INTRODUCTION

Anthropogenic eutrophication of the Baltic Sea is of great concern. Of the Baltic Sea sub-basins, the Gulf of Finland is the most eutrophicated and has been suffering from severe cyanobacterial blooms caused by excessive nutrient loading (Pitkänen & Tamminen 1995). Nitrogen loading in particular is difficult to control, and the total nitrogen (TN) load per area in the Gulf of Finland is the highest in the entire Baltic Sea (HELCOM 2009). Nitrogen is removed from aquatic systems by microbially mediated anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA) at 2 coastal sites in the Gulf of Finland. In addition to the estimation of in situ rates, we assessed the potential for nitrification and denitrification in different seasons. The nitrification and nitrogen removal processes were maximal during the warm summer months, when the sediment organic content was highest. In colder seasons, the rates of nitrification and nitrate reduction measured under in situ conditions decreased, but the potential for nitrification remained equal to, or higher than, that during the warm months. The rates of denitrification and nitrification were usually higher in the accumulation basin, where the organic content of the sediment was higher, but the transportation area, despite lower denitrification rates and potential, typically had a higher potential for nitrification than the accumulation basin. Anammox and DNRA were not important nitrate sinks in any of the seasons sampled. The results also show that the rates of denitrification in the coastal Gulf of Finland sediment have decreased, and that benthic denitrification might be a less important sink for fixed nitrogen than previously assumed.

KEY WORDS: Sediment · Nitrification · Denitrification · Anammox · DNRA · Baltic Sea · Gulf of Finland

ABSTRACT: The Baltic Sea is one of the most eutrophic marine areas in the world. The role of nitrogen as a eutrophating nutrient in the Baltic Sea has remained controversial owing to a lack of understanding of nitrogen cycling in the area. We investigated the seasonal variation in sediment nitrification, denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA) at 2 coastal sites in the Gulf of Finland. In addition to the estimation of in situ rates, we assessed the potential for nitrification and denitrification in different seasons. The nitrification and nitrogen removal processes were maximal during the warm summer months, when the sediment organic content was highest. In colder seasons, the rates of nitrification and nitrate reduction measured under in situ conditions decreased, but the potential for nitrification remained equal to, or higher than, that during the warm months. The rates of denitrification and nitrification were usually higher in the accumulation basin, where the organic content of the sediment was higher, but the transportation area, despite lower denitrification rates and potential, typically had a higher potential for nitrification than the accumulation basin. Anammox and DNRA were not important nitrate sinks in any of the seasons sampled. The results also show that the rates of denitrification in the coastal Gulf of Finland sediment have decreased, and that benthic denitrification might be a less important sink for fixed nitrogen than previously assumed.
oxidizing bacteria (NOB). Nitrification is often one of the most important factors controlling the rates of nitrogen removal.

Sediments are important sites for nitrogen cycling because the sharp gradient of O₂ in the sediment allows oxic nitrification and anoxic denitrification and anammox to operate in close proximity (Jenkins & Kemp 1984, Jensen et al. 1993, 1994). Anoxic conditions, which commonly prevail at the sediment surface in the Gulf of Finland (HELCOM 2009), decrease nitrogen removal by closing down the coupled nitrification–denitrification pathway, which is the most important pathway for nitrogen removal (Tuominen et al. 1998, Hietanen & Kuparinen 2008). When the sulfide oxidation rates and/or organic loading are high, and the concentrations of O₂ and NO₃⁻ are low, dissimilatory nitrate reduction to ammonium (DNRA) can become an important pathway for nitrate reduction (Tiedje et al. 1982, Rysgaard et al. 1996, Christensen et al. 2000, Burgin & Hamilton 2007). In terms of eutrophication, DNRA is a harmful process because it maintains nitrogen in a bioavailable form. Karlsson et al. (2005) studied laminated Baltic Sea sediment and observed NO₃⁻-consumption in the sediment that could not be explained by denitrification, suggesting that DNRA could be an important nitrate-reduction pathway enhancing the vicious cycle of eutrophication in the Baltic Sea.

To expand our understanding of nitrogen cycling in the Gulf of Finland, we investigated the seasonal variability in nitrification, nitrification potentials, and the NO₃⁻-reduction pathways in the coastal Gulf of Finland accumulation basin and shallow transportation area.

MATERIALS AND METHODS

Study area. Two coastal stations in the northwestern Gulf of Finland, Storfjärden (59° 51.31’ N, 23° 18.81’ E) and Muncken (59° 51.14’ N, 23° 14.70’ E), were sampled for sediment nitrification and nitrate-reduction pathways in September 2007 (only Storfjärden), April, August, and November 2008, and April, August, and December 2009. Storfjärden represents a typical outer archipelago accumulation basin (Niemi 1975) in which the sediment is soft mud. It is 33 m in depth and is typically thermally stratified from June to September. The highest temperatures in the bottom water are measured in late autumn, when the thermal stratification breaks, and lowest in early spring, after the break-up of ice. Muncken is located ~1 km from Storfjärden and is closer to the river outflow from Pojo Bay. It is a shallow (11 m) transportation area where the sediment is soft mud mixed with a small proportion of fine-grained sand. The highest sedimentation rates in the area occur in May after the spring bloom, when ~80% of the sedimenting carbon reaches the bottom (Tallberg & Heiskanen 1998).

Sampling and incubation. The temperature and salinity were recorded using a CTD probe (SIS CTD plus 100). The sediment was collected in acrylic cores, using a Gemini (∅ 80 mm, height 80 cm) or Gemax (∅ 90 mm, height 80 cm) twin corer with sediment and water in each core. The concentrations of O₂, NO₃⁻ and NH₄⁺ in the bottom water were measured by withdrawing a water sample from ~5 cm above the sediment surface in a core. The sediment’s organic content (as a loss on ignition, LOI, in %) and porosity were determined from the topmost 1 cm of the sediment. Each large core was sub-sampled into 3 or 4 smaller acrylic plastic cores (∅ 26 mm, height 20 cm), so that there was ~5 cm of sediment and 13 cm of water in each sub-sample. The remaining volume in the core was filled by caps, leaving no air in the cores. The sub-samples were transported in cooler boxes to the shore within 2 h for further treatments.

The intact sediment cores for measuring denitrification, anammox and DNRA were enriched with potassium nitrate (K¹⁵NO₃, 99% ¹⁵N) to final concentrations of 40, 80, 120 and 160 μM ¹⁵NO₃⁻ in the overlying water (n = 4 per concentration; Table 1).

The intact sediment cores for measuring nitrification were enriched with ammonium chloride (¹⁵NH₄Cl, 99% ¹⁵N) to final concentrations of 100 μM, 200 and 300 μM ¹⁵NH₄⁺ (n = 4 per concentration; Table 1).

Table 1. Experimental set-up. Dₜ = denitrification based on NO₃⁻ from the overlying water; Dₚ = denitrification coupled to nitrification; anammox = anaerobic ammonium oxidation; Nₚ = nitrification producing NO₃⁻ to the water column; ¹⁵N₂ = potential coupled denitrification–denitrification; DNRA = dissimilatory nitrate reduction to ammonium; IPT = isotope-pairing technique; r-IPT = revised isotope-pairing technique; IRMS = isotope ratio mass spectrometry; SPINMAS = an automated sample preparation unit for inorganic nitrogen species coupled to a quadrupole mass spectrometer

<table>
<thead>
<tr>
<th>Processes measured</th>
<th>Dₜ, Dₚ, anammox</th>
<th>Nₚ, ¹⁵N₂</th>
<th>DNRA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>IPT, r-IPT</td>
<td>¹⁵NH₄⁺ oxidation</td>
<td>¹⁵NO₃⁻ reduction to ¹⁵NH₄⁺</td>
</tr>
<tr>
<td><strong>Substrate tracer added</strong></td>
<td>¹⁵NO₃⁻</td>
<td>¹⁵NH₄⁺</td>
<td>¹⁵NO₃⁻</td>
</tr>
<tr>
<td><strong>Products measured</strong></td>
<td>²⁹N₂ &amp; ³⁰N₂</td>
<td>¹⁵NO₃⁻, ²⁹N₂ &amp; ³⁰N₂</td>
<td>¹⁵NH₄⁺</td>
</tr>
<tr>
<td><strong>Analysis methods</strong></td>
<td>IRMS</td>
<td>SPINMAS, IRMS</td>
<td>NH₃ diffusion combined with IRMS</td>
</tr>
</tbody>
</table>
All cores were incubated for 4 h at the *in situ* temperature with mixing provided by magnetic stirring bars in the caps. After incubation, the sediment was mixed with the overlying water and the cores were submerged in ice-cold water and allowed to settle for ~3 min. A water sample (12 ml) for isotopic analysis of N₂ was withdrawn from the top of the core into a gas-tight glass vial (Exetainer, Labco Scientific) containing 0.5 ml zinc chloride (ZnCl₂) (100% w/v). The N₂ samples from September 2007 were analyzed at Iso-Analytical Laboratories in Crewe, Cheshire, UK, and the samples from 2008 and 2009 were analyzed at the National Environmental Research Institute (NERI), University of Aarhus, Silkeborg, Denmark. The remainder of the core content was centrifuged (237 × g, 10 min) and the supernatant was filtered (pre-washed syringe filter, Acrodisc) and frozen immediately.

The concentration of NH₄⁺ was measured in April 2009 (Muncken only) or December 2009 (both stations). For the remaining samples was done in NERI, using a combined analytical Laboratories in Crewe, Cheshire, UK, and the samples from September 2007 were analyzed at Iso-Analytical Laboratories. The total sediment nitrification rate includes the nitrification providing NO₃⁻ to the water column (Nₐ; ¹⁵NH₄⁺ oxidation samples) and the nitrification coupled to denitrification (Dₐ; isotope-pairing technique [IPT], see below). Dₐ was used as an estimate of coupled nitrification–denitrification instead of Dₐ (= N₂ + 2 × N₂) production from the ¹⁵NH₄⁺ oxidation samples because the IPT was less prone to biases caused by substrate limitation. Nₐ was calculated by plotting the ¹⁵NO₃⁻ production from ¹⁵NH₄⁺ oxidation samples against the total NH₄⁺ concentration and extrapolating linearly the NO₃⁻ production to the ambient NH₄⁺ level. If no linear significant correlation was found, Nₐ was the average of ¹⁵NO₃⁻ productions.

The denitrification rate was calculated using the IPT (Nielsen 1992). The total nitrate-reduction rates were calculated by combining denitrification based on NO₃⁻ from nitrification (Dₕ; IPT), denitrification based on NO₃⁻ in the sediment-overlying water (Dₐ; IPT), and the DNRA rate (Dₑ; IPT). The traditional IPT is not a reliable denitrification measurement technique if anammox is present. Anammox produces ¹⁵N₂ by combining the added ¹⁵NO₃⁻ and ambient ¹⁴NH₄⁺. This violates the binomial distribution of ²⁸N₂, ²⁹N₂, and ³⁰N₂ productions, which is the fundamental assumption of IPT. When anammox was detected (November 2008 and December 2009), denitrification and anammox were calculated according to the revised IPT (r-IPT; Sigman et al. 1997).
Risgaard-Petersen et al. 2003, 2004). The results of the IPT data are also unreliable when denitrification is not nitrate-limited (Nielsen 1992, Steingruber et al. 2001). On these occasions (Muncken, April 2008 and 2009; Storfjärden, April 2009) denitrification coupled to nitrification was calculated from nitrification samples, in which we assumed that the NO$_3^-$ produced by nitrification diffused at similar 15/14N ratios to the bottom water and to the denitrification layer so that coupled nitrification–denitrification could be expressed as:

$$\text{Coupled nitrification – denitrification} = \frac{N_d}{r}$$

(1)

where $r$ is the 15/14N ratio of the NO$_3^-$ effluxing from the sediment (Jäntti et al. unpubl.). By using this method, the assessment of $D_n$ is not possible, and consequently $D_n$ data are not available when this method was applied. The nitrification potential was assessed from the nitrification samples by combining the production of 15NO$_3^-$ and 15N$_2$ at the given 15NH$_4^+$ concentration, assuming that the added 15NH$_4^+$ was the only substrate for nitrification. This assumption was made because the concentration of 15NH$_4^+$ added was at least 20-fold higher than the ambient NH$_4^+$ concentration in the water. Statistical analyses were done with the SPSS statistical package. Correlations between the environmental variables and the process rates were done by using the non-parametric Spearman correlation coefficient ($r_S$) analysis and were defined as significant when $p < 0.05$.

### RESULTS

The physical and chemical environmental characteristics in the bottom water followed a seasonal cycle at both stations. High temperatures in August co-occurred with maximum NH$_4^+$ concentration and LOI% as well as the year’s lowest O$_2$ and NO$_3^-$ concentrations. When the temperatures began to decrease, the LOI% and NH$_4^+$ concentrations decreased and both NO$_3^-$ and O$_2$ concentrations increased. With the exception of December 2009, the LOI% was always higher at Storfjärden than at Muncken (Table 2). Two exceptional environmental variations occurred during the sampling period: the period January–April 2008 was unusually warm and no ice cover formed in the Gulf of Finland, and in December 2009, an upwelling event on the day before sampling increased the bottom water salinity from 6.4 to 7.0, and decreased the temperature of the bottom water from 7.0 to 6.3°C at Storfjärden.

In spring, nitrification and denitrification typically showed low rates that nearly doubled in late summer and again decreased during the winter (Figs. 1 & 2). An exception to this pattern was observed in Storfjärden in April 2009 when the highest nitrification rate of the entire sampling period was observed (689.1 μmol N m$^{-2}$ d$^{-1}$; Fig. 1). Although 15NO$_3^-$ was not measured in April 2009 (Muncken only) or December 2009 (both stations), assessment of $N_w$ was possible from the total NO$_3^-$ fluxes because, in April 2009, NO$_3^-$ was effluxing in Muncken from all 15NH$_4^+$ concentrations indicating

![Fig. 1. Total nitrification rates and SE, comprising nitrification coupled to denitrification ($D_n$; isotope-pairing technique) and nitrification producing NO$_3^-$ to the water column ($N_w$; 15NH$_4^+$ oxidation). NA = data not available](image)

### Table 2. Physical and chemical characteristics in the bottom water and sediment surface

<table>
<thead>
<tr>
<th></th>
<th>Temp (°C)</th>
<th>Salinity (mg l $^{-1}$)</th>
<th>O$_2$ (μM)</th>
<th>NH$_4^+$ (μM)</th>
<th>NO$_3^-$ (μM)</th>
<th>LOI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storfjärden</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept 07</td>
<td>8.9</td>
<td>6.3</td>
<td>5.3</td>
<td>4.5</td>
<td>2.8</td>
<td>22.0</td>
</tr>
<tr>
<td>Apr 08</td>
<td>2.4</td>
<td>5.4</td>
<td>13.2</td>
<td>2.5</td>
<td>2.6</td>
<td>17.7</td>
</tr>
<tr>
<td>Aug 08</td>
<td>16.2</td>
<td>5.9</td>
<td>6.5</td>
<td>6.2</td>
<td>1.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Nov 08</td>
<td>6.4</td>
<td>6.5</td>
<td>13.6</td>
<td>0.3</td>
<td>5.3</td>
<td>10.8</td>
</tr>
<tr>
<td>Apr 09</td>
<td>0.8</td>
<td>5.3</td>
<td>12.5</td>
<td>0.1</td>
<td>6.9</td>
<td>13.2</td>
</tr>
<tr>
<td>Aug 09</td>
<td>5.7</td>
<td>6.6</td>
<td>5.7</td>
<td>1.8</td>
<td>3.5</td>
<td>31.6</td>
</tr>
<tr>
<td>Dec 09</td>
<td>6.3</td>
<td>7.0</td>
<td>9.3</td>
<td>1.4</td>
<td>4.0</td>
<td>8.6</td>
</tr>
<tr>
<td><strong>Muncken</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Apr 08</td>
<td>2.7</td>
<td>5.3</td>
<td>13.1</td>
<td>0</td>
<td>1.1</td>
<td>7.5</td>
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<tr>
<td>Aug 08</td>
<td>16.6</td>
<td>5.9</td>
<td>7.2</td>
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<td>Nov 08</td>
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<tr>
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<td>4.6</td>
<td>5.5</td>
<td>11.5</td>
<td>0.1</td>
<td>4.3</td>
<td>8.6</td>
</tr>
</tbody>
</table>
nitrification of the added $^{15}$NH$_4^+$ was efficient in December 2009, NO$_3^-$ influxing to the sediment in all $^{15}$NH$_4^+$ concentrations indicating that all NO$_3^-$ produced by nitrification was consumed by NO$_3^-$ reduction. Consequently no $^{15}$NO$_3^-$ efflux was assumed (Figs. 1 & 3). The denitrification rates were typically higher at Storfjärden than at Muncken, and denitrification was, with the exception of August 2009 in Storfjärden, tightly coupled with nitrification, nitrification providing more than 95% of the NO$_3^-$ for the process (Fig. 2). Anammox was detected in November 2008 at both sampling stations, and in December 2009 at Storfjärden. In November 2008, anammox totalled 18% (Storfjärden) and 26% (Muncken) of the N$_2$ production and in December 2009 27% of the N$_2$ production. At the times when anammox was detected, the total N$_2$ production was low, and consequently the anammox rates were low (Fig. 2). DNRA was always detected when measured and, with the exception of April 2008 in Muncken, comprised 5 to 20% of the total NO$_3^-$ reduction (Fig. 2). Although the DNRA comprised nearly half of the total NO$_3^-$ reduction in April 2008 in Muncken, the overall nitrate-reduction rates were low, hence the DNRA rates were not high.

Production of $^{15}$N$_2$ from $^{15}$NH$_4^+$ oxidation samples increased significantly linearly with $^{15}$NH$_4^+$ availability, indicating NH$_4^+$ limitation of the first step of the coupled nitrification–denitrification pathway. A similar trend was also observed in both April samplings in Muncken, but the increase was not significantly linear (Fig. 3). The only sampling time when $D_n$ measured with the IPT was significantly higher than the $^{15}$N$_2$ production from $^{15}$NH$_4^+$ oxidation samples was August 2008 at both stations (Fig. 3). At that time the $^{15}$N labeling of the NH$_4^+$ pool in the nitrification layer was probably not complete owing to high NH$_4^+$ availability in the sediment, as indicated by the relatively high NH$_4^+$ concentration in bottom water and the LOI% value compared to other values observed in the area (Table 2). As a consequence, the $^{15}$NH$_4^+$ added was not the only substrate for nitrification, which reduced the $^{15}$NO$_3^-$ and subsequent $^{15}$N$_2$ production from $^{15}$NH$_4^+$ oxidation samples. N$_w$ occurred more commonly in Muncken than in Storfjärden, and when N$_w$ occurred in Storfjärden, the production of $^{15}$NO$_3^-$ did not increase with increasing $^{15}$NH$_4^+$ availability, whereas at Muncken, the production of $^{15}$NO$_3^-$ increased linearly with $^{15}$NH$_4^+$ availability in April 2008, November 2008 and April 2009. In August 2009, the increase was almost significantly linear ($p = 0.058$; Fig. 3).

In Storfjärden, there was a significant positive correlation between total denitrification and LOI% ($r_S = 0.893$), bottom water NH$_4^+$ concentration and total denitrification ($r_S = 0.786$), and temperature and $D_n$ ($r_S = 0.829$) — indicating that denitrification was stimulated when the sediment organic content and water temperatures increased. In Muncken, neither LOI% nor temperature correlated with denitrification. There was a negative correlation in Storfjärden between anammox and denitrification ($r_S = -0.757$) and anammox and LOI% ($r_S = -0.757$), suggesting that anammox was inhibited when denitrification rates and sediment organic content were high. Also, a significant negative correlation ($r_S = -0.829$) was found between O$_2$ concentration and DNRA rates in Storfjärden.

**DISCUSSION**

The highest nitrification potentials always occurred in April and late autumn (Fig. 3) when the primary production rates are lower than in August. This is in line with several previous studies that report high nitrification rates during low primary production (Focht & Verstraete 1977, Kemp et al. 1990, Caffrey et al. 2007). The clearest reasons for the high nitrification potential are low sedimentation rates causing low decomposition and subsequent low O$_2$ consumption rates, which in turn increase O$_2$ availability for nitrification. However,
the NH$_4^+$ availability was low, and this limited nitrification. The nitrification rates measured under in situ conditions were highest, with the exception of Storfjärden in April 2009, during summer (Fig. 1). The high estimates of summer in situ nitrification rates were probably a result of high NH$_4^+$ availability due to enhanced mineralization rates caused by the higher organic content of the sediment (Table 2). The limiting factor for nitrification must have been low O$_2$ availability because heterotrophic bacteria, which are abundant when the organic content is high, have higher affinities for O$_2$ than do nitrifiers (Verhagen & Laanbroek 1991, Caffrey et al. 1993, Sloth et al. 1995, Strauss & Lambert 2000, Starry et al. 2005).

There was a significant positive correlation between LOI% and denitrification rates, and LOI% and denitrification potential in Storfjärden. The most obvious reason for the correlation between LOI% and denitrification in Storfjärden is that denitrifying bacteria are heterotrophic (Payne 1973) and depend on organic carbon as their energy source. Also, when organic carbon availability was high, the estimated in situ nitrification rates were high, which provided NO$_3^-$ for denitrification (Figs. 1 & 2). The results agree with several previous studies that report high denitrification rates in sediment with an elevated organic content (Tuominen et al. 1998, Barnes & Owens 1999, Dong et al 2000, Hietanen & Kuparinen 2008). Muncken is a transportation area, where organic matter never accumulates, causing low variation in LOI% values (4%), and as a consequence, LOI% and denitrification did not correlate.
Denitrification was more frequently limited by the availability of NO$_3^-$ in Storfjärden than in Muncken. When $^{15}$NO$_3^-$ was produced in the nitrification samples at Storfjärden, it was, with the exception of April and August 2009, immediately taken up by denitrification and, as a result, no $^{15}$NO$_3^-$ diffused into the water column (Fig. 3). At Muncken, denitrification was more saturated by the availability of NO$_3^-$; this was indicated by the fact that $^{15}$NO$_3^-$ from the nitrification samples diffused frequently into the bottom water rather than being reduced by denitrification (Fig. 3). Instead of NO$_3^-$ limitation, denitrification was probably limited by carbon availability, because less carbon was available in Muncken than in Storfjärden (Table 2).

The nitrification potentials at Storfjärden were higher in 2009 than in 2008 (Fig. 3). One factor causing interannual variation in the nitrogen processes was a lack of ice cover in the winter of 2008 due to an exceptionally warm winter; this possibly caused the earlier development and sedimentation of a spring bloom, increasing the organic content of the sediment and possibly favoring heterotrophic bacteria over nitrifying bacteria in April 2008. In December 2009, the day before sampling, Storfjärden experienced a minor upwelling event which may have influenced nitrification. Sudden increases in salinity can cause an efflux of NH$_4^+$ from the sediment—due to ion pairing of NH$_4^+$ with saltwater anions, and the blocking of ion-exchange sites by seawater cations (Gardner et al. 1991, Seitzinger et al. 1991)—which would increase the availability of NH$_4^+$ to nitrifiers and stimulate nitrification.

There are no data available for direct comparison of nitrification rates in the Baltic Sea sediments and, overall, we found only a few nitrification measurements made with intact sediment cores quantifying both nitrification producing NO$_3^-$ to the water column and nitrification coupled to denitrification. Of the studies that were, to some extent, comparable in terms of measurement technique, the nitrification rates measured at the Danish coasts were at least 10-fold higher (Henriksen et al. 1981, Sloth et al. 1992), and at the Island of Sylt, Germany (Jensen et al. 1996) and a Danish lake (Rysgaard et al. 1993) ~2-fold higher than those measured here (Fig. 1). At Chesapeake Bay (USA), the rates were nearly 20-fold higher in spring, but decreased to zero during the summer months (Jenkins & Kemp 1984). All in all, the nitrification rates in the coastal Gulf of Finland were low, compared with rates measured in more southerly locations.

Although the nitrification rates and nitrification potentials, particularly at Storfjärden, were higher in 2009 (Figs. 1 & 3), the denitrification rates at both stations were slightly lower in 2009 than in 2008 (Fig. 2). The explanation for the lower rates in 2009 might be lower water temperature. The lack of ice cover in 2008 allowed earlier penetration of light to the surface water, and a faster warm-up of the water, stimulating all biological processes very early in the spring of that year. Also, in August 2009, the water column in Storfjärden had not yet mixed by the sampling time (unlike the situation in 2008), causing an almost 10ºC difference in the bottom water temperatures (Table 2); this may have decreased the denitrification rates. This is also supported by the significant positive correlation between temperature and denitrification. Compared to the rates measured in Storfjärden in the period 2003 to 2004 (Hietanen & Kuparinen 2008), denitrification was almost 50% lower. However, because the previous data had not been gathered using r-IPT in all seasons, they are directly comparable only for spring and summer rates. Still, even assuming that anammox would have contributed 10% to the N$_2$ production during those seasons when r-IPT was not applied, the denitrification rates had decreased (Hietanen & Kuparinen 2008). We found no apparent reason for the decreased rates from the long-term monitoring data (2002 to 2009; O$_2$, temperature, salinity, chlorophyll a) of the area, but we discovered a high abundance of the invasive polychaete worm Marenzelleria spp. (Bick & Burckhardt 2008) in the samples at all times. Marenzelleria spp. are recent invaders in the Gulf of Finland and were not present in such high numbers in the period 2003 to 2004, compared with the present study. These animals do not have a major impact on denitrification (Hietanen et al. 2007) but they tolerate low-oxygen conditions (Schiedek 1997, Hahlbeck et al.2000), and the increase in their abundance might be an indication that the sampling area has recently experienced hypoxia to an extent greater than that seen in the period 2003 to 2004.

In general, the rates of denitrification in the coastal Gulf of Finland are low compared to more southerly locations. For example, the rates were 20-fold lower than in the Colne estuary, UK (Dong et al. 2000, 2009), and approximately 100-fold lower than in the Rowley River estuary, USA (Tobias et al. 2003), Humber estuary, UK (Barnes & Owens 1999) and Thames estuary, UK (Trimmer et al. 2000). However, in most of these sites the NO$_3^-$ concentrations were substantially higher, which may explain the large difference in the rates. When compared to sites in the same latitude, our rates were lower than the denitrification rates measured in the open Gulf of Finland sediments (Tuominen et al. 1998) and in the Archipelago Sea (Silvennoinen et al. 2007), but higher than in the eastern Gulf of Finland (Gran & Pitkänen 1999) and in the Gulf of Bothnia (Stockenberg & Johnstone 1997).

Anammox was previously reported in the sampling area in May and August 2003 (Hietanen & Kuparinen 2008). In the present study, we tested for anammox at all sampling times, and it was detected only in late
autumn when the total N₂ production rates and the anammox rates were very low. Anammox rates negatively correlated with LOI% and denitrification, hence the appearance of anammox may have been due to a decrease in the availability of organic carbon needed for denitrification, when anammox did not have to compete for NO₃⁻ with denitrifying bacteria (Thamdrup & Dalsgaard 2002, Dalsgaard et al. 2005, Engström et al. 2005). The presence of anammox can also be explained by low water temperatures because anammox appears to be favored in colder environments (Rysgaard et al. 2004, Trimmer et al. 2005).

Overall, the present study confirms the previous results by Hietanen & Kuparinen (2008) that anammox has a minor role as a nitrogen-removing process in the coastal Gulf of Finland.

Direct measurements of coupled nitrification–DNRA are impossible with stable isotope-based methods because the substrate and the end product of the pathway are the same. DNRA is typically measured by adding ¹⁵NO₃⁻ and monitoring the production of ¹⁵NH₄⁺, as was also done in the present study. However, due to the addition of ¹⁵NO₃⁻, the substrate availability for DNRA increased and the ¹⁵NH₄⁺ production is only a measure of DNRA potential. Scaling the DNRA rate to the ambient NO₃⁻ levels is challenging. The actual ¹⁵/¹⁴N ratio of the NO₃⁻ consumed by DNRA is difficult to estimate because the ¹⁵N label of the NO₃⁻ pool is constantly diluted by ¹⁴NO₃⁻ produced by nitrification. In the calculations presented by Christensen et al. (2000), the ¹⁵/¹⁴N ratio of the NO₃⁻ consumed was proposed to be similar to the ¹⁵/¹⁴N ratio of the N₂ produced by denitrification, because DNRA and denitrification occur at the same anoxic sediment depth right below the oxic layer. To estimate DNRA rates accurately with this technique, the ¹⁴NH₄⁺ production should increase in parallel with the ¹⁵N content of the N₂ produced by denitrification, which was not the case in the present study. Alternatively, Dong et al. (2009) proposed that the ¹⁵/¹⁴N ratio of the NO₃⁻ consumed is simply the ratio of the ambient ¹⁴NO₃⁻ and the added ¹⁵NO₃⁻, and the isotopes are consumed by DNRA in the same ratio as they are available in the water phase. Because the DNRA was not nitrate-limited, and the IPT calculation formulas could not be applied in all seasons due to lack of NO₃⁻ limitation in denitrification, we used the calculation scheme of Dong et al. (2009). The DNRA rates measured were comparable to those in 2 lowland lakes (Nizzoli et al. 2010) and a wetland (Scott et al. 2008), but were at least 10-fold lower in comparison to marine sediments (Jorgensen 1989, Rysgaard et al. 1996). High rates of DNRA have been reported from sediments that have high concentrations of sulfide and/or a high organic content, causing a low redox potential (Burgin & Hamilton 2007), and a negative correlation between O₂ concentration and DNRA rates was also found in the present study. The low rates of DNRA measured in this study were probably a result of a permanently oxidized top layer of sediment, creating an environment with low concentrations of sulfide and a high redox potential at the sediment layer where NO₃⁻ is available.

The results shown here emphasize the limited capacity of the coastal Gulf of Finland sediments to purify the nitrogen load from the land. It has been estimated that the total nitrogen load from the drainage basin to the sampling area is 40 to 50 tonnes of nitrogen in each month (Malve et al. 2000). By assuming that the denitrification rates in the entire river outflow area (22.5 km²) are equal to denitrification rates measured in Muncken, denitrification removes ~2% of the nitrogen load. If the denitrification rates in the river outflow area are approximately the same as those in Storfjärden, denitrification removes 2.8% of the nitrogen load. Depositional areas, similar to Storfjärden, have been estimated to cover only 25 to 35% of the Gulf of Finland (H. Kankaanpää & H. Vallius pers. comm.); hence the former estimate might be more accurate. If the rates are expanded to the entire Gulf of Finland (29600 km²)—assuming the 25% areal coverage of the depositional areas and 75% coverage of transportation areas—denitrification and anammox removed ~16 kt N yr⁻¹, which is substantially less than the 39 to 45 kt estimates of Tuominen et al. (1998) and Hietanen & Kuparinen (2008). Moreover, the 16 kt N yr⁻¹ is probably an overestimate because, in the period 2008 to 2009, the Gulf of Finland had vast anoxic areas. The bottom-water O₂ conditions in the Gulf of Finland are highly regulated by salt water inflows from the Baltic Sea main basins that strengthen the stagnation of the water column, which, in turn, prevents mixing of the water column. In the period 2008 to 2009, the water column stagnation was strong, which may also explain the decreased denitrification rates.

It is noteworthy that when the loading from land is highest, during spring, the natural capacity of the sediments to purify the nitrogen load is at its lowest. The increasing winter rains predicted for the northern Baltic Sea, due to climate change (HELCOM 2007), can increase the annual nitrogen load substantially. High nitrogen loading, particularly during winter, creates more intense spring blooms, which, in turn, can increase the area of anoxic seafloor because most of the sedimenting material in the Gulf of Finland originates from the spring bloom (Tallberg & Heiskanen 1998, Conley et al. 2009). Therefore, the control of nitrogen loading from the entire drainage basin is essential in the battle against eutrophication.
CONCLUSION

Nitrification and denitrification in the coastal Gulf of Finland follows a seasonal pattern, with the highest rates occurring during late summer and low rates during late fall and spring. When the denitrification rates were at their highest, denitrification was limited by the nitrification rate but the \( \text{NH}_4^+ \) availability did not limit nitrification. In the accumulation basin, nitrification was occasionally limited by \( \text{NH}_4^+ \) availability, and the nitrification potentials were typically lower than in the transportation area. When nitrification was stimulated by adding excess \( \text{NH}_4^+ \) to the bottom water, denitrification generally reduced all of the \( \text{NO}_3^- \) that was produced to \( \text{N}_2 \). In the transportation basin, nitrification was frequently limited by \( \text{NH}_4^+ \) availability, and when nitrification was stimulated by adding \( \text{NH}_4^+ \), the \( \text{NO}_3^- \) produced could not be utilized by denitrification, causing \( \text{NO}_3^- \) to efflux to the bottom water. The magnitudes of the alternative \( \text{NO}_3^- \) reduction pathways, anammox and DNRA, in the area were small. The rates of DNRA remained low in all seasons, and anammox appeared only in late autumn when the nitrate-reduction rates altogether were low. The denitrification rates in the Gulf of Finland have decreased from the values measured in the early 2000s; this suggests that sediment denitrification might be a less important sink for fixed nitrogen than was previously assumed. It is also noteworthy that the natural capacity of the coastal sediments to mitigate the nitrogen load was at its lowest during spring, when the flux of nitrogen from the drainage basin is highest.

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