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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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*.

26

1 **Abstract**

2

3 Benthic processes were measured at a coastal deposition area in the northern Baltic

4 Sea, covering all seasons. The N_2 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 N_2 production. The low percentage did,

11 however, result in a significant error in the total N_2 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 Introduction

The Gulf of Finland is a eutrophic, highly seasonal sub-estuary of the Baltic Sea. It is directly connected to the Baltic Proper at its western end and is under the influence of the Neva River at the eastern end. In the easternmost part of the Gulf, primary production is limited by phosphorus availability, whereas the central and western parts are nitrogen-limited (Kivi et al. 1993, Pitkänen & Tamminen, 1995). Nutrient loading into the Gulf has decreased in recent decades, due to the active protection of the Gulf and economic changes (depression) in the surrounding states of Russia and Estonia (Pitkänen et al. 2001). Still, 120 kt of nitrogen enter the Gulf of Finland every year (Kiirikki et al. 2003). Denitrification, the sequential reduction of nitrate to nitrogen gas, is a process that removes nitrogen from the aquatic ecosystem. Mass balance calculations (Perttilä et al. 1995) as well as ecosystem models (Kiirikki et al. 2006) indicate that about 70 kt nitrogen is denitrified in the Gulf of Finland annually. Denitrification has been extensively measured in the open Baltic Sea depositional areas (Tuominen et al. 1998). However, the rates measured have been lower than predicted, and based on these measurements, denitrification has been calculated to remove only 45 kt of nitrogen from the Gulf of Finland annually. This estimate is based solely on measurements performed in the open Baltic Sea depositional areas, assuming that denitrification proceeds at the same rate throughout the entire basin. The shallower coastal areas are hypothesized to be sites of more intense denitrification due to differences in temperature and nitrate and carbon input. So far, no data have been published concerning coastal denitrification in the Gulf of Finland, except from the inner Neva estuary, where it was very low (Gran & Pitkänen, 1999). 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, 59º51'21, 23º15'56), 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

1 (Heiskanen & Leppänen, 1995, Heiskanen & Tallberg, 1999). In an intensive study in

2 1992, the total primary sedimentation at the station from March to October was 34 g

3 C m^{-2} (Heiskanen & Tallberg, 1999), of which phytoplankton carbon contributed 8.3 g

4 C m^{-2} (Tallberg & Heiskanen, 1998).

5 In the present study, samples were collected throughout the year in 1-2-week periods,

6 with several sampling days in each period (Table 1). Temperature and salinity of the

7 near-bottom water were recorded daily using a CTD probe (SIS CTD plus 100,

8 Klausdorf/Schwentine, Germany). The sediment was sampled with a Gemini twin

9 corer (ID of the cores 80 mm, length 80 cm). The oxygen and nitrate concentrations in

10 the overlying water were measured from a single core, about 2 cm above the sediment

11 surface, on every sampling day. The sediment dry weight and organic content (loss on

12 ignition) were measured from a single core once every sampling period from the

13 topmost 1 cm, and C% and N% were measured in the same samples in all except the

14 April 2004 sampling period. The oxygen profiles in the sediment were measured in

15 October, December and April in undisturbed subsample cores (see denitrification),

16 using Clark-type oxygen microelectrodes having 100-µm tips (OX-100, Unisense

17 A/S, Denmark), giving a spatial resolution of about 200 µm.

18

19 **2.2   Denitrification and anaerobic ammonium oxidation**
20

21 Denitrification was measured every sampling day, using the isotope pairing technique

22 (IPT; Nielsen, 1992). Three replicate subsamples were taken in clear plastic (acrylic)

23 cores (diameter 2.6 cm, height 9 cm) so that about half the core was filled with the

24 sediment and half with the water from above. The samples were enriched with

25 K^{15}NO$_3$ (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 $^{15}NO_3^-$ concentration used in the incubations (Nielsen, 1992).

12 Briefly, in sediments where denitrification is the only N_2 -producing process, the

13 estimated dinitrogen ($^{28}N_2$) production rate, based on naturally occurring $^{14}NO_3^-$, is

14 independent of the incubation concentration of the added $^{15}NO_3^-$, whereas the

15 production rate of labelled compounds ($^{29}N_2$ and $^{30}N_2$) is linearly dependent on the

16 concentration added. If anammox is present, both the $^{28}N_2$ production rate and the

17 production rate of labelled compounds correlate positively with the $^{15}NO_3^-$

18 concentration used in the experiment. If this is the case, the true rate of $^{28}N_2$

19 production must be estimated from the $^{14}NO_3^-/^{15}NO_3^-$ ratios in the reducing zone and

20 the ratios of labelled compounds produced at different $^{15}NO_3^-$ incubation

21 concentrations, using revised equations (r-IPT; Risgaard-Petersen et al., 2003, 2004b).

22 The resulting new $^{28}N_2$ production estimate is lower than the estimate calculated using

23 the classical IPT, since part of the observed $^{29}N_2$ production is attributed to anammox

24 instead of denitrification. Three sets of samples were incubated with different

25 concentrations of $^{15}NO_3^-$ (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.

4

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-µl 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

1   incorporation was converted to carbon production according to Simon & Azam

2   (1989), using the measured isotope dilution factor.

3

4   **2.4   Diffusive oxygen consumption in sediment**

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6   The sediment oxygen consumption was calculated in August, October and December

7   from the concentration change during incubation in plastic (acrylic) chambers similar

8   to those used for the denitrification measurements, but equipped additionally with a

9   sampling tube at the side, 1 cm higher than the middle of the chamber. A series of 3-6

10 samples were incubated in the dark, with one sample killed at the beginning of the

11 incubation and thereafter every 45 minutes. The oxygen concentration was measured

12 using Winkler titration. Oxygen diffusion into the chambers through the walls was

13 measured in August. Sterile (0.2-μm filtered) seawater was bubbled with nitrogen gas

14 until the ambient 90-μM O_2 concentration was attained. The incubation chambers

15 were filled with the bubbled seawater, incubated in the coldroom the same way as the

16 samples and the concentration increase in the sterile water was followed over time by

17 Winkler titration. Diffusion of oxygen into the chambers through the walls was 50 μM

18 $O_2 \, h^{-1}$. This was taken into account in calculating the oxygen consumption rates in

19 August. During all the other measuring periods the ambient oxygen concentration was

20 approximately at saturation level and, according to Fick's First law, no diffusion into

21 the chambers was expected. After incubation the sediment samples were sieved (mesh

22 size 100 μm) to check for the presence of macrofauna. The few samples with animals,

23 mainly *Macoma baltica* (L.), were omitted from the final calculations. Therefore the

24 calculated rates represent diffusive oxygen consumption, not total sediment uptake.

25

1

2 **3   Results**

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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 µM O_2) in the bottom water, and stratified by temperature and

10 salinity in August 2003, with a clearly lowered oxygen level (95 µM 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 O_2 concentration.

5

6 **3.3 Contribution of anammox to nitrogen reduction**
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8 In the experiments with increasing $^{15}NO_3^-$ concentrations, both $^{14}NO_3^-$ reduction ($^{28}N_2$

9 production) and $^{15}NO_3^-$ reduction ($^{29}N_2$ and $^{30}N_2$ 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 N_2 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 N_2 production rate by

15 80% in May and 150% in August, at the routinely used 100 μM $^{15}NO_3^-$ 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 N_2

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

1   given here for the N_2 production could still be overestimates. On the other hand, if the

2   percentage of anammox would for some reason decrease from October to April, these

3   new values would be underestimates of the true N_2 production. The effect is clear

4   when the annual N_2 production in the Gulf of Finland is calculated by multiplying the

5   rates measured at the coastal station (which are similar to the rates measured in the

6   open sea (Tuominen et al., 1998)) for the entire area of the Gulf of Finland (29 600

7   km^2). Using 10% anammox, 90% denitrification for May (measured), October,

8   December and April and 15% anammox, 85% denitrification for August (measured)

9   gives 39 100 t N removed per year (Figure 2B). Using the same data so that 10%

10   anammox, 90% denitrification are used for May (measured) and 15% anammox, 85%

11   denitrification for August (measured), and no anammox is assumed for October,

12   December and April when it was not measured, gives 43 100 t N. If anammox is

13   totally overlooked and only the results from classical IPT are used, denitrification

14   removes 53 800 t N annually from the Gulf of Finland (Figure 2A).

15

16   **3.4   Seasonal and short-term variability in benthic bacterial production**
17

18   Benthic leucine uptake became saturated at about 4 µM in all seasons. The isotope

19   dilution, measured in all but the May samples, varied from no dilution (factor 1) in

20   April to the highest dilution (factor 5.2) in December. Benthic bacterial production

21   was low in the early spring and increased towards the end of the year (Figure 1).

22   Assuming no isotope dilution in May 2003 (none occurred in April 2004), bacterial

23   production increased from 6.5 mmol C m^{-2} d^{-1} in May to 84 mmol C m^{-2} d^{-1} in

24   December. It varied in a statistically significant manner ($\alpha = 0.05$, $p < 0.01$) daily

25   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.

4

3.5   Sediment oxygen consumption

5

6

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 mmol O_2 m^{-2} d^{-1} at 95 μM O_2). There was no difference

11 between the rates in October and December (averages 10.1 and 11.5 mmol O_2 m^{-2} d^{-1}

12 at 310 and 380 μM 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.

24

1   **4   Discussion**

2

3   **Seasonal variation in N$_2$ production**

4   Rates of N$_2$ production at the coastal depositional bottom studied, 90-400 μmol N m^{-2}

5   d^{-1} (exception: one day in December 550 μmol N m^{-2} d^{-1}), were slightly lower than the

6   rates measured in the central Gulf of Finland (100-650 μmol N m^{-2} d^{-1}, Tuominen et

7   al., 1998). The N$_2$ production, of which denitrification contributed 85-90%, followed

8   an annual cycle similar to that of the open Baltic Sea areas, with low spring values

9   and higher late summer-early winter rates. The driving forces behind the seasonal

10   variation in denitrification in the open Baltic Sea areas are presumably organic carbon

11   (sedimentation), temperature, nitrate concentration and oxygen availability in the

12   sediments. Carbon sedimentation has a twofold impact, since it increases the activity

13   of the denitrifying heterotrophic bacteria through substrate availability, but also

14   causes a decline in the oxygen concentration. Sedimentation of carbon in the study

15   area was much higher than in the open Gulf of Finland (study area 34 g C m^{-2} in an 8-

16   month study (Heiskanen & Tallberg, 1999), vs. station GF2, 21 g C m^{-2} in a 6-month

17   study (Leivuori & Vallius, 1998)). Still, denitrification was lower in the coastal area.

18   The carbon content of the sediment was measured in May, August, October and

19   December 2003, with values decreasing from 6.5% in the spring to 5.5% in

20   December. At the same time, denitrification tended to increase. Therefore, the

21   absolute carbon sedimentation and content alone could not explain the observed

22   denitrification rates. Instead, a key to the denitrification dynamics may be the

23   availability of carbon compounds suitable for the heterotrophic denitrifying bacteria.

24   In the study area, most of the annual sedimenting carbon reaches the seafloor

25   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 (N_2 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 O_2

3   concentration (through nitrification) apparently co-limit the rates. In December, when

4   bacterial production and sediment oxygen consumption peaked, the N_2 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 O_2 availability in the sediment, and therefore the anoxic denitrification

11   process does not simply increase with decreasing O_2 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 O_2

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

1 higher O_2 concentrations and consumption rates were measured (Figure 2B, Table 1).

2 A direct linear relationship between denitrification and sediment oxygen demand

3 exists in a wide variety of estuarine, freshwater and continental shelf sediments

4 (Seitzinger, 1987, Seitzinger & Giblin, 1996). A positive correlation between oxygen

5 consumption and Dn (but not Dw) could also be seen in the present study in the late

6 summer- winter period. Kemp et al. (1990) found that the nitrification rates in

7 Chesapeake Bay were negligible at O_2 concentrations < 125 μM, because the

8 sediment O_2 consumption exceeded O_2 diffusion into the sediment and restricted

9 nitrification to the sediment surface. In Storfjärden, the O_2 concentration dropped to

10 95 μM in August, but the sediment did not become totally anoxic, because the O_2

11 consumption also decreased. Clearly the oxygen deficiency did not totally block

12 nitrification at the concentrations observed, since the denitrification values were high

13 in August. Previous exposure to low O_2 concentrations or even anoxia can also cause

14 adaptations in nitrifying communities, so that bacteria repeatedly experiencing such

15 conditions have higher affinity for O_2 than bacteria from permanently oxic

16 environments (Bodelier et al. 1996). This may also be the case in the Storfjärden area.

17

18 **Spatial variation in N_2 production**

19 So far, all the published denitrification data from the Gulf of Finland have been

20 obtained from depositional areas. Extrapolation from these to the entire Gulf resulted

21 in an estimate of 30% removal of the annual nitrogen loading to the Gulf of Finland

22 (Tuominen et al., 1998, this study). However, only 25-35% of the Gulf of Finland

23 bottom can be classified as depositional areas (H. Kankaanpää, Finnish Institute of

24 Marine Research; H. Vallius, Geological Survey of Finland, pers. comm.) and thus the

25 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 N_2 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 N_2 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 was 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

1   macrofauna was limited to a few small *Macoma baltica* mussels. These were found

2   occasionally (not even daily) in the samples, and the results from these samples were

3   always omitted from the calculations. Therefore the intact core method probably gave

4   a reliable approximation of the N_2 production in the study area. The calculated

5   contribution of 10-15% caused a dramatic 80-150% overestimate in the N_2 production

6   when the classical IPT was used, due to the high $^{15}NO_3^-$ incubation concentration

7   used. This indicates a need to verify the measurements performed earlier in the Gulf

8   of Finland using the IPT and high $^{15}NO_3^-$ concentrations, since no data are yet

9   available on the bias anammox may have caused in the denitrification estimates.

10

11 **5   Conclusions**

12
13   Coastal denitrification, measured here for the first time in the northern Baltic Sea, is

14   of similar magnitude as denitrification in the open Baltic Sea depositonal areas. It

15   follows a clear seasonal cycle of low rates in early spring, high rates from late

16   summer to late autumn and diminishing rates again in winter. This variation is

17   strongly related to temperature and mineralization of the sedimented spring diatom

18   bloom, the main carbon source to the sediment in the basin studied. Anammox was

19   explored for the first time in the Gulf of Finland. It was measured in spring and

20   autumn and contributed less than 10% to the total N_2 production. The low percentage

21   did, however, cause a significant error in the total N_2 production rate estimate,

22   calculated using the classical IPT. Therefore we recommend that anammox be taken

23   into account in future research in Gulf of Finland sediment nitrogen cycling. Since no

24   data on the seasonal and spatial variability of anammox rates from the Gulf of Finland

25   are yet available, the true N_2 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 N_2 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 N_2

production in some areas. Since some of the highest N_2 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.

Acknowledgements

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Tables

2

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 (μmol N m^{-2} d^{-1}) in May and August 2003 at different $^{15}NO_3^-$

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 (μmol N m^{-2} d^{-1}) measured using classical IPT; grey

9 columns Dn, white columns Dw B) total N_2 production (μmol N m^{-2} d^{-1}) calculated

10 using r-IPT; black columns anammox, grey columns Dn, white columns Dw C)

11 benthic bacterial carbon production (mmol C m^{-2} d^{-1}) and D) sediment oxygen

12 consumption (mmol O_2 m^{-2} d^{-1}). Average and standard deviation.

13