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Denitrification in the river estuaries of the northern Baltic Sea

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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 µmol N m\(^{-2}\) d\(^{-1}\). The estuary bays at the Archipelago Sea and the Gulf of Bothnia had denitrification rates from 90 to 910 µmol N m\(^{-2}\) d\(^{-1}\) and from 230 - 320 µmol N m\(^{-2}\) 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
Gulf is in an ecologically good state. Annual nitrogen (N) loading to the Gulf was 74 100 tons in 2000 [1]. Since the 1990’s, increasing nitrate concentrations have been measured especially in Finnish coastal waters rivers entering the Gulf of Bothnia [2].

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

The fate of N entering the aquatic ecosystem depends on the prevailing conditions. Organic N compounds are decomposed by microbes to ammonium (NH$_4^+$), which can be taken up by primary producers, or nitrified by bacteria to nitrate (NO$_3^-$). NO$_3^-$ can be taken up or processed further in denitrification, anaerobic ammonium oxidation (anammox) or dissimilatory NO$_3^-$ reduction to NH$_4^+$ (DNRA). Of these processes, DNRA reduces NO$_3^-$ back to NH$_4^+$, still available to other organisms. N taken up by primary producers is bound to the biomass and later released back to the water ecosystem. Denitrification (the sequential reduction of NO$_3^-$ to nitrogen gas (N$_2$)) and anammox (oxidizing of NH$_4^+$ with nitrite (NO$_2^-$) to N$_2$) are the only processes that remove N permanently from the system. Denitrification, but not anammox, may have an effect on global warming via the gaseous intermediate, nitrous oxide (N$_2$O). N$_2$, the end product of denitrification, is abundant (78 %) in the atmosphere, whereas the intermediate N$_2$O is an effective greenhouse gas. The ratio of these gases produced by denitrification in aquatic ecosystems is affected by several environmental factors, e.g.
temperature, oxygen concentration and NO$_3^-$ availability [5,6,7]. In sediments, usually less than 5% of the gases produced in denitrification is N$_2$O [8]. Nevertheless, it has been suggested that in areas with high NO$_3^-$ load, such as river mouths and estuaries, production of N$_2$O may be enhanced [8].

Denitrification has been estimated to remove 30% of the annual N loading in the Gulf of Finland [9], and 23% in the Bothnian Bay [10]. Both of these estimates are based on results from the depositional areas of the open sea. Estuaries have been suggested to be effective sinks of N loading, reducing the load transported to the sea [8,11,12]. The few estimates of the filtering capacity published so far from the northern Baltic Sea seem to challenge this view (Hietanen S. and Kuparinen J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea. Submitted) [13,14]. More data is needed to understand the capacity of these ecosystems to reduce the N load entering 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 (Figure 1). Two projects, both part of the Baltic Sea Research Programme (BIREME) of the Academy of Finland (2003-2006) contributed to this work. Two $^{15}$N methods were applied in the studies. The CoastGas project (University of Kuopio, Finland and UFZ Leipzig-Halle, Germany) measured NO$_3^-$ removal in the river mouths using a flow-through method, and the SEGUE-N project (University of 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.

24 Denitrification measurements in the laboratory experiments

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

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

The water reservoir was vacuumed three times to remove N₂ from the gas phase of the water and flushed each time with gas mixture consisting of Ar/O₂ 80/20 (v/v) (AGA, Finland) to improve the sensitivity of concentration analyses of N₂ formed in denitrification. Distilled water, amended with in situ concentrations of sulphate (0.2 mM) and chloride (0.5 mM) (added as MgSO₄ and CaCl₂), was used as inflow water. The sediments were incubated at 30µM of ¹⁵NO₃⁻ (60 at %). The fluxes of NO₃⁻ and gases were measured from the difference between concentrations in the in- and outflowing water. During the experiment the NO₃⁻ concentrations and the isotopic composition (¹⁵N/¹⁴N) of the NO₃ of the in- and outflowing water were determined.
three times in every week (4th, 5th and 6th incubation days). Samples were stored at -20°C prior to analyses. NO$_3^-$ and SO$_4^{2-}$ concentrations were measured with ion chromatography (Dionex DX-130, Sunnyvale, CA, with an anion column A59-HC, 12 mM Na$_2$CO$_3$ as an eluent). The N isotopic composition (at %) of the NO$_3^-$ was determined with R/CF-QMS (Reaction Continuous Flow Quadrupole Mass Spectrometer) [17].

The fluxes of dissolved inorganic carbon (DIC) and dissolved gases CH$_4$, N$_2$O and N$_2$ were determined from the concentration differences between the in- and outflowing water and by taking into account the flow rates and sediment surface area (69 cm$^2$). The water samples for DIC, CH$_4$ and N$_2$O were balanced for 1 day with an Ar headspace, and then analyzed with a GC (Agilent 6890N, Agilent Technologies Deutschland GmbH, Waldbronn, Germany) equipped with a peristaltic pump (Minipuls 3, Gilson Inc., Middleton, WI, USA) and an autosampler (Gilson autosampler 222XL, Gilson Inc., Middleton, WI, USA). The gas concentrations in the original sample were calculated according to Henry’s law (modified from McAuliffe et al. 1971 [18]). The N$_2$ samples were stored in vacuumed 12 ml exetainers (Labco, Co., UK) in concentrated salt solution (NaCl). Concentration and isotopic composition ($^{15}$N/$^{14}$N) of N$_2$ were measured by a specifically configurated Gas Chromatography Quadrupole Mass Spectrometer coupling (GC-QMS) (QP 2000, Schimadzu Corp., [19]) The masses 28, 29 and 30 were measured and the peaks were calibrated against normal air (78 % N$_2$) for concentration measurements. The contamination of samples by N$_2$ in the laboratory atmosphere was prevented by flushing the injection system and the sample loop of the GC with He flow before
injection of the sample. The amount of N$_2$ derived from denitrification was calculated according to non-random distribution of the masses 28, 29 and 30 [20,21,22].

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

Field measurements (Ahvenkoskenlahti Bay and Paimionlahti Bay)

Sediment was sampled either with a single or twin gravity corer, both having an inner diameter of 80 mm. Oxygen and NO$_3^-$ concentrations in the overlying water were measured about 2 cm above the sediment surface. Denitrification was measured using the isotope pairing technique [15]. Three replicate sub-samples were taken in clear plastic (acrylic) cores (ø 2.6 cm, height 9 cm) so that about half of the core was filled with the sediment and half with the overlying water. Samples were enriched with K$^{15}$NO$_3$ (98% labelling, Cambridge Isotope Laboratories, MA, USA) to a final concentration of 100 µM of $^{15}$NO$_3^-$ in the water phase and incubated, with a magnetic stirrer on the lid of the cores, at in situ temperature in darkness for 3 hours. The biological activity was terminated with ZnCl$_2$, and the samples were mixed. Sub-samples of sediment-water slurry were sent in gas-tight 12 ml vials (Exetainer, Labco, UK) to the National Environmental Research Institute, Silkeborg, Denmark, for the analysis of the N$_2$ isotopic composition.
Data processing and statistical analyses

In the laboratory experiments the total denitrification ($D_{\text{tot}}$) was calculated as a sum of measured $\text{N}_2\text{O}$ and $\text{N}_2$. Calculation of $D_n$ (denitrification from coupled nitrification-denitrification) based on the differences in the $^{15}\text{NO}_3^-$ content in the output water (i.e. overlying water) and in the output $\text{N}_2$ (and $\text{N}_2\text{O}$). Denitrification based on the $\text{NO}_3^-$ in the overlying water ($D_w$) was calculated as the remaining part of the total denitrification.

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

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

3 Results

Denitrification rates in the rivers in the Bothnian Bay

The total denitrification rates in the four river mouths varied between 330 and 905 $\mu\text{mol N m}^{-2}\text{ d}^{-1}$ (Figure 1A). Denitrification was mainly based on the nitrate diffusing from the overlying water into the sediments ($D_w$), with only a minor share based on the coupled nitrification-denitrification ($D_n$) (Figure 1A). The variation in
denitrification rates within a site was high (e.g. the river Kalajoki, 906 ± 590 μmol N m⁻² d⁻¹, distance between replicate samples 500 - 1000 meters), and the denitrification rates between the rivers did not differ statistically significantly. The denitrification rate correlated positively (0.876, p=0.05) with the CH₄ efflux, which varied from 0.02 to 2.3 mmol m⁻² d⁻¹ (Table 2), and with oxygen consumption (0.550, p=0.01), which varied from 19 to 27 mmol m⁻² d⁻¹ (Table 2). It did not correlate with pH or with the fluxes of CO₂ or NH₄⁺ (Table 2). The N₂O effluxes varied from 11 to 16 μmol N m⁻² d⁻¹ (Table 2). The percentage of N₂O in the gaseous end products of denitrification did not exceed 5 %. Differences in the N₂O effluxes between the rivers were not statistically significant.

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

In Paimionlahti Bay, the denitrification rate varied tenfold within the estuary, from 90 μmol N m⁻² d⁻¹ in one station in the middle, to 910 μmol N m⁻² d⁻¹ in the outer end of the estuary. The bulk of denitrification was coupled to nitrification in the whole estuary, and the proportion of denitrification that was dependent on the NO₃⁻ in the water column (Dw) was at its highest in the middle estuary (Figure 1B), where the NO₃⁻ concentration was highest. Dw was positively correlated with the NO₃⁻ concentration (0.917, p=0.00) and negatively with the oxygen concentration (-0.871, p=0.000). The rates of coupled nitrification-denitrification (Dn) and total denitrification did not correlate with any of the environmental factors measured.

The denitrification rate in the Ahvenkoskenlahti Bay varied from 230 to 320 μmol N m⁻² d⁻¹. No clear gradient in the total denitrification could be seen within the basin, but 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 µmol N m$^{-2}$d$^{-1}$ [23]. The studies made in northern latitudes are few, but Garcia-Ruiz et al. [24] reported rates of 0-13 800 µmol N m$^{-2}$d$^{-1}$, from the Swale-Ouse river system in UK. In this study, the denitrification rates in the rivers were lower (330-910 µmol N m$^{-2}$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 µmol N m$^{-2}$d$^{-1}$) [10]. They fall into same range with the values measured from the open Gulf of Bothnia (0-940 µmol N m$^{-2}$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
varied between 250 and 300 µmol N m$^{-2}$d$^{-1}$ \cite{9}, which is lower than the rates measured in the river sediments in this study, and also lower than the rates measured in the Paimionlahti Bay (90-910 µmol N m$^{-2}$d$^{-1}$, average 460 µmol N m$^{-2}$d$^{-1}$), southern Gulf of Bothnia.

In the Gulf of Finland, denitrification measurements have been done using the isotope pairing technique since the mid-90’s. Gran and Pitkänen \cite{14} found a gradient in the denitrification rates from the eastern Gulf of Finland, inner Neva estuary, towards the open Gulf. The denitrification rates were lowest (<10 µmol N m$^{-2}$d$^{-1}$) in the inner Neva estuary, higher in the outer estuary and the highest, up to 1260 µmol N m$^{-2}$d$^{-1}$, in the open Gulf of Finland. Tuominen et al. \cite{9} measured the highest denitrification rates of 150-650 µmol N m$^{-2}$d$^{-1}$ from open sea area of the Gulf of Finland, with lower rates in the eastern and western ends of the Gulf (100-400 µmol N m$^{-2}$d$^{-1}$). Thus, the denitrification rates in the Ahvenkoskenlahti Bay (Gulf of Finland) (230-320 µmol N m$^{-2}$d$^{-1}$, average 280 µmol N m$^{-2}$d$^{-1}$) are in the lower end reported for the Gulf. However, there can be some overestimation in the rates reported for the open Gulf of Finland, resulting from the potential effect of anammox in the estimates, whereas no anammox was detected in the Ahvenkoskenlahti Bay. The denitrification rates in the Ahvenkoskenlahti Bay were much higher than the rates outside the bay, towards the open Gulf of Finland (Figure 1C). The stations at the estuary were located on accumulation bottoms, with high carbon and nitrogen content per sediment volume, whereas the stations on the transportation/transient accumulation bottoms outside the estuary had very low dry matter and low carbon and nitrogen content per sediment volume. In the Paimionlahti Bay, the inner estuary rates were slightly (but significantly, p=0.01) lower than those immediately outside the estuary (Figure 1B).
As the highly variable denitrification rate (260-620 µmol N m⁻² d⁻¹, with a single station showing a rate of 90 µmol N m⁻² d⁻¹) did not correlate with any of the environmental factors measured (depth, temperature, salinity, oxygen and nitrite/nitrate concentration, total carbon, nitrogen and loss on ignition in the sediment) likewise showing high variation, it is unclear why the rates outside the estuary basin were higher (720-910 µmol N m⁻² d⁻¹).

Although the total denitrification rates fall into the same range in the laboratory experiments and in the field measurements, the ratio of Dw/Dn was remarkably different. In the laboratory experiments, the share of Dn was always less than 10%, whereas in the field measurements the share was 50-85% in Ahvenkoskenlahti and 65-95% in Paimionlahti. Similar results – small share of Dn - have been reported earlier from flow-through systems as well [29,23]. In the laboratory experiments, the sediment surface is continuously provided with NO₃⁻, mimicking the natural conditions in river ecosystems. Dw correlates with the NO₃⁻ concentration, and the high NO₃⁻ concentrations in river waters (Table 1) are likely to sustain high Dw in the river sediments. The NO₃⁻ concentrations were much higher in the rivers than in the two estuaries (Table 1), and probably explain the lower share of Dn in the river sediments. Low share of Dn has been reported from river and estuary systems using the isotope pairing technique, too. [e.g. 30,31]. In river sediments NO₃⁻ is easily available in the denitrification zone due to more efficient penetration and thus the importance of nitrification as NO₃⁻ source is diminished. Another factor affecting the share of Dn is the oxygen concentration in the overlying water of the sediments. Low oxygen concentration lowers the oxygen penetration depth, thereby enhancing Dw by shortening the distance NO₃⁻ needs to diffuse into the denitrifying zone in the
sediment. Oxygen deficiency also lowers nitrification rate, and thereby Dn rate [32]. In the laboratory experiments the oxygen demand was high, but no anoxia developed due to continuous feeding of the system with oxic water. Also in the field measurements oxygen was not limiting nitrification and Dn, as the rates were high and did not correlate with oxygen concentration.

7  

$N_2O$ production in denitrification

The $N_2O$ effluxes measured in the river sediments (manipulation experiments) were lower than the rates reported for rivers in the literature [33,34,35]. In the river Swale-Ouse, NE England, the lowest effluxes, measured at the highest upstream sites [36], were more than ten times the effluxes measured in this study. The $N_2O$ effluxes have not been measured from the rivers of the northern Baltic Sea before. In shallow profundal sediments of freshwater lake of the same latitude, the effluxes in aerobic conditions were of the same magnitude (up to 17µmol $N_2O$-N m$^{-2}$d$^{-1}$) [37] as measured from the rivers in this study. Seitzinger et al. [8] reported that in eutrophic water ecosystems, up to 5% of the gases produced in denitrification are released as $N_2O$. Of the rivers studied in the manipulation experiment, none showed such a high ratio. The $N_2O$ production rates measured were low, and therefore the N removal by denitrification did not, so far, have a significant climatic impact. Increasing $NO_3^-$ concentrations in the river water would, however, probably enhance $N_2O$ over $N_2$. Ratios as high as 80% have been measured from very eutrophic rivers in NE-England [38] and very high $N_2O$ concentrations exist in estuaries around the world [39,5], also in the southern Baltic Sea [40].

Could anammox cause inaccuracies in the $N_2$ production rate estimates?
Until recently, denitrification was seen as the only process removing fixed nitrogen from the water ecosystem. However, a decade ago, another nitrogen removing process, anammox (anaerobic ammonium oxidation), was discovered in wastewater treatment plants [41,42], and later also in marine sediments [43,44,45,46]. In a recent study, anammox was found in a coastal station of the northern Gulf of Finland, too (Hietanen S. and Kuparinen J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea. Submitted). The discovery of the anammox process in these sediments challenges the previous measurements made in the area, as the coexistence of anammox and denitrification compromises the central assumptions behind the method used in denitrification measurements, and causes overestimates in the N\(_2\) production. Therefore, the true N\(_2\) production rates can not be reliably calculated without knowing the share of anammox in the total N\(_2\) production. In the coastal Gulf of Finland, anammox contributed 10-15% to the total N\(_2\) production, with the effect that the N\(_2\) production was overestimated by 80-150% (Hietanen S. and Kuparinen J. Seasonal and short-term variation in denitrification and anammox at a coastal station on the Gulf of Finland, Baltic Sea. Submitted). It has been studied also the Ahvenkoskenlahti Bay, and found to be negligible (<1%) (Hietanen, S. Anammox in the sediments of the Gulf of Finland. Submitted). Thus, there is no bias in the N\(_2\) production rates presented here for that estuary. Anammox was not measured at the other field measurement area, the Paimionlahti Bay, where much higher denitrification rates were found, nor in the river sediments used in the laboratory experiments. Therefore, it is possible that the denitrification rates presented here for these areas are overestimates. Anammox has so far been measured only in two different river ecosystems, the temperate Thames estuary [45] and a subtropical Logan and Albert river system in Australia [47]. In both
of these locations, the highest contribution of anammox to the overall N\textsubscript{2} production (8-9%), as well as the highest rates, were measured upstream, with decreasing rates towards the river mouth and open sea, where anammox was found to be negligible. In addition, the relative contribution of anammox to the overall nitrogen reduction has often been found to be minor in coastal environments, and to increase with depth, as the rate of denitrification decreases [43,48,49]. Based on these published findings and those measured from Ahvenkoskenlahti Bay and the coastal station at the Gulf of Finland, we have assumed that in the Paimionlahti Bay and the river sediments, the anammox activity is negligible, and our denitrification estimates therefore valid.

**Importance of nitrogen removal by denitrification**

We evaluated the efficiency of the nitrogen removal in the studied estuaries. In the laboratory experiments, the calculations of N removal are based on Dw, due to the small share of Dn. The NO\textsubscript{3}\textsuperscript{-} removal by denitrification was calculated as a ratio of output labelled gaseous nitrogen species to the input of labelled NO\textsubscript{3}\textsuperscript{-}. Dw accounted for 17-22 % total NO\textsubscript{3}\textsuperscript{-} removal. The overall NO\textsubscript{3}\textsuperscript{-} removal, which includes DNRA and assimilation in addition to denitrification, was 21-27%. According to these results, denitrification was always the most important process removing nitrate, if the assumption of negligible anammox is correct. When estimating the role of denitrification in reducing the total nitrogen load *in situ*, one has to bear in mind that only a share of total nitrogen is in the form of nitrate in the river waters (Table 1). No information exists yet about the seasonal variation of denitrification in these ecosystems, nor does about the possible differences in the rates between accumulation and transportation bottoms. Stockenberg and Johnstone [23] have suggested that the denitrification rate on transportation and erosion areas is only 30% of that on the
accumulation areas. The studied sediments were collected from accumulation bottoms only, and the share of accumulation bottoms has not been mapped in these rivers. Therefore, the estimate given only applies for the environmental conditions prevailing during the experiment.

The two estuaries studied differed greatly from each other in their capacity to remove nitrogen entering the bay. In Ahvenkoskenlahti Bay in August 2004, assuming that the denitrification rate was similar throughout the basin, the average rate of 280 µmol N m$^{-2}$ d$^{-1}$ (Dw 86 µmol N m$^{-2}$ d$^{-1}$) removed 1.7 % of NO$_3^-$ loading (by Dw) and 3.6 % of the total N loading (by Dtot) reaching the bay. The share of accumulation bottom in the study area is 58 % (Heikki Pitkänen, SYKE, unpubl.). Using the lower rates for the transportation and erosion areas [23] gives only 1.2 % reduction to the nitrate and 1% reduction to the total nitrogen loading in August 2004. In Paimionlahti Bay the average denitrification rate of 460 µmol N m$^{-2}$ d$^{-1}$ (Dw 70 µmol N m$^{-2}$ d$^{-1}$) was high enough to remove all of the nitrogen loading reaching the bay in September 2003. The year 2003 was exceptionally dry, so the nitrogen removal was calculated also using the loading data of more typical conditions in September 2004. If the denitrification rate was similar in 2004, 4.5 % of nitrate loading and 19 % of total nitrogen loading were removed from the water in the estuary in 2004. The amount of accumulation areas has not been mapped in the Paimionlahti Bay, and the given value is likely to be an overestimate.

The residence time has been indicated as having a major effect in the estuarine retention capacity in several different estuaries [30,50,51,52,53]. In Ahvenkoskenlahti Bay, the discharge is high year round, and the estimated residence time fluctuates
between 10 and 14 days (average 12 days). In the larger and deeper Paimionlahti Bay, the flow is 20 times lower and more pulsed, and the calculated residence time varies from 2 to 19 years (average 7 years). Clearly, more nitrogen is removed as it stays longer in the estuary, and, therefore, Paimionlahti Bay is more efficient in removing nitrogen than Ahvenkoskenlahti Bay.

Estuaries and river mouths as N filters in northern Baltic

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


anammox activity and microscale distribution of nitrite in subtropical mangrove


ammonium oxidation by nitrite (anammox): Implications for N₂ production in

independently estimated by the denitrification rate and mass balance methods: a


53. Nixon, S.W., Ammerman, J.W., Atkinson, L.P., Berounsky, V.M., Billen, G.,
and phosphorus at the land-sea margin of the North Atlantic Ocean.
*Biogeochemistry* 35, 141-180.


1 Illustrations and tables

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

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Drainage basin km²</th>
<th>Cultivated field %</th>
<th>Peatlands %</th>
<th>Forests %</th>
<th>Lakes %</th>
<th>Mean flow m³s⁻¹</th>
<th>N load tons</th>
<th>NO₃-N µM</th>
<th>DIN/N₉₀ %</th>
<th>NO₃/DIN %</th>
<th>Temp °C</th>
<th>O₂ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Temmesjoki Estuary</td>
<td>1 184</td>
<td>15</td>
<td>2</td>
<td>82</td>
<td>0.5</td>
<td>11</td>
<td>520</td>
<td>80</td>
<td>48</td>
<td>88</td>
<td>14-18</td>
<td>ND</td>
</tr>
<tr>
<td>River Siikajoki Estuary</td>
<td>4 318</td>
<td>8</td>
<td>3</td>
<td>87</td>
<td>2</td>
<td>60</td>
<td>1 700</td>
<td>20</td>
<td>26</td>
<td>92</td>
<td>15-20</td>
<td>ND</td>
</tr>
<tr>
<td>River Pyhajoki Estuary</td>
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<td>10</td>
<td>0</td>
<td>85</td>
<td>5</td>
<td>40</td>
<td>1 620</td>
<td>25</td>
<td>23</td>
<td>95</td>
<td>15-19</td>
<td>ND</td>
</tr>
<tr>
<td>River Kalajoki Estuary</td>
<td>4 247</td>
<td>16</td>
<td>0</td>
<td>82</td>
<td>2</td>
<td>50</td>
<td>4 000</td>
<td>70</td>
<td>44</td>
<td>98</td>
<td>13-20</td>
<td>ND</td>
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<tr>
<td>Paimionlahti Bay</td>
<td>1 088</td>
<td>42</td>
<td>4</td>
<td>45</td>
<td>2.7</td>
<td>7</td>
<td>950</td>
<td>2 - 24</td>
<td>100</td>
<td>99</td>
<td>8-11</td>
<td>65-250</td>
</tr>
<tr>
<td>Ahvenkoskenlahti Bay</td>
<td>37 158</td>
<td>9</td>
<td>11</td>
<td>56</td>
<td>19</td>
<td>175</td>
<td>3 540</td>
<td>3 - 10</td>
<td>29</td>
<td>88</td>
<td>8-18</td>
<td>170-280</td>
</tr>
</tbody>
</table>
Table 2. Oxygen demand (mmol m\(^{-2}\)d\(^{-1}\)) and fluxes of nitrous oxide (µmol N m\(^{-2}\)d\(^{-1}\)), methane, carbon dioxide and inorganic nitrogen species from sediment to the water (mmol m\(^{-2}\)d\(^{-1}\)) and pH in the manipulation experiments (average and standard deviation). DIC dissolved inorganic carbon. N number of replicates.

<table>
<thead>
<tr>
<th>River</th>
<th>Estuary</th>
<th>N</th>
<th>O(_2) demand</th>
<th>N(_2)O-N</th>
<th>CH(_4)-C</th>
<th>DIC</th>
<th>NO(_3)-N</th>
<th>NH(_4)-N</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Temmesjoki</td>
<td>3</td>
<td>19 ± 2.0</td>
<td>15.1 ± 3.4</td>
<td>0.8 ± 0.7</td>
<td>37 ± 4.5</td>
<td>-0.3 ± 0.2</td>
<td>3.7 ± 1.5</td>
<td>5.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>River Siikajoki</td>
<td>4</td>
<td>20 ± 2.5</td>
<td>11.4 ± 5.6</td>
<td>0.02 ± 0.01</td>
<td>16 ± 2.0</td>
<td>-0.3 ± 0.2</td>
<td>3.0 ± 0.8</td>
<td>5.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>River Pyhajoki</td>
<td>4</td>
<td>24 ± 4.1</td>
<td>13.6 ± 4.3</td>
<td>0.6 ± 0.5</td>
<td>20 ± 4.11</td>
<td>-0.4 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>5.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>River Kalajoki</td>
<td>4</td>
<td>27 ± 5.8</td>
<td>15.6 ± 6.7</td>
<td>2.3 ± 2.2</td>
<td>21 ± 5.7</td>
<td>-0.4 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>5.5 ± 0.1</td>
<td></td>
</tr>
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</table>
Figure legends

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