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1 **Seasonal and short-term variation in denitrification and anammox at a coastal**
2 **station on the Gulf of Finland, Baltic Sea**

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21 consumption, Baltic Sea

22
23
24 This paper has not been submitted elsewhere in similar or identical form, nor will it be
25 during the first three months after its submission to *Hydrobiologia*.

1 **Abstract**

2

3 Benthic processes were measured at a coastal deposition area in the northern Baltic
4 Sea, covering all seasons. The N₂ production rates, 90-400 μmol N m⁻² d⁻¹, were
5 highest in autumn-early winter and lowest in spring. Heterotrophic bacterial
6 production peaked unexpectedly late in the year, indicating that in addition to the
7 temperature, the availability of carbon compounds suitable for the heterotrophic
8 bacteria also plays a major role in regulating the denitrification rate. Anaerobic
9 ammonium oxidation (anammox) was measured in spring and autumn and contributed
10 10% and 15%, respectively, to the total N₂ production. The low percentage did,
11 however, result in a significant error in the total N₂ production rate estimate,
12 calculated using the isotope pairing technique. Anammox must be taken into account
13 in the Gulf of Finland in future sediment nitrogen cycling research.

14

15

1 **1 Introduction**

2

3 The Gulf of Finland is a eutrophic, highly seasonal sub-estuary of the Baltic Sea. It is

4 directly connected to the Baltic Proper at its western end and is under the influence of

5 the Neva River at the eastern end. In the easternmost part of the Gulf, primary

6 production is limited by phosphorus availability, whereas the central and western

7 parts are nitrogen-limited (Kivi et al. 1993, Pitkänen & Tamminen, 1995). Nutrient

8 loading into the Gulf has decreased in recent decades, due to the active protection of

9 the Gulf and economic changes (depression) in the surrounding states of Russia and

10 Estonia (Pitkänen et al. 2001). Still, 120 kt of nitrogen enter the Gulf of Finland every

11 year (Kiirikki et al. 2003). Denitrification, the sequential reduction of nitrate to

12 nitrogen gas, is a process that removes nitrogen from the aquatic ecosystem. Mass

13 balance calculations (Perttilä et al. 1995) as well as ecosystem models (Kiirikki et al.

14 2006) indicate that about 70 kt nitrogen is denitrified in the Gulf of Finland annually.

15 Denitrification has been extensively measured in the open Baltic Sea depositional

16 areas (Tuominen et al. 1998). However, the rates measured have been lower than

17 predicted, and based on these measurements, denitrification has been calculated to

18 remove only 45 kt of nitrogen from the Gulf of Finland annually. This estimate is

19 based solely on measurements performed in the open Baltic Sea depositional areas,

20 assuming that denitrification proceeds at the same rate throughout the entire basin.

21 The shallower coastal areas are hypothesized to be sites of more intense

22 denitrification due to differences in temperature and nitrate and carbon input. So far,

23 no data have been published concerning coastal denitrification in the Gulf of Finland,

24 except from the inner Neva estuary, where it was very low (Gran & Pitkänen, 1999).

25 Another natural process removing fixed nitrogen from the aquatic ecosystem,

1 anaerobic ammonium oxidation (anammox), has recently been found in marine
2 sediments also (Dalsgaard & Thamdrup, 2002, Thamdrup & Dalsgaard, 2002,
3 Trimmer et al. 2003). No information on the importance of this process, in which
4 ammonium is oxidized with nitrite to form nitrogen gas, is available from the Baltic
5 Sea area.

6 To estimate seasonal and short-term variation in coastal nitrogen removal and carbon
7 cycling, we measured denitrification and benthic bacterial production, oxygen
8 consumption and oxygen penetration into the sediment at a coastal station in the
9 northern Gulf of Finland. The processes were measured in May, August, October and
10 December 2003 and in April 2004. The contribution of anammox to nitrogen
11 reduction was estimated in May and August.

12

13 **2 Methods**

14

15 **2.1 Study area and sampling**

16

17 Samples were collected from a coastal station in the northern Gulf of Finland
18 (Tvärminne Storfjärden, 59°51'21, 23°15'56), representing a typical outer archipelago
19 depositional area consisting of soft mud. The water depth at the sampling station is 33
20 m, and the water column usually is thermally stratified from June to September. The
21 highest bottom water temperatures, up to 13 °C, are found in late autumn when
22 thermal stratification breaks, and the lowest, below 2 °C, in early spring when the
23 water column has yet to stabilize after ice-out. Sedimentation at the station shows a
24 typical pattern of about 80% of the sedimenting carbon reaching the bottom at the end
25 of the spring bloom in May, with little sedimentation during the rest of the year

(Heiskanen & Leppänen, 1995, Heiskanen & Tallberg, 1999). In an intensive study in 1992, the total primary sedimentation at the station from March to October was 34 g C m⁻² (Heiskanen & Tallberg, 1999), of which phytoplankton carbon contributed 8.3 g C m⁻² (Tallberg & Heiskanen, 1998). In the present study, samples were collected throughout the year in 1-2-week periods, with several sampling days in each period (Table 1). Temperature and salinity of the near-bottom water were recorded daily using a CTD probe (SIS CTD plus 100, Klausdorf/Schwentine, Germany). The sediment was sampled with a Gemini twin corer (ID of the cores 80 mm, length 80 cm). The oxygen and nitrate concentrations in the overlying water were measured from a single core, about 2 cm above the sediment surface, on every sampling day. The sediment dry weight and organic content (loss on ignition) were measured from a single core once every sampling period from the topmost 1 cm, and C% and N% were measured in the same samples in all except the April 2004 sampling period. The oxygen profiles in the sediment were measured in October, December and April in undisturbed subsample cores (see denitrification), using Clark-type oxygen microelectrodes having 100-µm tips (OX-100, Unisense A/S, Denmark), giving a spatial resolution of about 200 µm.

18

19 **2.2 Denitrification and anaerobic ammonium oxidation**

20

Denitrification was measured every sampling day, using the isotope pairing technique (IPT; Nielsen, 1992). Three replicate subsamples were taken in clear plastic (acrylic) cores (diameter 2.6 cm, height 9 cm) so that about half the core was filled with the sediment and half with the water from above. The samples were enriched with K¹⁵NO₃ (98 atom.% labelling; Cambridge Isotope Laboratories, MA, USA) to a final

1 concentration of 100 μM and incubated, with a magnetic stirrer on the lid of the cores,
2 at *in situ* temperature in darkness for 3 hours. Microbial activity was then terminated
3 by adding 1 ml of 100% ZnCl_2 , the samples were mixed, and subsamples of the
4 sediment-water slurry were transferred into gastight 12 ml glass vials (Exetainer;
5 Labco, High Wycombe, UK). These were sent to the National Environmental
6 Research Institute, Silkeborg, Denmark, for analysis of N_2 isotopic composition, using
7 a gas chromatographic column coupled to a triple-collector isotopic mass ratio
8 spectrometer (RoboPrep G^+ in line with Tracer-Mass, Europa Scientific, Crewe, UK).
9 In May and August, the validity of the IPT at this coastal site was evaluated by testing
10 a major assumption behind the method, namely the independency of the rate of
11 denitrification of the $^{15}\text{NO}_3^-$ concentration used in the incubations (Nielsen, 1992).
12 Briefly, in sediments where denitrification is the only N_2 -producing process, the
13 estimated dinitrogen ($^{28}\text{N}_2$) production rate, based on naturally occurring $^{14}\text{NO}_3^-$, is
14 independent of the incubation concentration of the added $^{15}\text{NO}_3^-$, whereas the
15 production rate of labelled compounds ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) is linearly dependent on the
16 concentration added. If anammox is present, both the $^{28}\text{N}_2$ production rate and the
17 production rate of labelled compounds correlate positively with the $^{15}\text{NO}_3^-$
18 concentration used in the experiment. If this is the case, the true rate of $^{28}\text{N}_2$
19 production must be estimated from the $^{14}\text{NO}_3^-/^{15}\text{NO}_3^-$ ratios in the reducing zone and
20 the ratios of labelled compounds produced at different $^{15}\text{NO}_3^-$ incubation
21 concentrations, using revised equations (r-IPT; Risgaard-Petersen et al., 2003, 2004b).
22 The resulting new $^{28}\text{N}_2$ production estimate is lower than the estimate calculated using
23 the classical IPT, since part of the observed $^{29}\text{N}_2$ production is attributed to anammox
24 instead of denitrification. Three sets of samples were incubated with different
25 concentrations of $^{15}\text{NO}_3^-$ (40, 100 and 400 μM) using randomized blocks design. The

1 results from these experiments were first analysed for significant differences between
2 rates in different concentrations using one-way randomized blocks ANOVA ($\alpha =$
3 0.05) and then further recalculated according to r-IPT.

5 **2.3 Benthic bacterial production**

6
7 Benthic bacterial production was measured every sampling day (except one) in the
8 topmost 1 cm of the sediment, using the leucine incorporation method modified for
9 sediment samples (Hietanen et al. 1999). In short, 100- μ l sediment samples were
10 slurried with filtered near-bottom water in microcentrifuge tubes. At the beginning of
11 every sampling period, the saturation level of the ^{14}C -leucine (3.7 MBq/ml, Perkin
12 Elmer Life Sciences, MA, USA) was measured by incubating samples with different
13 ^{14}C -leucine concentrations. The measured saturating concentration was then used in
14 all the following incubations, run in three replicates and one blank. The samples were
15 incubated at *in situ* temperature for 40-60 minutes (tested for linearity of the uptake)
16 and killed with 10% TCA. The unincorporated isotope was removed by repeated
17 centrifugation after washing once with 80% ethanol and twice with 10% TCA. The
18 samples were then suspended in a scintillation cocktail (Instagel Plus; Packard
19 Instruments, Frankfurt, Germany) and gel was formed by adding water according to
20 the manufacturer's instructions. Radioactivity was recorded in a scintillation counter
21 (WALLAC 1414 LSC; Wallac, Turku, Finland). Isotope dilution was measured once
22 every sampling period, except in May 2003, by adding increasing amounts of
23 unlabelled leucine to samples incubated with a constant amount of ^{14}C -leucine (at
24 saturation level) and using the reciprocal plot for calculation (Findlay, 1993). Leucine

incorporation was converted to carbon production according to Simon & Azam (1989), using the measured isotope dilution factor.

2.4 Diffusive oxygen consumption in sediment

The sediment oxygen consumption was calculated in August, October and December from the concentration change during incubation in plastic (acrylic) chambers similar to those used for the denitrification measurements, but equipped additionally with a sampling tube at the side, 1 cm higher than the middle of the chamber. A series of 3-6 samples were incubated in the dark, with one sample killed at the beginning of the incubation and thereafter every 45 minutes. The oxygen concentration was measured using Winkler titration. Oxygen diffusion into the chambers through the walls was measured in August. Sterile (0.2- μm filtered) seawater was bubbled with nitrogen gas until the ambient 90- μM O_2 concentration was attained. The incubation chambers were filled with the bubbled seawater, incubated in the coldroom the same way as the samples and the concentration increase in the sterile water was followed over time by Winkler titration. Diffusion of oxygen into the chambers through the walls was 50 μM $\text{O}_2 \text{ h}^{-1}$. This was taken into account in calculating the oxygen consumption rates in August. During all the other measuring periods the ambient oxygen concentration was approximately at saturation level and, according to Fick's First law, no diffusion into the chambers was expected. After incubation the sediment samples were sieved (mesh size 100 μm) to check for the presence of macrofauna. The few samples with animals, mainly *Macoma baltica* (L.), were omitted from the final calculations. Therefore the calculated rates represent diffusive oxygen consumption, not total sediment uptake.

1

2 **3 Results**

3

4 **3.1 Annual variation in hydrography and oxygen conditions**

5

6 The temperature in the near-bottom water varied from 1.1 °C to 6.5 °C between the
7 sampling periods, showing the typical annual dynamics of a coastal station. The water
8 column was completely mixed in the first sampling period in May 2003, with good
9 oxygen conditions (330 $\mu\text{M O}_2$) in the bottom water, and stratified by temperature and
10 salinity in August 2003, with a clearly lowered oxygen level (95 $\mu\text{M O}_2$). By October
11 2003, the stratification had deteriorated and the mixing had replenished the depleted
12 oxygen stores (Table 1). The average depth of oxygen penetration into the sediment
13 was 3.2 mm in October (SD 0.3), 3.6 mm in December (SD 0.7) and 2.5 mm in April
14 (SD 1.3).

15

16 **3.2 Seasonal and short-term variability in denitrification**

17

18 There was wide and statistically significant variation in denitrification activity
19 between seasons, whereas the short-term variation between the days within a
20 sampling period was not significant, except in December when a value from one day
21 was exceptionally high (ANOVA, $\alpha = 0.05$, $p < 0.01$). Denitrification was highest in
22 October-December and lowest in April and May (Figure 2B). The bulk of
23 denitrification was always coupled with nitrification (Figure 2B). Coupled
24 nitrification-denitrification (Dn) was positively correlated ($p < 0.05$) with sediment
25 oxygen consumption and bacterial carbon production, as well as with temperature and

1 nitrate concentration. Denitrification based on the nitrate available in the water
2 column (Dw) correlated positively with nitrate availability and bacterial carbon
3 production, but was not significantly correlated with temperature. Neither Dn nor Dw
4 were correlated with the O₂ concentration.

6 **3.3 Contribution of anammox to nitrogen reduction**

8 In the experiments with increasing ¹⁵NO₃⁻ concentrations, both ¹⁴NO₃⁻ reduction (²⁸N₂
9 production) and ¹⁵NO₃⁻ reduction (²⁹N₂ and ³⁰N₂ production) correlated with the
10 concentration added (May p = 0.00 and 0.00, August p = 0.01 and 0.00, respectively,
11 Figure 1). Therefore the contribution of anammox was calculated according to the r-
12 IPT. The rate of anammox was about 10 μmol N m⁻² d⁻¹, corresponding to 10% of the
13 total N₂ production rate in May, and about 30 μmol N m⁻² d⁻¹, corresponding to 14%
14 in August. The anammox caused the IPT to overestimate the N₂ production rate by
15 80% in May and 150% in August, at the routinely used 100 μM ¹⁵NO₃⁻ incubation
16 concentration (Figures 2A and 2B). Since we have no reason to believe that anammox
17 would disappear for the rest of the year, the results from the IPT (shown uncorrected
18 in Figure 2A) were also corrected for the effect of anammox outside the measured
19 seasons. We used a conservative estimate of 10% contribution to the total N₂
20 production (*ra*) as measured in May to recalculate the results from October, December
21 and April (Figure 2B), because we have no information on the seasonality of the
22 process in the Baltic Sea. However, if the increase in relative importance from May to
23 August were correlated with the overall increase in activity caused by the rising
24 temperature and proceeding of the mineralization of the sedimented spring bloom, the
25 higher *ra* levels could have lasted for the rest of the year and, consequently, the values

given here for the N_2 production could still be overestimates. On the other hand, if the percentage of anammox would for some reason decrease from October to April, these new values would be underestimates of the true N_2 production. The effect is clear when the annual N_2 production in the Gulf of Finland is calculated by multiplying the rates measured at the coastal station (which are similar to the rates measured in the open sea (Tuominen et al., 1998)) for the entire area of the Gulf of Finland (29 600 km^2). Using 10% anammox, 90% denitrification for May (measured), October, December and April and 15% anammox, 85% denitrification for August (measured) gives 39 100 t N removed per year (Figure 2B). Using the same data so that 10% anammox, 90% denitrification are used for May (measured) and 15% anammox, 85% denitrification for August (measured), and no anammox is assumed for October, December and April when it was not measured, gives 43 100 t N. If anammox is totally overlooked and only the results from classical IPT are used, denitrification removes 53 800 t N annually from the Gulf of Finland (Figure 2A).

3.4 Seasonal and short-term variability in benthic bacterial production

Benthic leucine uptake became saturated at about 4 μM in all seasons. The isotope dilution, measured in all but the May samples, varied from no dilution (factor 1) in April to the highest dilution (factor 5.2) in December. Benthic bacterial production was low in the early spring and increased towards the end of the year (Figure 1). Assuming no isotope dilution in May 2003 (none occurred in April 2004), bacterial production increased from 6.5 $mmol\ C\ m^{-2}\ d^{-1}$ in May to 84 $mmol\ C\ m^{-2}\ d^{-1}$ in December. It varied in a statistically significant manner ($\alpha = 0.05$, $p < 0.01$) daily within a season except in April 2004 (when there were only 3 sampling days) and

1 between the seasons as well. Benthic bacterial carbon production correlated with
2 denitrification (Dn and Dw), sediment oxygen consumption, temperature and nitrate
3 concentration.

5 **3.5 Sediment oxygen consumption**

7 Oxygen consumption did not vary in a statistically significant manner ($\alpha = 0.05$,
8 $p > 0.01$) daily within a season except in October ($p < 0.01$), but varied between the
9 seasons (Figure 1), showing the lowest respiration rates in low oxygen concentrations
10 in August (average $5.4 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$ at $95 \text{ } \mu\text{M O}_2$). There was no difference
11 between the rates in October and December (averages 10.1 and $11.5 \text{ mmol O}_2 \text{ m}^{-2} \text{ d}^{-1}$
12 at 310 and $380 \text{ } \mu\text{M O}_2$). Oxygen consumption correlated positively with anammox
13 and Dn (but not with Dw), benthic bacterial carbon production, temperature and
14 oxygen concentration. Unfortunately it was only measured in August, October and
15 December, leaving the low-activity season, early spring, unaccounted for.
16 In August, live animals were found in some of the subsamples almost every day. In
17 these samples the average oxygen consumption was twice the diffusive oxygen
18 consumption. Surprisingly, while the oxygen concentration increased towards the end
19 of the year (Table 1), the amount of live macrofauna decreased, so that in October
20 only 8 animals were found in the 63 subsamples and in December only 3 in 45
21 subsamples. In these rare samples with animals the oxygen consumption was
22 increased by about 40% and 75%, respectively, compared with the diffusive oxygen
23 uptake.

4 Discussion

Seasonal variation in N₂ production

Rates of N₂ production at the coastal depositional bottom studied, 90-400 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$ (exception: one day in December 550 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$), were slightly lower than the rates measured in the central Gulf of Finland (100-650 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$, Tuominen et al., 1998). The N₂ production, of which denitrification contributed 85-90%, followed an annual cycle similar to that of the open Baltic Sea areas, with low spring values and higher late summer-early winter rates. The driving forces behind the seasonal variation in denitrification in the open Baltic Sea areas are presumably organic carbon (sedimentation), temperature, nitrate concentration and oxygen availability in the sediments. Carbon sedimentation has a twofold impact, since it increases the activity of the denitrifying heterotrophic bacteria through substrate availability, but also causes a decline in the oxygen concentration. Sedimentation of carbon in the study area was much higher than in the open Gulf of Finland (study area 34 g C m^{-2} in an 8-month study (Heiskanen & Tallberg, 1999), vs. station GF2, 21 g C m^{-2} in a 6-month study (Leivuori & Vallius, 1998)). Still, denitrification was lower in the coastal area. The carbon content of the sediment was measured in May, August, October and December 2003, with values decreasing from 6.5% in the spring to 5.5% in December. At the same time, denitrification tended to increase. Therefore, the absolute carbon sedimentation and content alone could not explain the observed denitrification rates. Instead, a key to the denitrification dynamics may be the availability of carbon compounds suitable for the heterotrophic denitrifying bacteria. In the study area, most of the annual sedimenting carbon reaches the seafloor immediately after the spring bloom, in the form of diatoms (Heiskanen & Leppänen,

1 1995, Tallberg & Heiskanen, 1998). Tallberg (1999) found that 68% of the spring
2 diatoms disappeared within 1 month in 9-12 °C lake sediments. Mineralization of the
3 diatom cells at the coastal station is most likely slower and can support benthic
4 processes up to late summer. The increasing leucine isotope dilution factors suggested
5 that there were abundant mineralization products available for the bacteria in autumn,
6 indicating increased substrate supply for the heterotrophic denitrification bacteria.
7 Why denitrification peaked in October rather than in December, when the bacterial
8 carbon production did, may be related to the decreasing temperature.
9 Being microbial processes, both denitrification and anammox are temperature-
10 dependent, but apparently respond differently to changes in environmental
11 temperature. In the permanently cold (< -1°C year-round) sediments of Greenland,
12 anammox was maximal at 12 °C, whereas denitrification exhibited maximum activity
13 at 24 °C (Rysgaard et al., 2004). In the Skagerrak, where the annual temperature
14 variation is from 4 °C to 6 °C, the optimal temperature for anammox was 15 °C and
15 the activity decreased sharply above 25 °C, whereas denitrification had a wider
16 optimal range (15-32 °C) and was still detectable at 45 °C (Dalsgaard & Thamdrup,
17 2002). In the open Gulf of Finland the annual variation is limited to a few degrees
18 around 3 °C, and the denitrification rates measured showed no temperature
19 dependency (Tuominen et al. 1998). In the study area, temperatures ranged from near
20 zero in spring to 6.5 °C in autumn, and all the measured process rates (N₂ production,
21 bacterial production and oxygen consumption) correlated positively with temperature.
22 The denitrification rate doubled and anammox rate tripled from May to August, while
23 at the same time the temperature rose from 2 °C to 3 °C. Denitrification nearly
24 doubled again and anammox rate increased by 30% (since lower *ra* levels were used
25 in the calculations) from August to October, while the temperature doubled to 6 °C.

1 This suggests that although the increase in the rate in summer may have been related
2 to the small increases in temperature, other factors such as carbon and O₂
3 concentration (through nitrification) apparently co-limit the rates. In December, when
4 bacterial production and sediment oxygen consumption peaked, the N₂ production
5 rates were already decreasing, as was the temperature.

6 The availability of nitrate in the bottom water had only a minor effect on the
7 denitrification rate. While the uncoupled denitrification (Dw) was enhanced by higher
8 nitrate concentrations, the amount of total denitrification was closely connected to the
9 nitrate formed by nitrification in the sediment (Dn). Nitrification, in turn, is largely
10 regulated by O₂ availability in the sediment, and therefore the anoxic denitrification
11 process does not simply increase with decreasing O₂ concentration. In Storfjärden, the
12 percentage of Dw in total denitrification was about 5% for May, October and
13 December, and as low as 0.4% in April, but increased to 10% in August when the O₂
14 concentration was low. This indicates either that the nitrification/denitrification ratio
15 decreased or that nitrification was concentrated in a shallower layer closer to the
16 sediment surface, leading to nitrate diffusion into the water column and thereby
17 uncoupling from denitrification. Unfortunately, there were no measurements of
18 oxygen penetration in August. The surface of the sediment, however, was oxidized in
19 all sampling seasons, as demonstrated by the light brown layer covering the darker,
20 deeper, reduced layers. This oxidized layer was thinner, but still present, in August.

21 We calculated the approximate oxygen penetration depth, using the oxygen
22 consumption rate, oxygen concentration in the bottom water and oxygen diffusion
23 coefficient into the sediment in August; the depth was about 1.6-1.9 mm, which is half
24 the depth measured during good oxygen conditions in the bottom water. Similarly, the
25 Dn rates were 40-50% lower than the rates in October and December when much

higher O₂ concentrations and consumption rates were measured (Figure 2B, Table 1). A direct linear relationship between denitrification and sediment oxygen demand exists in a wide variety of estuarine, freshwater and continental shelf sediments (Seitzinger, 1987, Seitzinger & Giblin, 1996). A positive correlation between oxygen consumption and Dn (but not Dw) could also be seen in the present study in the late summer- winter period. Kemp et al. (1990) found that the nitrification rates in Chesapeake Bay were negligible at O₂ concentrations < 125 µM, because the sediment O₂ consumption exceeded O₂ diffusion into the sediment and restricted nitrification to the sediment surface. In Storfjärden, the O₂ concentration dropped to 95 µM in August, but the sediment did not become totally anoxic, because the O₂ consumption also decreased. Clearly the oxygen deficiency did not totally block nitrification at the concentrations observed, since the denitrification values were high in August. Previous exposure to low O₂ concentrations or even anoxia can also cause adaptations in nitrifying communities, so that bacteria repeatedly experiencing such conditions have higher affinity for O₂ than bacteria from permanently oxic environments (Bodelier et al. 1996). This may also be the case in the Storfjärden area.

Spatial variation in N₂ production

So far, all the published denitrification data from the Gulf of Finland have been obtained from depositional areas. Extrapolation from these to the entire Gulf resulted in an estimate of 30% removal of the annual nitrogen loading to the Gulf of Finland (Tuominen et al., 1998, this study). However, only 25-35% of the Gulf of Finland bottom can be classified as depositional areas (H. Kankaanpää, Finnish Institute of Marine Research; H. Vallius, Geological Survey of Finland, pers. comm.) and thus the present estimates for the Gulf of Finland may be biased, since no approximation of the

1 variability between different (transport or erosion) bottoms can be given. Stockenberg
2 & Johnstone (1997) measured denitrification in the Bothnian Bay in accumulation as
3 well as transportation areas, finding that the denitrification capacity of transportation
4 bottoms was only 30% of that of the accumulation basins. If this is the case in the
5 Gulf of Finland as well, the nitrogen removal via denitrification and anammox would
6 only cover 15-20% of the annual loading, further illustrating the discrepancy between
7 the measured and modelled removal rates.

8

9 **Anaerobic ammonium oxidation (anammox)**

10 In the present study, the presence of anammox was explored for the first time in the
11 Gulf of Finland. Anammox produced 10-15% of the total N₂ production at the coastal
12 station studied. The relative contribution of anammox to the overall nitrogen
13 reduction is often minor in coastal environments with high denitrification rates and
14 increases with depth, as the rate of denitrification decreases (Thamdrup & Dalsgaard,
15 2002, Risgaard-Petersen et al., 2004a, Engström et al., 2005). Accordingly, a higher
16 relative contribution in this study could have been expected in May, when
17 denitrification was low, than in August, when denitrification was maximal. However,
18 the absolute rate of anammox tripled from May to August, while the denitrification
19 rate doubled. A concomitant increase of 1 °C (from 2 °C to 3 °C) at these low
20 temperatures may have had a larger effect on anammox than on denitrification
21 (Dalsgaard & Thamdrup, 2002, Rysgaard et al., 2004), causing an increase in the
22 relative percentage of anammox from 10% to 15% of the total N₂ production. A trend
23 similar to the one observed here – increase in importance of anammox with increasing
24 overall activity – was described in the Thames Estuary (Trimmer et al., 2003) and in
25 subtropical mangrove sediments in Australia (Meyer et al., 2005). In the Thames

1 Estuary, anammox was positively correlated with the organic carbon content, whereas
2 in the subtropical mangrove sediments, nitrite and nitrate availability regulated
3 anammox activity. Organic carbon may control the autotrophic anammox
4 community in the Thames Estuary by enhancing heterotrophic denitrification, which
5 in turn produces nitrite for the anammox bacteria (Trimmer et al., 2003). At low
6 nitrate concentration (below 10 μM), not only the relative contribution but also the
7 absolute rate of anammox diminishes, probably as a consequence of competition for
8 substrate with heterotrophic nitrate and nitrite reducers (Trimmer et al., 2005). In the
9 present study the concentrations of combined nitrate and nitrite were most of the year
10 below 2 μM and never exceeded 3 μM , which supports the findings of low anammox
11 activities in the area.

12 Two methods are currently used to estimate the contribution of anammox to the total
13 N_2 production in sediments where both processes exist. The site-specific, intact core
14 method (r-IPT, Risgaard-Petersen et al. 2003, 2004b) used in this study gives the best
15 estimates of anammox and denitrification activity because it does not call for
16 destruction of the natural stratification in the sediment. It also measures the activity of
17 the entire sediment core instead of a selected layer. However, it requires minimal
18 sediment heterogeneity, because high scatter in the raw data can mask significant
19 differences in rates (Trimmer et al., 2006). The slurry incubations method (Thamdrup
20 & Dalsgaard, 2002) is based on collection of the active surface/subsurface layer of
21 sediment for anoxic incubation with different combinations of labelled and unlabelled
22 nitrogen compounds (NO_2^- , NO_3^- , NH_4^+). The method breaks the chemical
23 stratification of the sediment, and the processes are not necessarily measured in the
24 layer in which they are most active. Therefore, it is prone to giving underestimates of
25 the processes (Trimmer et al., 2006.) At the coastal station studied here, the

macrofauna was limited to a few small *Macoma baltica* mussels. These were found occasionally (not even daily) in the samples, and the results from these samples were always omitted from the calculations. Therefore the intact core method probably gave a reliable approximation of the N₂ production in the study area. The calculated contribution of 10-15% caused a dramatic 80-150% overestimate in the N₂ production when the classical IPT was used, due to the high ¹⁵NO₃⁻ incubation concentration used. This indicates a need to verify the measurements performed earlier in the Gulf of Finland using the IPT and high ¹⁵NO₃⁻ concentrations, since no data are yet available on the bias anammox may have caused in the denitrification estimates.

5 Conclusions

Coastal denitrification, measured here for the first time in the northern Baltic Sea, is of similar magnitude as denitrification in the open Baltic Sea depositional areas. It follows a clear seasonal cycle of low rates in early spring, high rates from late summer to late autumn and diminishing rates again in winter. This variation is strongly related to temperature and mineralization of the sedimented spring diatom bloom, the main carbon source to the sediment in the basin studied. Anammox was explored for the first time in the Gulf of Finland. It was measured in spring and autumn and contributed less than 10% to the total N₂ production. The low percentage did, however, cause a significant error in the total N₂ production rate estimate, calculated using the classical IPT. Therefore we recommend that anammox be taken into account in future research in Gulf of Finland sediment nitrogen cycling. Since no data on the seasonal and spatial variability of anammox rates from the Gulf of Finland are yet available, the true N₂ production rates cannot be reliably estimated. Both N

budgets and models, however, indicate that some 70 000-86 000 t N are removed from the Gulf of Finland annually (Perttilä et al., 1995, Savchuk 2005, Kiirikki et al., 2006). The N₂ production rates measured in this project release 39 100 t N annually, leaving 30 900- 46 900 t N to be removed by other processes, or by more efficient N₂ production in some areas. Since some of the highest N₂ production values have been measured from coastal and river estuarine basins (Silvennoinen et al., 2007), these seem likely places to begin looking for the "mysteriously disappearing nitrogen". The high spatial and temporal variability in the sediment processes, caused by heterogeneity in the bottom topography, flow rates, environmental conditions and interannual changes in these, leave room for speculation. Clearly, there are still large gaps in our understanding of the nitrogen dynamics of the Gulf of Finland.

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2

1

2 **Tables**

3

4 Table 1. Sampling periods, number of measurements and environmental conditions 5
 5 cm above the sediment surface within periods.

	May-03	Aug-03	Oct-03	Dec-03	Apr-04
Denitrification	3	8	8	8	3
Anammox	1	1	0	0	0
Benthic bacterial production	3	8	8	7	3
Sediment oxygen consumption	0	7	8	8	0
O ₂ profiles	0	0	9	21	3
Temperature °C	2.0	3.0	5.9	4.4	2.6
Salinity	6.35	6.83	6.62	6.46	5.82
O ₂ μM	330	95	310	380	390
NO ₂ ⁻ +NO ₃ ⁻ -N μM	0.29	1.40	1.64	2.26	0.29

6

7

1 **Figure captions**

2

3 Figure 1. N_2 production ($\mu\text{mol N m}^{-2} \text{d}^{-1}$) in May and August 2003 at different $^{15}\text{NO}_3^-$
4 incubation concentrations, calculated using the classical IPT equations (diamonds),
5 and using the r-IPT with r_{14} estimated from concentration series (triangles). Average
6 and standard deviation.

7

8 Figure 2. A) Denitrification ($\mu\text{mol N m}^{-2} \text{d}^{-1}$) measured using classical IPT; grey
9 columns Dn, white columns Dw B) total N_2 production ($\mu\text{mol N m}^{-2} \text{d}^{-1}$) calculated
10 using r-IPT; black columns anammox, grey columns Dn, white columns Dw C)
11 benthic bacterial carbon production ($\text{mmol C m}^{-2} \text{d}^{-1}$) and D) sediment oxygen
12 consumption ($\text{mmol O}_2 \text{m}^{-2} \text{d}^{-1}$). Average and standard deviation.

13