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Denitrification in the river estuaries of the northern Baltic Sea

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Dr. Anu Liikanen¹ (PhD in environmental sciences) (anu.liikanen@uku.fi, tel. +358 40 7238551) is a post doctoral researcher at the Department of Environmental Sciences, University of Kuopio, Finland. She has expertise in studying biogeochemical cycles of elements carbon, nitrogen and phosphorus in aquatic sediments and wetlands. Since her dissertation on "Greenhouse gas and nutrient dynamics in lake sediment and water column in changing environment" she has

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4 Prof. C. Florian Stange³ (florian.stange@ufz.de, tel. +49 345-558-5418) is
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8 has worked for nearly 10 years on modelling of soil processes. He started with
9 modelling of herbicide transport in macroporous soils and since his PhD work at the
10 Fraunhofer Institute for Atmospheric Environmental Research he is involved in
11 investigation of production, consumption and transport of trace gases in soils. Topics of
12 his current research: separation of trace gas production during heterotrophic and
13 autotrophic nitrification, studies regarding the emission ratio of N₂O/N₂ influenced by
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15
16 Dr. Rolf Russow³ (PhD (Dr.rer.nat.) in physical chemistry) (isotopes@russow-lpz.de,
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21 Topics of his research: kinetic and heterogeneous catalysis in organic chemistry,
22 biological nitrogen fixation, N transformation processes in biological systems, trace
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1 Prof. Jorma Kuparinen² (jorma.kuparinen@helsinki.fi, tel. +358 57820) has a
2 professorship at the University of Helsinki in marine biology. His research experience
3 extends over the last 27 years. His main interest has been on microbial ecology with
4 specific focus on the carbon cycle. His field studies have often been supplemented
5 with experimental studies in microcosm including manipulations with environmental
6 factors such as nitrogen, phosphorus and carbon. Rate measurements, based on
7 isotope incorporation techniques (³H, ¹⁴C, ³²P and ¹⁵N) as well as biomass estimated
8 by various microscopic techniques have been applied and developed in the studies.

9

10 Prof. Pertti J. Martikainen¹ (Pertti.Martikainen@uku.fi, tel. +358 17163586) has a
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15 carbon and methane dynamics of tundra. He has 180 articles in international refereed
16 journals on microbial ecology and biogeochemistry of greenhouse gases.

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Abstract

Estuaries have been suggested to have an important role in reducing the nitrogen load transported to the sea. We measured denitrification rates in six estuaries of the northern Baltic Sea. Four of them were river mouths in the Bothnian Bay (northern Gulf of Bothnia), and two were estuary bays, one in the Archipelago Sea (southern Gulf of Bothnia) and the other in the Gulf of Finland. Denitrification rates in the four river mouths varied between 330 and 905 $\mu\text{mol N m}^{-2} \text{d}^{-1}$. The estuary bays at the Archipelago Sea and the Gulf of Bothnia had denitrification rates from 90 to 910 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ and from 230 -320 $\mu\text{mol N m}^{-2} \text{d}^{-1}$, respectively. Denitrification removed 3.6 - 9.0 % of the total nitrogen loading in the river mouths and in the estuary bay in the Gulf of Finland, where the residence times were short. In the estuary bay with a long residence time, in the Archipelago Sea, up to 4.5% of nitrate loading and 19% of nitrogen loading were removed before entering the Sea. According to our results, the sediments of the fast flowing rivers and the estuary areas with short residence times have a limited capacity to reduce the nitrogen load to the Baltic Sea.

1 Introduction

The Baltic Sea is a large brackish water basin suffering from eutrophication. The drainage area of the sea is four times larger than the sea itself, and populated by 85 million people. The Gulf of Bothnia is the northernmost basin of the Baltic Sea. Shallow sills at the Archipelago Sea prevent the inflow of the more saline water. This isolation from the rest of the Baltic Sea, together with the large freshwater inflow, makes the area less saline than the northern Baltic Sea in general. Primary production in the Gulf of Bothnia is phosphorus limited and, unlike the rest of the Baltic Sea, The

1 Gulf is in an ecologically good state. Annual nitrogen (N) loading to the Gulf was 74
2 100 tons in 2000 [1]. Since the 1990's, increasing nitrate concentrations have been
3 measured especially in Finnish coastal waters rivers entering the Gulf of Bothnia [2].
4

5 The Gulf of Finland is an estuary-like area, directly connected to the Baltic Proper at
6 its western end, and under the influence of the river Neva at the eastern end. In
7 contrast to the Gulf of Bothnia, it is heavily eutrophied. Nutrient loading into the Gulf
8 of Finland has been decreasing in the last decades, due to the active protection of the
9 Gulf of Finland and economic depression in the surrounding states Russia and Estonia
10 [3]. However, 120 000 tons of N still enter the Gulf of Finland every year [4].
11

12 The fate of N entering the aquatic ecosystem depends on the prevailing conditions.
13 Organic N compounds are decomposed by microbes to ammonium (NH_4^+), which can
14 be taken up by primary producers, or nitrified by bacteria to nitrate (NO_3^-). NO_3^- can
15 be taken up or processed further in denitrification, anaerobic ammonium oxidation
16 (anammox) or dissimilatory NO_3^- reduction to NH_4^+ (DNRA). Of these processes,
17 DNRA reduces NO_3^- back to NH_4^+ , still available to other organisms. N taken up by
18 primary producers is bound to the biomass and later released back to the water
19 ecosystem. Denitrification (the sequential reduction of NO_3^- to nitrogen gas (N_2)) and
20 anammox (oxidizing of NH_4^+ with nitrite (NO_2^-) to N_2) are the only processes that
21 remove N permanently from the system. Denitrification, but not anammox, may have
22 an effect on global warming via the gaseous intermediate, nitrous oxide (N_2O). N_2 , the
23 end product of denitrification, is abundant (78 %) in the atmosphere, whereas the
24 intermediate N_2O is an effective greenhouse gas. The ratio of these gases produced by
25 denitrification in aquatic ecosystems is affected by several environmental factors, e.g.

1 temperature, oxygen concentration and NO_3^- availability [5,6,7]. In sediments, usually
2 less than 5% of the gases produced in denitrification is N_2O [8]. Nevertheless, it has
3 been suggested that in areas with high NO_3^- load, such as river mouths and estuaries,
4 production of N_2O may be enhanced [8].

5

6 Denitrification has been estimated to remove 30 % of the annual N loading in the Gulf
7 of Finland [9], and 23 % in the Bothnian Bay [10]. Both of these estimates are based
8 on results from the depositional areas of the open sea. Estuaries have been suggested
9 to be effective sinks of N loading, reducing the load transported to the sea [8,11,12].

10 The few estimates of the filtering capacity published so far from the northern Baltic
11 Sea seem to challenge this view (Hietanen S. and Kuparinen J. Seasonal and short-
12 term variation in denitrification and anammox at a coastal station on the Gulf of
13 Finland, Baltic Sea. Submitted) [13,14]. More data is needed to understand the
14 capacity of these ecosystems to reduce the N load entering the sea.

15

16 We measured denitrification rates in six estuaries of the northern Baltic Sea. Four of
17 them were river mouths in the Bothnian Bay (northern Gulf of Bothnia), and two were
18 estuary bays, one in the Archipelago Sea (southern Gulf of Bothnia) and the other in
19 the Gulf of Finland (Figure 1). Two projects, both part of the Baltic Sea Research
20 Programme (BIREME) of the Academy of Finland (2003-2006) contributed to this
21 work. Two ^{15}N methods were applied in the studies. The CoastGas project (University
22 of Kuopio, Finland and UFZ Leipzig-Halle, Germany) measured NO_3^- removal in the
23 river mouths using a flow-through method, and the SEGUE-N project (University of
24 Helsinki, Finland) measured denitrification in the two estuary bays using the isotope

pairing technique [15]. The rates measured were compared to the local N loading, in order to estimate the filtering capacity of the estuaries.

2 Materials and Methods

Area descriptions

In the Bothnian Bay, the study sites were at river mouths of Temmesjoki, Siikajoki, Pyhäjoki and Kalajoki (Figure 1A). The catchments of these rivers contain mainly peatlands and forests, and water systems contain only few lakes. Therefore, the river water is rich in organic matter. The N load to the rivers is at its highest in April - May, diminishes rapidly towards summer months and peaks again slightly in late autumn.

In the Archipelago Sea the study site was at the Paimionlahti Bay, an estuary of the river Paimionjoki (Figure 1A). The discharges of both freshwater and N compounds to the bay are remarkably pulsed, with half of the N loading reaching the estuary in April - May, and most of the other half late in the autumn. The flow diminishes substantially for the summer months. In the Gulf of Finland, the study site was at the Ahvenkoskenlahti Bay, which is a semi-enclosed estuary receiving loading from the rivers Kymijoki and Taasianjoki (Figure 1A). The discharge, as well as the N loading, fluctuate only modestly from season to season, and are usually highest in April - May.

In contrast to the rivers in the Bothnian Bay, the catchments of rivers Kymijoki and Taasianjoki have low coverage of peatlands, but are significantly affected by agriculture. The Paimionlahti Bay has a catchment with low lake content, while the catchment of the Ahvenkoskenlahti Bay contains several lakes.

Denitrification measurements in the laboratory experiments

(the rivers Temmesjoki, Siikajoki, Pyhäjoki and Kalajoki)

1 Intact sediment cores were taken from three sampling sites in the river Temmesjoki
2 and four sites in the rivers Siikajoki, Pyhäjoki and Kalajoki in August 2004. The
3 sediment cores were incubated in a laboratory microcosm for three weeks. Samples
4 for the determination of denitrification were collected six times from each core during
5 the last two weeks.

6

7 Sediments were taken directly to transparent acrylic cores (ø 94 mm, height 650 mm).
8 The height of the intact sediments in the cores was ≤ 200 mm. The sediment cores
9 were placed into laboratory microcosm equipped with continuous water flow [16].
10 The microcosm was situated in a dark, temperature controlled room at 15 °C, similar
11 to the *in situ* temperatures in river waters during sampling (13-20 °C, Table 1). Water
12 was pumped from an 80-liter water reservoir over the cores by a peristaltic pump
13 (Ismatec, BVK-MS/CA8-6, Glattbrugg-Zürich, Switzerland) at the rate of 50 ml h⁻¹.
14 Overlying water in the cores was gently stirred with a rotating magnet to prevent
15 stratification [16].

16

17 The water reservoir was vacuumed three times to remove N₂ from the gas phase of the
18 water and flushed each time with gas mixture consisting of Ar/O₂ 80/20 (v/v) (AGA,
19 Finland) to improve the sensitivity of concentration analyses of N₂ formed in
20 denitrification. Distilled water, amended with in situ concentrations of sulphate (0.2
21 mM) and chloride (0.5 mM) (added as MgSO₄ and CaCl₂), was used as inflow water.
22 The sediments were incubated at 30 μM of ¹⁵NO₃⁻ (60 at %). The fluxes of NO₃⁻ and
23 gases were measured from the difference between concentrations in the in- and
24 outflowing water. During the experiment the NO₃⁻ concentrations and the isotopic
25 composition (¹⁵N/¹⁴N) of the NO₃ of the in- and outflowing water were determined

1 three times in every week (4th, 5th and 6th incubation days). Samples were stored at -20
2 °C prior to analyses. NO₃⁻ and SO₄²⁻ concentrations were measured with ion
3 chromatography (Dionex DX-130, Sunnyvale, CA, with an anion column A59-HC, 12
4 mM Na₂CO₃ as an eluent). The N isotopic composition (at ‰) of the NO₃⁻ was
5 determined with R/CF-QMS (Reaction Continuous Flow Quadrupole Mass
6 Spectrometer) [17].

7

8 The fluxes of dissolved inorganic carbon (DIC) and dissolved gases CH₄, N₂O and N₂
9 were determined from the concentration differences between the in- and outflowing
10 water and by taking into account the flow rates and sediment surface area (69 cm²).
11 The water samples for DIC, CH₄ and N₂O were balanced for 1 day with an Ar
12 headspace, and then analyzed with a GC (Agilent 6890N, Agilent Technologies
13 Deutschland GmbH, Waldbronn, Germany) equipped with a peristaltic pump
14 (Minipuls 3, Gilson Inc., Middleton, WI, USA) and an autosampler (Gilson
15 autosampler 222XL, Gilson Inc., Middleton, WI, USA). The gas concentrations in the
16 original sample were calculated according to Henry's law (modified from McAuliffe
17 et al. 1971 [18]). The N₂ samples were stored in vacuumed 12 ml exetainers (Labco,
18 Co., UK) in concentrated salt solution (NaCl). Concentration and isotopic
19 composition (¹⁵N/¹⁴N) of N₂ were measured by a specifically configured Gas
20 Chromatography Quadrupole Mass Spectrometer coupling (GC-QMS) (QP 2000,
21 Shimadzu Corp., [19]) The masses 28, 29 and 30 were measured and the peaks were
22 calibrated against normal air (78 % N₂) for concentration measurements. The
23 contamination of samples by N₂ in the laboratory atmosphere was prevented by
24 flushing the injection system and the sample loop of the GC with He flow before

1 injection of the sample. The amount of N_2 derived from denitrification was calculated
2 according to non-random distribution of the masses 28, 29 and 30 [20,21,22].

3
4 At the end of each incubation week the oxygen concentrations of overlying water
5 (1cm above the sediment surface) were measured with an oxygen electrode
6 (Dissolved Oxygen Meter Oxi 330 with Dissolved oxygen Probe Cellox 325, WTW,
7 Weilheim, Germany), and pH (0.5 cm below the sediment surface) was measured with
8 an electrode (Microprocessor pH meter pH 320, WTW, Germany, with Hamilton pH
9 electrode, Bonaduz, Switzerland).

10 11 *Field measurements (Ahvenkoskenlahti Bay and Paimionlahti Bay)*

12 Sediment was sampled either with a single or twin gravity corer, both having an inner
13 diameter of 80 mm. Oxygen and NO_3^- concentrations in the overlying water were
14 measured about 2 cm above the sediment surface. Denitrification was measured using
15 the isotope pairing technique [15]. Three replicate sub-samples were taken in clear
16 plastic (acrylic) cores ($\varnothing$ 2.6 cm, height 9 cm) so that about half of the core was filled
17 with the sediment and half with the overlying water. Samples were enriched with
18 $K^{15}NO_3$ (98% labelling, Cambridge Isotope Laboratories, MA, USA) to a final
19 concentration of 100 μM of $^{15}NO_3^-$ in the water phase and incubated, with a magnetic
20 stirrer on the lid of the cores, at *in situ* temperature in darkness for 3 hours. The
21 biological activity was terminated with $ZnCl_2$, and the samples were mixed. Sub-
22 samples of sediment-water slurry were sent in gas-tight 12 ml vials (Exetainer, Labco,
23 UK) to the National Environmental Research Institute, Silkeborg, Denmark, for the
24 analysis of the N_2 isotopic composition.

1 *Data processing and statistical analyses*

2 In the laboratory experiments the total denitrification (D_{tot}) was calculated as a sum
3 of measured N_2O and N_2 . Calculation of D_{n} (denitrification from coupled
4 nitrification-denitrification) based on the differences in the $^{15}\text{NO}_3^-$ content in the
5 output water (i.e. overlying water) and in the output N_2 (and N_2O). Denitrification
6 based on the NO_3^- in the overlying water (D_{w}) was calculated as the remaining part of
7 the total denitrification.

8

9 In the field measurements, the share of D_{w} was calculated from the ratio of $^{14}\text{NO}_3^-$
10 and $^{15}\text{NO}_3^-$ concentrations at the nitrate reducing zone and the D_{tot} [15]. D_{n} was then
11 calculated as the difference between D_{tot} and D_{w} .

12

13 Statistical analyses were done with SPSS statistical package (SPSS Inc. USA). The
14 normal distribution of the variables was tested with the Kolmogorov-Smirnov Test
15 and correlations of N_2 effluxes to environmental variables with Spearman correlation
16 coefficients. The statistical significance of differences in denitrification and $\text{N}_2\text{O}/\text{N}_2$
17 between rivers were tested with Kruskal-Wallis' post hoc -test, suitable for non-
18 parametric data.

19

20 **3 Results**

21 *Denitrification rates in the rivers in the Bothnian Bay*

22 The total denitrification rates in the four river mouths varied between 330 and 905
23 $\mu\text{mol N m}^{-2} \text{d}^{-1}$ (Figure 1A). Denitrification was mainly based on the nitrate diffusing
24 from the overlying water into the sediments (D_{w}), with only a minor share based on
25 the coupled nitrification-denitrification (D_{n}) (Figure 1A). The variation in

1 denitrification rates within a site was high (e.g. the river Kalajoki, $906 \pm 590 \mu\text{mol N}$
2 $\text{m}^{-2}\text{d}^{-1}$, distance between replicate samples 500 - 1000 meters), and the denitrification
3 rates between the rivers did not differ statistically significantly. The denitrification
4 rate correlated positively (0.876 , $p=0.05$) with the CH_4 efflux, which varied from 0.02
5 to $2.3 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Table 2), and with oxygen consumption (0.550 , $p=0.01$), which
6 varied from 19 to $27 \text{ mmol m}^{-2} \text{ d}^{-1}$ (Table 2). It did not correlate with pH or with the
7 fluxes of CO_2 or NH_4^+ (Table 2). The N_2O effluxes varied from 11 to $16 \mu\text{mol N m}^{-2}\text{d}^{-1}$
8 (Table 2). The percentage of N_2O in the gaseous end products of denitrification did
9 not exceed 5% . Differences in the N_2O effluxes between the rivers were not
10 statistically significant.

11

12 *Denitrification in the Bays in the Archipelago Sea and the Gulf of Finland*

13 In Paimionlahti Bay, the denitrification rate varied tenfold within the estuary, from 90
14 $\mu\text{mol N m}^{-2} \text{ d}^{-1}$ in one station in the middle, to $910 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ in the outer end of
15 the estuary. The bulk of denitrification was coupled to nitrification in the whole
16 estuary, and the proportion of denitrification that was dependent on the NO_3^- in the
17 water column (Dw) was at its highest in the middle estuary (Figure 1B), where the
18 NO_3^- concentration was highest. Dw was positively correlated with the NO_3^-
19 concentration (0.917 , $p=0.00$) and negatively with the oxygen concentration (-0.871 ,
20 $p=0.000$). The rates of coupled nitrification-denitrification (Dn) and total
21 denitrification did not correlate with any of the environmental factors measured.

22

23 The denitrification rate in the Ahvenkoskenlahti Bay varied from 230 to $320 \mu\text{mol N}$
24 $\text{m}^{-2} \text{ d}^{-1}$. No clear gradient in the total denitrification could be seen within the basin, but
25 Dn increased from about 50% at the innermost station to about 80% towards the outer

end of the basin (Figure 1C). Outside the basin, however, denitrification was lower than in the basin itself, and the share of Dn was lower, reflecting the changes in the sediment quality (higher water content and lower concentration of total carbon, nitrogen and sulphur per sediment volume, data not shown).

The denitrification rates in the estuaries of the Gulf of Finland and Archipelago Sea were in the same range in the rivers (Figure 1). The possible statistical differences were not tested due to the differences in the methodology.

4 Discussion

Denitrification rates

Denitrification rates reported from the rivers range up to 18 000 $\mu\text{mol N m}^{-2}\text{d}^{-1}$ [23]. The studies made in northern latitudes are few, but Garcia-Ruiz et al. [24] reported rates of 0- 13 800 $\mu\text{mol N m}^{-2}\text{d}^{-1}$, from the Swale-Ouse river system in UK. In this study, the denitrification rates in the rivers were lower (330-910 $\mu\text{mol N m}^{-2}\text{d}^{-1}$) than those measured from other river ecosystems, but higher than the values measured in the open sea area of the Bothnian Bay (120-160 $\mu\text{mol N m}^{-2}\text{d}^{-1}$) [10]. They fall into same range with the values measured from the open Gulf of Bothnia (0-940 $\mu\text{mol N m}^{-2}\text{d}^{-1}$) [10]. In those studies, denitrification was measured using the acetylene blockage method, now known to have some serious flaws (inhibition of nitrification [25], reversal of blockage by sulphide [26], incomplete blockage by acetylene [27], scavenging of intermediate NO [28]). Therefore, the earlier denitrification estimates from the open Gulf of Bothnia [10] may be too low. If that is the case, the rates measured in the river estuaries are, in fact, lower than those measured in the open sea. In the Bothnian Sea, denitrification rate, measured using the isotope pairing technique

1 [15] varied between 250 and 300 $\mu\text{mol N m}^{-2}\text{d}^{-1}$ [9], which is lower than the rates
2 measured in the river sediments in this study, and also lower than the rates measured
3 in the Paimionlahti Bay (90-910 $\mu\text{mol N m}^{-2}\text{d}^{-1}$, average 460 $\mu\text{mol N m}^{-2}\text{d}^{-1}$), southern
4 Gulf of Bothnia.

5

6 In the Gulf of Finland, denitrification measurements have been done using the isotope
7 pairing technique since the mid-90's. Gran and Pitkänen [14] found a gradient in the
8 denitrification rates from the eastern Gulf of Finland, inner Neva estuary, towards the
9 open Gulf. The denitrification rates were lowest ($<10 \mu\text{mol N m}^{-2}\text{d}^{-1}$) in the inner
10 Neva estuary, higher in the outer estuary and the highest, up to 1260 $\mu\text{mol N m}^{-2}\text{d}^{-1}$, in
11 the open Gulf of Finland. Tuominen et al. [9] measured the highest denitrification
12 rates of 150-650 $\mu\text{mol N m}^{-2}\text{d}^{-1}$ from open sea area of the Gulf of Finland, with lower
13 rates in the eastern and western ends of the Gulf (100-400 $\mu\text{mol N m}^{-2}\text{d}^{-1}$). Thus, the
14 denitrification rates in the Ahvenkoskenlahti Bay (Gulf of Finland) (230-320 $\mu\text{mol N}$
15 $\text{m}^{-2}\text{d}^{-1}$, average 280 $\mu\text{mol N m}^{-2}\text{d}^{-1}$) are in the lower end reported for the Gulf.
16 However, there can be some overestimation in the rates reported for the open Gulf of
17 Finland, resulting from the potential effect of anammox in the estimates, whereas no
18 anammox was detected in the Ahvenkoskenlahti Bay. The denitrification rates in the
19 Ahvenkoskenlahti Bay were much higher than the rates outside the bay, towards the
20 open Gulf of Finland (Figure 1C). The stations at the estuary were located on
21 accumulation bottoms, with high carbon and nitrogen content per sediment volume,
22 whereas the stations on the transportation /transient accumulation bottoms outside the
23 estuary had very low dry matter and low carbon and nitrogen content per sediment
24 volume. In the Paimionlahti Bay, the inner estuary rates were slightly (but
25 significantly, $p=0.01$) lower than those immediately outside the estuary (Figure 1B).

1 As the highly variable denitrification rate ($260\text{--}620 \mu\text{mol N m}^{-2}\text{d}^{-1}$, with a single
2 station showing a rate of $90 \mu\text{mol N m}^{-2}\text{d}^{-1}$) did not correlate with any of the
3 environmental factors measured (depth, temperature, salinity, oxygen and
4 nitrite/nitrate concentration, total carbon, nitrogen and loss on ignition in the
5 sediment) likewise showing high variation, it is unclear why the rates outside the
6 estuary basin were higher ($720\text{--}910 \mu\text{mol N m}^{-2}\text{d}^{-1}$).

7

8 Although the total denitrification rates fall into the same range in the laboratory
9 experiments and in the field measurements, the ratio of D_w/D_n was remarkably
10 different. In the laboratory experiments, the share of D_n was always less than 10%,
11 whereas in the field measurements the share was 50–85% in Ahvenkoskenlahti and
12 65–95% in Paimionlahti. Similar results – small share of D_n - have been reported
13 earlier from flow-through systems as well [29,23]. In the laboratory experiments, the
14 sediment surface is continuously provided with NO_3^- , mimicking the natural
15 conditions in river ecosystems. D_w correlates with the NO_3^- concentration, and the
16 high NO_3^- concentrations in river waters (Table 1) are likely to sustain high D_w in the
17 river sediments. The NO_3^- concentrations were much higher in the rivers than in the
18 two estuaries (Table 1), and probably explain the lower share of D_n in the river
19 sediments. Low share of D_n has been reported from river and estuary systems using
20 the isotope pairing technique, too. [e.g. 30,31]. In river sediments NO_3^- is easily
21 available in the denitrification zone due to more efficient penetration and thus the
22 importance of nitrification as NO_3^- source is diminished. Another factor affecting the
23 share of D_n is the oxygen concentration in the overlying water of the sediments. Low
24 oxygen concentration lowers the oxygen penetration depth, thereby enhancing D_w by
25 shortening the distance NO_3^- needs to diffuse into the denitrifying zone in the

1 sediment. Oxygen deficiency also lowers nitrification rate, and thereby Dn rate [32].
2 In the laboratory experiments the oxygen demand was high, but no anoxia developed
3 due to continuous feeding of the system with oxic water. Also in the field
4 measurements oxygen was not limiting nitrification and Dn, as the rates were high
5 and did not correlate with oxygen concentration.

6 7 *N₂O production in denitrification*

8 The N₂O effluxes measured in the river sediments (manipulation experiments) were
9 lower than the rates reported for rivers in the literature [33,34,35]. In the river Swale-
10 Ouse, NE England, the lowest effluxes, measured at the highest upstream sites [36],
11 were more than ten times the effluxes measured in this study. The N₂O effluxes have
12 not been measured from the rivers of the northern Baltic Sea before. In shallow
13 profundal sediments of freshwater lake of the same latitude, the effluxes in aerobic
14 conditions were of the same magnitude (up to 17 μmol N₂O-N m⁻²d⁻¹) [37] as
15 measured from the rivers in this study. Seitzinger et al. [8] reported that in eutrophic
16 water ecosystems, up to 5% of the gases produced in denitrification are released as
17 N₂O. Of the rivers studied in the manipulation experiment, none showed such a high
18 ratio. The N₂O production rates measured were low, and therefore the N removal by
19 denitrification did not, so far, have a significant climatic impact. Increasing NO₃⁻
20 concentrations in the river water would, however, probably enhance N₂O over N₂.
21 Ratios as high as 80 % have been measured from very eutrophic rivers in NE-England
22 [38] and very high N₂O concentrations exist in estuaries around the world [39,5], also
23 in the southern Baltic Sea [40].

24
25 *Could anammox cause inaccuracies in the N₂ production rate estimates?*

1 Until recently, denitrification was seen as the only process removing fixed nitrogen
2 from the water ecosystem. However, a decade ago, another nitrogen removing
3 process, anammox (anaerobic ammonium oxidation), was discovered in wastewater
4 treatment plants [41,42], and later also in marine sediments [43,44,45,46]. In a recent
5 study, anammox was found in a coastal station of the northern Gulf of Finland,
6 too(Hietanen S. and Kuparinen J. Seasonal and short-term variation in denitrification
7 and anammox at a coastal station on the Gulf of Finland, Baltic Sea. Submitted). The
8 discovery of the anammox process in these sediments challenges the previous
9 measurements made in the area, as the coexistence of anammox and denitrification
10 compromises the central assumptions behind the method used in denitrification
11 measurements, and causes overestimates in the N_2 production. Therefore, the true N_2
12 production rates can not be reliably calculated without knowing the share of anammox
13 in the total N_2 production. In the coastal Gulf of Finland, anammox contributed 10-
14 15% to the total N_2 production, with the effect that the N_2 production was
15 overestimated by 80-150% (Hietanen S. and Kuparinen J. Seasonal and short-term
16 variation in denitrification and anammox at a coastal station on the Gulf of Finland,
17 Baltic Sea. Submitted). It has been studied also the Ahvenkoskenlahti Bay, and found
18 to be negligible (<1%) (Hietanen, S. Anammox in the sediments of the Gulf of
19 Finland. Submitted). Thus, there is no bias in the N_2 production rates presented here
20 for that estuary. Anammox was not measured at the other field measurement area, the
21 Paimionlahti Bay, where much higher denitrification rates were found, nor in the river
22 sediments used in the laboratory experiments. Therefore, it is possible that the
23 denitrification rates presented here for these areas are overestimates. Anammox has so
24 far been measured only in two different river ecosystems, the temperate Thames
25 estuary [45] and a subtropical Logan and Albert river system in Australia [47]. In both

1 of these locations, the highest contribution of anammox to the overall N_2 production
2 (8-9%), as well as the highest rates, were measured upstream, with decreasing rates
3 towards the river mouth and open sea, where anammox was found to be negligible. In
4 addition, the relative contribution of anammox to the overall nitrogen reduction has
5 often been found to be minor in coastal environments, and to increase with depth, as
6 the rate of denitrification decreases [43,48,49]. Based on these published findings and
7 those measured from Ahvenkoskenlahti Bay and the coastal station at the Gulf of
8 Finland, we have assumed that in the Paimionlahti Bay and the river sediments, the
9 anammox activity is negligible, and our denitrification estimates therefore valid.

11 *Importance of nitrogen removal by denitrification*

12 We evaluated the efficiency of the nitrogen removal in the studied estuaries. In the
13 laboratory experiments, the calculations of N removal are based on D_w , due to the
14 small share of D_n . The NO_3^- removal by denitrification was calculated as a ratio of
15 output labelled gaseous nitrogen species to the input of labelled NO_3^- . D_w accounted
16 for 17-22 % total NO_3^- removal. The overall NO_3^- removal, which includes DNRA
17 and assimilation in addition to denitrification, was 21-27%. According to these
18 results, denitrification was always the most important process removing nitrate, if the
19 assumption of negligible anammox is correct. When estimating the role of
20 denitrification in reducing the total nitrogen load *in situ*, one has to bear in mind that
21 only a share of total nitrogen is in the form of nitrate in the river waters (Table 1). No
22 information exists yet about the seasonal variation of denitrification in these
23 ecosystems, nor does about the possible differences in the rates between accumulation
24 and transportation bottoms. Stockenberg and Johnstone [23] have suggested that the
25 denitrification rate on transportation and erosion areas is only 30% of that on the

1 accumulation areas. The studied sediments were collected from accumulation bottoms
2 only, and the share of accumulation bottoms has not been mapped in these rivers.
3 Therefore, the estimate given only applies for the environmental conditions prevailing
4 during the experiment.

5
6 The two estuaries studied differed greatly from each other in their capacity to remove
7 nitrogen entering the bay. In Ahvenkoskenlahti Bay in August 2004, assuming that
8 the denitrification rate was similar throughout the basin, the average rate of $280 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ (Dw $86 \mu\text{mol N m}^{-2} \text{ d}^{-1}$) removed 1.7 % of NO_3^- loading (by Dw) and 3.6 %
9 of the total N loading (by Dt_{tot}) reaching the bay. The share of accumulation bottom in
10 the study area is 58 % (Heikki Pitkänen, SYKE, unpubl.). Using the lower rates for
11 the transportation and erosion areas [23] gives only 1.2 % reduction to the nitrate and
12 1% reduction to the total nitrogen loading in August 2004. In Paimionlahti Bay the
13 average denitrification rate of $460 \mu\text{mol N m}^{-2} \text{ d}^{-1}$ (Dw $70 \mu\text{mol N m}^{-2} \text{ d}^{-1}$) was high
14 enough to remove all of the nitrogen loading reaching the bay in September 2003. The
15 year 2003 was exceptionally dry, so the nitrogen removal was calculated also using
16 the loading data of more typical conditions in September 2004. If the denitrification
17 rate was similar in 2004, 4.5 % of nitrate loading and 19 % of total nitrogen loading
18 were removed from the water in the estuary in 2004. The amount of accumulation
19 areas has not been mapped in the Paimionlahti Bay, and the given value is likely to be
20 an overestimate.

21
22
23 The residence time has been indicated as having a major effect in the estuarine
24 retention capacity in several different estuaries [30,50,51,52,53]. In Ahvenkoskenlahti
25 Bay, the discharge is high year round, and the estimated residence time fluctuates

1 between 10 and 14 days (average 12 days). In the larger and deeper Paimionlahti Bay,
2 the flow is 20 times lower and more pulsed, and the calculated residence time varies
3 from 2 to 19 years (average 7 years). Clearly, more nitrogen is removed as it stays
4 longer in the estuary, and, therefore, Paimionlahti Bay is more efficient in removing
5 nitrogen than Ahvenkoskenlahti Bay.

6

7 *Estuaries and river mouths as N filters in northern Baltic*

8 In 1988, Seitzinger [8] introduced a “rule of thumb” of 40-50% removal of N by
9 denitrification in estuaries, indicating them as important filters for the N loading
10 transported towards the sea, and therefore having a considerable role in slowing down
11 eutrophication of seas. Since then, various studies have either supported [e.g. 12] or
12 opposed [51,53,54,55,56] this claim. Only few studies have been made in the Baltic
13 Sea area. A lowland stream entering the Kattegat was found to remove less than 1%
14 of the annual loading, although during low discharge in summer, the removal was
15 temporarily up to 60% of the loading [30]. Similarly, a small, shallow estuary opening
16 to the Kattegat denitrified only 2% of the annual loading [50]. In the Swedish east
17 coast, a coastal embayment was found to remove 5-11% of wastewater N input, with
18 denitrification being the most important mechanism for removal [11]. A study of
19 several rivers entering the Gulf of Bothnia revealed them to be ineffective sinks of N
20 [13]. All of these studies, as well as the results presented here, confirm the pattern of
21 increasing efficiency with decreasing discharge, suggested by Nixon (1996). In
22 conclusion, according to our results, the sediments of the fast flowing rivers and the
23 estuary areas with short residence times of both the Gulf of Bothnia and the Gulf of
24 Finland are inefficient filters of N load. This emphasizes the role of reduction of

anthropogenic N loading to the high latitude rivers and estuaries in order to avoid further eutrophication of susceptible sea areas.

References and notes

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1 Illustrations and tables

2 Table 1. Description of the study areas. ND not determined. DIN dissolved inorganic nitrogen.

						Annual		During sampling				
	Drainage basin km ²	Cultivated field %	Peatlands %	Forests %	Lakes %	Mean flow m ³ s ⁻¹	N load tons	NO ₃ -N μM	DIN/N _{tot} %	NO ₃ /DIN %	Temp °C	O ₂ μM
River Temmesjoki Estuary	1 184	15	2	82	0.5	11	520	80	48	88	14-18	ND
River Siikajoki Estuary	4 318	8	3	87	2	60	1 700	20	26	92	15-20	ND
River Pyhajoki Estuary	3 724	10	0	85	5	40	1 620	25	23	95	15-19	ND
River Kalajoki Estuary	4 247	16	0	82	2	50	4 000	70	44	98	13-20	ND
Paimionlahti Bay	1 088	42	4	45	2.7	7	950	2 - 24	100	99	8-11	65-250
Ahvenkoskenlahti Bay	37 158	9	11	56	19	175	3 540	3 - 10	29	88	8-18	170-280

1 Table 2. Oxygen demand ($\text{mmol m}^{-2}\text{d}^{-1}$) and fluxes of nitrous oxide ($\mu\text{mol N m}^{-2}\text{d}^{-1}$), methane, carbon dioxide and inorganic nitrogen species
2 from sediment to the water ($\text{mmol m}^{-2}\text{d}^{-1}$) and pH in the manipulation experiments (average and standard deviation). DIC dissolved inorganic
3 carbon. N number of replicates.

	N	O ₂ demand	N ₂ O-N	CH ₄ -C	DIC	NO ₃ -N	NH ₄ -N	pH
River Temmesjoki								
Estuary	3	19 ± 2.0	15.1 ± 3.4	0.8 ± 0.7	37 ± 4.5	-0.3 ± 0.2	3.7 ± 1.5	5.4 ± 0.1
River Siikajoki								
Estuary	4	20 ± 2.5	11.4 ± 5.6	0.02 ± 0.01	16 ± 2.0	-0.3 ± 0.2	3.0 ± 0.8	5.3 ± 0.1
River Pyhajoki								
Estuary	4	24 ± 4.1	13.6 ± 4.3	0.6 ± 0.5	20 ± 4.11	-0.4 ± 0.2	0.9 ± 0.1	5.6 ± 0.2
River Kalajoki								
Estuary	4	27 ± 5.8	15.6 ± 6.7	2.3 ± 2.2	21 ± 5.7	-0.4 ± 0.2	1.2 ± 0.4	5.5 ± 0.1

1 **Figure legends**

2

3 **Figure 1. A)** Denitrification in the rivers Temmesjoki (1), Siikajoki (2), Pyhäjoki (3)
4 Kalajoki (4), in the Paimionlahti Bay (5) and in the Ahvenkoskenlahti Bay (6). Black
5 columns; total denitrification (D_{tot}). Numbers; the percentage of coupled nitrification-
6 denitrification (D_n) of the total denitrification (D_{tot}). **B)** Black columns; D_n , white
7 columns; D_w in the Paimionlahti Bay and **C)** in the Ahvenkoskenlahti Bay. Averages
8 and standard deviations shown. The circled sites are inside the estuary.

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