,24	172,24	1,722
12,5	171,74	171,74	1,717
13	171,24	171,24	1,712
13,5	170,74	170,74	1,707
14	170,24	170,24	1,702
14,5	169,74	169,74	1,697
15	169,24	169,24	1,692
15,5	168,74	168,74	1,687
16	168,24	168,24	1,682
16,5	167,74	167,74	1,677
17	167,24	167,24	1,672
17,5	166,74	166,74	1,667

Appendix 3: Table of NO_x^- concentrations in the overlying water of experimental cores of the Kreidesegler after tracer addition.

Sample	**Treatment**	**Dilution factor**	**Absorption**	**Absorption corrected**	**NO_x^- [µmol/L]**
87.1	Background	1	0,134	0,117	4,1
88.1	Background	1	0,067	0,05	1,8
89.1	Reference cs b	1	0,131	0,114	4,0
90.1	Referenz cs b	1	0,105	0,088	3,1
91.1	25 µmol/l b	4	0,35	0,333	45,9
92.1	25 µmol/l b	4	0,223	0,206	28,5
93.1	25 µmol/l b	4	0,301	0,284	39,2
94.1	50 µmol/l b	10	0,35	0,333	114,8
95.1	50 µmol/l b	10	0,36	0,343	118,3
96.1	50 µmol/l b	10	0,209	0,192	66,4

97.1	100 µmol/l b	33,33	0,21	0,193	222,4
98.1	100 µmol/l b	20	0,141	0,124	86,0
99.1	100 µmol/l b	20	0,16	0,143	99,1
100.1	150 µmol/l b	20	0,182	0,165	114,2
101.1	150 µmol/l b	33,33	0,143	0,126	145,6
102.1	150 µmol/l b	20	0,444	0,427	294,3
103.1	Ref ts b	1	0,125	0,108	3,7
104.1	Ref ts b	1	0,065	0,048	1,7
105.1	to b	5	0,172	0,155	26,8
106.1	to b	4	0,131	0,114	15,8
107.1	t1 b	4	0,213	0,196	27,1
108.1	t2 b	4	0,335	0,318	43,9
109.1	t3 b	4	0,215	0,198	27,4
110.1	t4 b	4	0,184	0,167	23,1
111.1	t5 b	4	0,167	0,15	20,8
112.1	t6 b	4	0,293	0,276	38,1
89.2	Referenz cs a	1	0,212	0,195	6,7
90.2	Referenz cs a	1	0,128	0,111	3,9
91.2	25 µmol/l a	2	0,379	0,362	25,0
92.2	25 µmol/l a	5	0,231	0,214	37,0
93.2	25 µmol/l a	4	0,175	0,158	21,9
94.2	50 µmol/l a	10	0,279	0,262	90,4
95.2	50 µmol/l a	10	0,259	0,242	83,6
97.2	100 µmol/l a	20	0,121	0,104	72,2
98.2	100 µmol/l a	20	0,204	0,187	129,3
99.2	100 µmol/l a	20	0,127	0,11	76,4
100.2	150 µmol/l a	33,33	0,1	0,083	96,3
101.2	150 µmol/l a	20	0,415	0,398	274,4
102.2	150 µmol/l a	20	0,388	0,371	255,8
103.2	Ref ZR a	1	0,246	0,229	7,9
104.2	Ref ZR a	1	0,211	0,194	6,7
105.2	t0 a	4	0,136	0,119	16,5
106.2	t0 a	4	0,129	0,112	15,5
107.2	t1 a	2	0,371	0,354	24,4
108.2	t2 a	2	0,32	0,303	20,9
109.2	t3 a	2	0,371	0,354	24,4
110.2	t4 a	2	0,297	0,28	19,3
111.2	t5 a	2	0,465	0,448	30,9
112.2	t6 a	2	0,376	0,359	24,8

Appendix 4: Table of NO_x^- concentrations in the overlying water of experimental cores of the Breitling after tracer addition.

Sample	Treatment	Dilution Factor	Absorption	Absorption corrected	NO_x^- [µmol/L]
115	Ref. Konz.	1	0,024	0,0065	0,09051
116	Ref. Konz.	1	0,026	0,0085	0,15439
117	25 µM	4	0,09	0,0725	8,7942
118	25 µM	4	0,096	0,0785	9,56076
119	25 µM	4	0,136	0,1185	14,67116

120	25 µM	4	0,14	0,1225	15,1822
121	50 µM	5	0,285	0,2675	42,13425
122	50 µM	5	0,412	0,3945	62,41615
123	50 µM	5	0,364	0,3465	54,75055
124	50 µM	5	0,231	0,2135	33,51045
125	100 µM	10	0,454	0,4365	138,2471
126	100 µM	10	0,468	0,4505	142,7187
127	100 µM	10	0,481	0,4635	146,8709
128	100 µM	10	0,488	0,4705	149,1067
129	150 µM	20	0,407	0,3895	246,4706
130	150 µM	20	0,349	0,3315	209,4202
131	150 µM	20	0,371	0,3535	223,4738
132	150 µM	20	0,371	0,3535	223,4738
133	Ref. Zeit	1	0,022	0,0045	0,02663
134	Ref. Zeit	1	0,033	0,0155	0,37797
135	t0	5	0,344	0,3265	51,55655
137	t1	5	0,149	0,1315	20,41505
138	t1	5	0,121	0,1035	15,94345
139	t2	5	0,141	0,1235	19,13745
140	t2	5	0,147	0,1295	20,09565
141	t3	5	0,235	0,2175	34,14925
142	t3	5	0,177	0,1595	24,88665
143	t4	5	0,271	0,2535	39,89845
144	t4	5	0,154	0,1365	21,21355
145	t5	5	0,263	0,2455	38,62085
146	t5	5	0,482	0,4645	73,59515
147	t6	5	0,19	0,1725	26,96275
148	t6	5	0,151	0,1335	20,73445
149	t7	5	0,207	0,1895	29,67765
150	t7	5	0,211	0,1935	30,31645
151	t8	5	0,314	0,2965	46,76555
152	t8	5	0,372	0,3545	56,02815
153	t9	5	0,168	0,1505	23,44935
154	t9	5	0,406	0,3885	61,45795

Appendix 5: Table of NO_x^- concentrations in the overlying water of experimental cores of Gollwitz 1 and Gollwitz 2 after tracer addition.

Sample	Station	Treatment	Dilution Factor	Absorption	Absorption corrected	NO_x^- [µmol/L]
B 258	Gollwitz 1	Referenz	1	0,032	0,006	0,07454
B 259	Gollwitz 1	Referenz	1	0,025	-0,001	0
B 260	Gollwitz 1	25 µmol	4	0,183	0,157	19,58992
B 261	Gollwitz 1	25 µmol	4	0,094	0,068	8,21928
B 262	Gollwitz 1	25 µmol	4	0,145	0,119	14,73504
B 263	Gollwitz 1	50 µmol	10	0,15	0,124	38,4346
B 264	Gollwitz 1	50 µmol	10	0,103	0,077	23,4228
B 265	Gollwitz 1	50 µmol	10	0,102	0,076	23,1034
B 266	Gollwitz 1	100 µmol	10	0,292	0,266	83,7894

B 267	Gollwitz 1	100 µmol	10	0,149	0,123	38,1152
B 268	Gollwitz 1	100 µmol	10	0,343	0,317	100,0788
B 269	Gollwitz 1	150 µmol	20	0,163	0,137	85,1736
B 270	Gollwitz 1	150 µmol	20	0,236	0,21	131,806
B 271	Gollwitz 1	150 µmol	20	0,197	0,171	106,8928
B 272	Gollwitz 1	25 µmol Glucose	4	0,15	0,124	15,37384
B 273	Gollwitz 1	25 µmol Glucose	4	0,202	0,176	22,01736
B 274	Gollwitz 1	25 µmol Glucose	4	0,116	0,09	11,03
B 275	Gollwitz 1	50 µmol Glucose	10	0,106	0,08	24,381
B 276	Gollwitz 1	50 µmol Glucose	10	0,069	0,043	12,5632
B 277	Gollwitz 1	50 µmol Glucose	10	0,192	0,166	51,8494
B 278	Gollwitz 1	100 µmol Glucose	20	0,308	0,282	177,7996
B 279	Gollwitz 1	100 µmol Glucose	10	0,189	0,163	50,8912
B 280	Gollwitz 1	100 µmol Glucose	10	0,405	0,379	119,8816
B 281	Gollwitz 1	150 µmol Glucose	20	0,24	0,214	134,3612
B 282	Gollwitz 1	150 µmol Glucose	20	0,17	0,144	89,6452
B 283	Gollwitz 1	150 µmol Glucose	20	0,106	0,08	48,762
B 286	Gollwitz 2	Referenz	1	0,033	0,007	0,10648
B 287	Gollwitz 2	Referenz	1	0,097	0,071	2,15064
B 288	Gollwitz 2	25 µmol	4	0,095	0,069	8,34704
B 289	Gollwitz 2	25 µmol	4	0,14	0,114	14,09624
B 290	Gollwitz 2	25 µmol	4	0,21	0,184	23,03944
B 291	Gollwitz 2	50 µmol	10	0,213	0,187	58,5568
B 292	Gollwitz 2	50 µmol	10	0,194	0,168	52,4882
B 293	Gollwitz 2	50 µmol	10	0,196	0,17	53,127
B 294	Gollwitz 2	100 µmol	10	0,391	0,365	115,41
B 295	Gollwitz 2	100 µmol	10	0,19	0,164	51,2106
B 296	Gollwitz 2	100 µmol	10	0,369	0,343	108,3832
B 297	Gollwitz 2	150 µmol	20	0,262	0,236	148,4148
B 298	Gollwitz 2	150 µmol	20	0,286	0,26	163,746
B 299	Gollwitz 2	150 µmol	20	0,124	0,098	60,2604
B 300	Gollwitz 2	25 µmol Glucose	4	0,201	0,175	21,8896
B 301	Gollwitz 2	25 µmol Glucose	4	0,107	0,081	9,88016
B 302	Gollwitz 2	25 µmol Glucose	4	0,086	0,06	7,1972
B 303	Gollwitz 2	50 µmol Glucose	10	0,094	0,068	20,5482
B 304	Gollwitz 2	50 µmol Glucose	10	0,09	0,064	19,2706
B 305	Gollwitz 2	100 µmol Glucose	10	0,165	0,139	43,2256
B 306	Gollwitz 2	100 µmol Glucose	10	0,148	0,122	37,7958
B 307	Gollwitz 2	150 µmol Glucose	20	0,11	0,084	51,3172
B 308	Gollwitz 2	100 µmol	10	0,156	0,13	40,351

		Glucose				
B 309	Gollwitz 2	150 µmol Glucose	20	0,132	0,106	65,3708

Appendix 6: Table of NO_x^- concentrations in the overlying water of the offshore experimental cores after tracer addition.

Sample	Station	Treatment	Delution Factor	Absorption	Absorption corrected	NO_x^- [µmol/L]
B161	NS1	t0 (10µM)	1	0,065	0,357	11,25
B162	NS1	t0 (10µM)	1	0,709	0,7105	22,55
B163	NS1	t1 (10µM)	1	0,381	0,3655	11,52
B164	NS1	t2 (10µM)	1	0,299	0,277	8,69
B165	NS1	t3 (10µM)	1	0,245	0,2315	7,23
B166	NS1	t4 (10µM)	1	0,172	0,1555	4,80
B167	NS1	t5 (10µM)	1	0,281	0,2675	8,38
B168	NS1	t6 (10µM)	1	0,24	0,226	7,06
B169	NS1	t7 (10µM)	1	0,08	0,066	1,94
B170	NS1	t8 (10 µM)	1	0,209	0,181	5,62
B171	NS1	t9 (10µM)	1	0,243	0,2335	7,30
B172	NS1	25 µM	5	0,136	0,1235	18,89
B173	NS1	25 µM	5	0,13	0,1155	17,61
B174	NS1	25 µM	5	0,144	0,133	20,41
B175	NS1	50 µM	10	0,13	0,1145	34,90
B176	NS1	50 µM	10	0,114	0,102	30,90
B177	NS1	50 µM	10	0,153	0,1405	43,21
B178	NS1	100 µM	10	0,22	0,214	66,72
B179	NS1	100 µM	10	0,295	0,2845	89,27
B180	NS1	100 µM	10	0,278	0,265	83,03
B181	NS1	150 µM	20	0,231	0,2205	137,60
B182	NS1	150 µM	20	0,266	0,2605	163,19
B183	NS1	150 µM	20	0,244	0,2245	140,16
B184	NS6	Background	1	0,229	0,215	6,70
B185	NS6	Background	1	0,213	0,202	6,29
B186	NS6	Referenz	1	0,216	0,1965	6,11
B187	NS6	Referenz	1	0,195	0,186	5,78
B188	NS6	25 µM	5	0,174	0,159	24,57
B189	NS6	25 µM	5	0,166	0,1525	23,53
B190	NS6	25 µM	5	0,159	0,142	21,85
B191	NS6	50 µM	10	0,141	0,129	39,54
B192	NS6	50 µM	10	0,166	0,1525	47,05
B193	NS6	50 µM	10	0,126	0,1125	34,26
B194	NS6	100 µM	10	0,28	0,266	83,35
B195	NS6	100 µM	10	0,239	0,2245	70,08
B196	NS6	100 µM	10	0,236	0,2225	69,44
B197	NS6	150 µM	20	0,262	0,244	152,63
B198	NS6	150 µM	20	0,214	0,2	124,49
B199	NS6	150 µM	20	0,171	0,161	99,54
B200	NS7	Background	1	0,222	0,2105	6,56
B201	NS7	Background	1	0,236	0,222	6,93

B202	NS7	Referenz	1	0,22	0,2045	6,37
B203	NS7	Referenz	1	0,226	0,2135	6,66
B204	NS7	25 µM	5	0,179	0,1705	26,40
B205	NS7	25 µM	5	0,17	0,1545	23,85
B206	NS7	25 µM	5	0,19	0,1735	26,88
B207	NS7	50 µM	10	0,17	0,1565	48,33
B208	NS7	50 µM	10	0,176	0,1595	49,29
B209	NS7	50 µM	10	0,15	0,139	42,73
B210	NS7	100 µM	10	0,282	0,271	84,95
B211	NS7	100 µM	10	0,343	0,3315	104,30
B212	NS7	100 µM	10	0,287	0,2715	85,11
B213	NS7	150 µM	20	0,273	0,26	162,87
B214	NS7	150 µM	20	0,217	0,202	125,77
B215	NS7	150 µM	20	0,255	0,242	94,60
B216	NS7	25 µM + Glucose	5	0,169	0,155	23,93
B217	NS7	25 µM + Glucose	5	0,185	0,1745	27,04
B218	NS7	25 µM + Glucose	5	0,179	0,16	24,73
B219	NS7	50 µM + Glucose	10	0,132	0,1145	34,90
B220	NS7	50 µM + Glucose	10	0,149	0,1385	42,57
B221	NS7	50 µM + Glucose	10	0,142	0,1275	39,06
B222	NS7	100 µM + Glucose	10	0,297	0,2865	89,91
B223	NS7	100 µM + Glucose	10	0,302	0,285	89,43
B224	NS7	100 µM + Glucose	10	0,269	0,26	81,43
B225	NS7	150 µM + Glucose	20	0,231	0,218	136,00
B226	NS7	150 µM + Glucose	20	0,258	0,2485	155,51
B227	NS7	150 µM + Glucose	20	0,242	0,233	145,60
B228	Arkona	Background	1	0,223	0,21	6,54
B229	Arkona	Background	1	0,21	0,199	6,19
B230	Arkona	Referenz	1	0,221	0,2105	6,56
B231	Arkona	Referenz	1	0,216	0,2015	6,27
B232	Arkona	25 µM	5	0,127	0,115	17,53
B233	Arkona	25 µM	5	0,132	0,129	19,77
B234	Arkona	25 µM	5	0,146	0,13	19,93
B235	Arkona	50 µM	10	0,115	0,102	30,90
B236	Arkona	50 µM	10	0,148	0,137	42,09
B237	Arkona	50 µM	10	0,113	0,1015	30,74
B238	Arkona	100 µM	10	0,351	0,338	106,38
B239	Arkona	100 µM	10	0,249	0,2365	73,92
B240	Arkona	100 µM	10	0,35	0,327	102,86
B241	Arkona	150 µM	20	0,274	0,2595	162,55
B242	Arkona	150 µM	20	0,197	0,183	113,61
B243	Arkona	150 µM	20	0,254	0,2405	150,39
B244	Arkona	25 µM + Glucose	5	0,145	0,1345	20,65
B245	Arkona	25 µM + Glucose	5	0,15	0,137	21,05

B246	Arkona	25 µM + Glucose	5	0,184	0,1755	27,20
B247	Arkona	50 µM + Glucose	10	0,185	0,173	53,61
B248	Arkona	50 µM + Glucose	10	0,143	0,132	40,50
B249	Arkona	50 µM + Glucose	10	0,145	0,127	38,90
B250	Arkona	100 µM + Glucose	10	0,321	0,3095	97,27
B251	Arkona	100 µM + Glucose	10	0,324	0,3135	98,54
B252	Arkona	100 µM + Glucose	10	0,315	0,307	96,47
B253	Arkona	150 µM + Glucose	20	0,235	0,2215	138,24
B254	Arkona	150 µM + Glucose	20	0,232	0,2255	140,80
B255	Arkona	150 µM + Glucose	20	0,229	0,211	131,52