Spatial and temporal variability of denitrification in the sediments of the northern Baltic Proper

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ABSTRACT: Denitrification in the sediments of the open northern Baltic Sea was measured using the isotope pairing method. The highest denitrification activity was found in the central Gulf of Finland, where denitrification varied between 150 and 650 μmol N m^{-2} d^{-1} The bulk of the denitrification was coupled to the NO_3^- production by nitrification. The mass of benthic fauna was found to be the most significant factor affecting the rate of denitrification. Denitrification rate was highest in late summer and early autumn. Calculated as an average for the Gulf of Finland, the denitrification rate obtained in the present study is 45 kt N yr^{-1}. This gives a release of ca 30% of the external N input by denitrification.

KEY WORDS: Denitrification · Baltic Sea Isotope pairing method Sediment

INTRODUCTION

Denitrification is the bacterial process in which NO_3^- and NO_2^- are successively reduced to gaseous N_2. The process occurs mainly in the absence of oxygen and is centered at the oxic-anoxic interface where denitrifying bacteria have access to NO_3^-. These kinds of interfaces appear both in sediment in the littoral and profundal zones and in the water above anoxic deeps. Denitrification removes N from aquatic ecosystems, and partly counteracts the development of eutrophication, especially in aquatic systems where N is the limiting nutrient for phytoplankton growth. In the past decades, the nutrient concentrations as well as phytoplankton growth have shown increasing trends in the northern Baltic Sea (Wulff et al. 1990, Perttilä et al. 1995, Rahm et al. 1996) due to a large input of nutrients by human activities. In recent years, it has been shown that N is the most limiting nutrient in the Baltic Proper and the Gulf of Finland (e.g. Granéli et al. 1990, Kivi et al. 1993), and P in the Bothnian Bay (e.g. Alasaarela et

al. 1986, Granéli et al. 1990). At the same time, however, discussion has been going on about the necessity of reducing N in waste water (Rinne 1988, Granéli et al. 1990, Seppänen 1992, Tamminen 1992a, b) with one open question being the intensity of the 'natural' purification by denitrification.

Denitrification has been calculated to efficiently remove N from the Baltic Sea ecosystem and therefore counteract eutrophication (Shaffer & Rönner 1984, Rönner 1985). Shaffer & Rönner (1984) calculated that 80 to 90% of denitrification takes place in sediments. However, direct measurements of denitrification in the open Baltic Sea have been carried out (and published) mostly with regard to the water column (Rönner & Sörensson 1985, Brettar & Rheinheimer 1991, 1992) with only 1 sediment study in the Gulf of Bothnia (Stockenberg & Johnstone 1997). All these studies used the acetylene blockage method. This method has some pitfalls, especially when applied to sediment samples, including the inhibition of nitrification providing NO_3^- for denitrification, and the reversal of the blockage of N_2O reductase by sulfide (Sørensen et al. 1987, Seitzinger et al. 1993, Lohse et al. 1996).

This study presents direct measurements of denitrification in the sediments of the open northern Baltic Sea.

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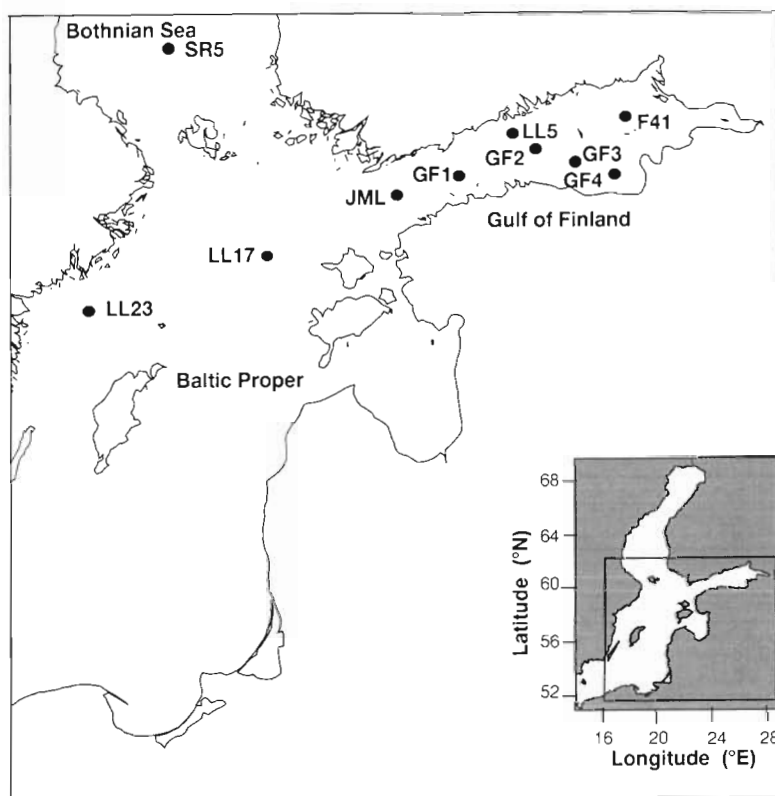**Present address: Academy of Finland, PO Box 99, FIN-00501 Helsinki, Finland

Fig. 1. The sampling stations in the northern Baltic Sea

The sampling stations represented depositional areas in the Gulf of Finland, in the Bothnian Sea and in the northern Baltic Proper. Denitrification was measured with the isotope pairing method (Nielsen 1992), which is a powerful method to study denitrification without major disturbances to the samples. In this method, sediment samples are incubated with $^{15}NO_3^-$, which, after diffusion to the denitrification zone, pairs with the indigenous $^{14}NO_3^-$. Total denitrification and denitrification based on the NO_3^- produced in the sediment by nitrification and on the NO_3^- diffusing from the water can then be calculated from the amount of $^{29}N_2$ and $^{30}N_2$ formed.

MATERIAL AND METHODS

Sediment samples were collected aboard RV 'Viktor Bujnickij' (in January 1996), RV 'Alkor' (in July 1997) and RV 'Aranda' (all other sampling occasions) during cruises in the northern Baltic Proper and southern Bothnian Sea in 1994 to 1997. The sampling stations represented depositional areas in the northern Baltic Sea (Winterhalter 1972, Kankaanpää et al. 1997) with depths ranging from 30 to 446 m (Figs. 1 & 2). Sediment was

sampled with a Gemini twin-corer (corer tube Ø 8 cm). Water samples for the analyses of NO_3^- and NO_2^- (Grasshoff 1983) and O_2 concentrations (Winkler titration) were collected from the near-bottom water (ca 1 to 2 m above the bottom) on the same days and analysed immediately aboard (except NO_3^- and NO_2^- on the RV 'Alkor' cruise, which were frozen and analysed later). Temperature and salinity were determined with a SeaBird CTD probe.

Sediment denitrification was assayed with the isotope pairing method (Nielsen 1992). In each Gemini twin-corer tube, 3 replicate incubation cores (plastic cylinders with a height of ca 100 mm and Ø 26 mm) were pushed into the sediment so that about half of the core length was filled with sediment and the rest with near-bottom water. The top of the incubation core was closed with a cap, the cores were gently lifted from the sediment, and the bottom was closed. In a temperature controlled room set at the near-bottom temperature, $K^{15}NO_3$ solution (99 atom%, Europa Scientific Ltd) was added to the water phase of the incubation cores near the sediment surface. The concentration used in normal incubations was 100 µM. The cores were then closed with caps equipped with magnetic stirring bars and incubated in the dark at in situ temperature with the stirring bars rotating slowly. After the incubation, the denitrification was stopped by adding 1 ml of $ZnCl_2$ (1 g ml^{-1}) to the water phase of the cores, and the cores were gently mixed using a glass stick. This procedure has been found by sequential mixing and resampling to cause only a 3% loss of $^{15}N_2$ (unpubl. results). Thereafter, part of the slurry was transferred using a 10 cm piece of Tygon tubing fitted to a plastic syringe into two 10 ml gas-tight exetainers containing 250 µl of the $ZnCl_2$ solution to prevent further microbial activity. In 1994-96 both of the 2 subsamples were
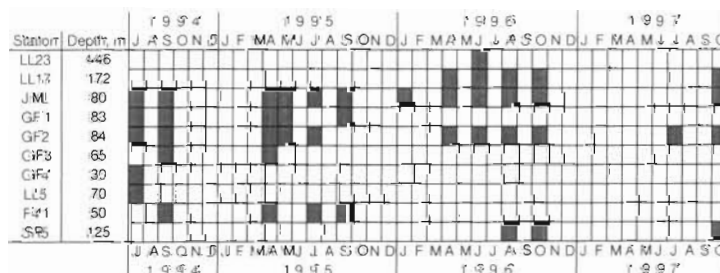

Fig. 2. Frequency of sampling for the stations

analysed, but in 1997 the second subsample was only analysed if there were problems with the analysis of the first one (since the variability between the subsamples was low, see 'Results'). After replacing 4 ml of the suspension in the exetainer with helium gas followed by vigorous shaking, the mass ratios of N_2 in subsamples of the gas phase were analysed using a mass spectrometer at the National Environmental Research Institute in Silkeborg, Denmark.

For the calculation of results, the water content (w, by drying at 70°C) and the porosity (p) of the samples were determined. The porosity was calculated as:

$$p = (w\rho)/[100 + w(\rho - 1)] \qquad (1)$$

where $\rho = 2.6$.

The denitrification rate based on the natural $^{14}NO_3^-$ was calculated from the ratios of $^{29}N_2$ ($^{14}N^{15}N$) and $^{30}N_2$ ($^{15}N^{15}N$) formed during the incubation. These were calculated by dividing the 29 and 30 currents given by the mass spectrometer by the current of 28. Atmospheric air was used as reference and analysed after every 5 or 6 samples. The average of the reference samples before and after the actual samples was subtracted from the results. The results so obtained were transferred into µM by multiplying them by the solubility of N_2 to sea water at *in situ* temperature and salinity (Kester 1975).

The denitrification rate based on the added $^{15}NO_3^-$ (D_{15}) is the sum of all ^{15}N species formed (Nielsen 1992):

$$D_{15} = (^{14}N^{15}N) + 2(^{15}N^{15}N) \qquad (2)$$

When the added $^{15}NO_3^-$ and the natural $^{14}NO_3^-$ are uniformly mixed in the sample, the *in situ* denitrification rate based on natural $^{14}NO_3^-$ (D_{14}) is:

$$D_{14} = D_{15}(^{14}N^{15}N)/[2(^{15}N^{15}N)] \qquad (3)$$

The denitrification rate obtained was changed into µmol N m^{-2} d^{-1} by multiplying D_{14} by the total water volume of the sample (= volume of water phase + volume of sediment × porosity) and by dividing by the surface area of the sample and the incubation time.

D_{14} can be divided into denitrification based on NO_3^- diffusing into the sediment from the overlying water (D_w) and on NO_3^- produced in the sediment by nitrification (D_n) (Nielsen et al. 1996). D_w is calculated using the ratio of $^{14}NO_3^-$ and $^{15}NO_3^-$ available in the water phase:

$$D_w = D_{15}a/b \qquad (4)$$

where a = natural concentration of NO_3^- in the near-bottom water, and b = added concentration of $^{15}NO_3^-$
D_n is thereby:

$$D_n = D_{14} - D_w \qquad (5)$$

The optimal incubation time was determined by incubating samples for 1, 3 and 5 h. The time series incubations were performed at Stns F41, GF1 and JML. The first-order kinetics and the minimization of the formation of unmeasurable $^{14}N^{14}N$ pairs were ensured by incubating the samples with increasing concentrations of $^{15}NO_3^-$ (10 to 100 µM) and by plotting the D_{15} and D_n results obtained with the concentration used (Nielsen 1992). These experiments were performed at Stns LL17, JML and GF2.

The sources of variation in the denitrification measurements were identified by comparing coefficients of variation between ^{15}N measurements from a single incubation core (2 subsamples), between the 3 incubation cores collected from a single Gemini core and between the Gemini cores.

RESULTS

Methodological tests

The time series incubations showed that the denitrification was linear for at least 5 h at Stns F41 and JML, whereas at GF1 large variation in results was found after 5 h incubation (Fig. 3). Sediment oxygen consumption measured during the same cruises as the denitrification was between 7 and 30% (on 4 occasions 30 to 40%) during 3 h incubation (Haahti & Karjala unpubl. results). This gives the upper limit for incubation time in the denitrification measurements since a major decline in the O_2 concentration during the incubation may affect the denitrification rates directly or through changes in the nitrification rates. Consequently, 3 h incubations were used in all of the later samplings.

The kinetic experiments revealed that the saturation of D_{14} was achieved using $^{15}NO_3^-$ addition of ca 100 µM (Fig. 4a, c). Thus, at that concentration, the production of the unmeasurable $^{14}N^{14}N$ was minimized. The addition of 100 µM $^{15}NO_3^-$ (which is a very high concentration compared to the natural concentration) did not affect the first-order kinetics of denitrification, which can be seen from the linear increase in D_{15} as the $^{15}NO_3^-$ concentration increased (Fig. 4b, d). Consequently, for these types of sediments, 100 µM of $^{15}NO_3^-$ was the ideal concentration. Stn LL17 had very little O_2 (see Fig. 5) which theoretically explains why D_{15} became nonlinear above 50 µM and why D_n consequently decreased (Fig. 4e, f).

The subsamples taken from a single incubation core for the ^{15}N analysis showed little variation (coefficient of variation 9.9%). Consequently, in 1997 only 1 subsample was measured. The coefficient of variation between incubation cores pushed into 1 single Gemini core was 26.2%, and between cores collected from a
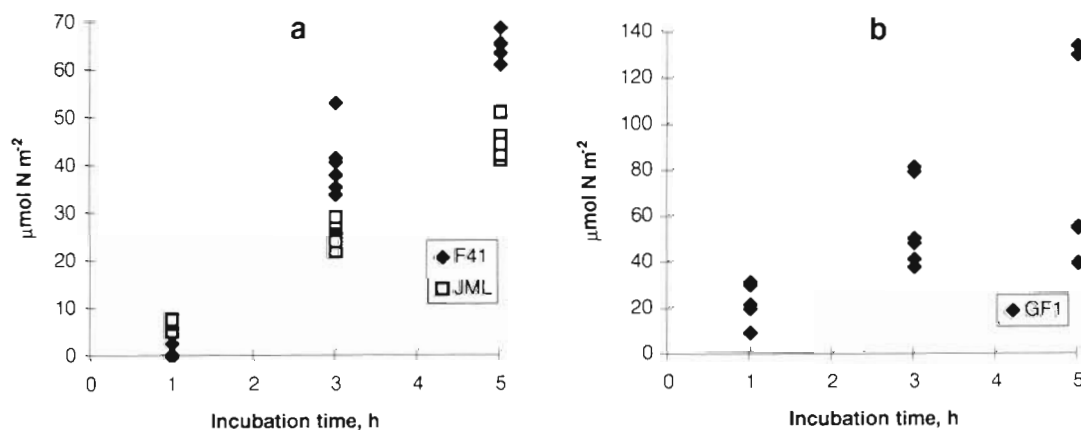
Fig. 3. Results from the time series incubations for samples collected from Stns (a) F41, JML and (b) GF1

single station 34.1%. Thus, a large part of the total variation was detected within 1 corer tube suggesting that the variability on the sea floor occurred on a cm scale.

Hydrographical data

The salinity in the near-bottom water was 5.5 to 6.5 in the Bothnian Sea and in the eastern Gulf of Finland. Westwards of this in the Gulf of Finland, the salinity increased to ca 7.5–8.5 at Stn GF2 and to 8.5–9.5 at JML at the entrance to the Gulf of Finland. In the northern Baltic Proper (Stns LL17 and LL23), the salinity was 10 to 10.5. The temperature varied between 0.8 and 4.9°C, with the lowest temperatures at the shallowest stations.

The O_2 and NO_3^- concentrations near the bottom are shown in Fig. 5. The O_2 concentration was lowest at Stns LL23 and LL17 in the northern Baltic Proper (Fig. 5e, f), and highest at SR5 in the Bothian Sea (Fig. 5f) and at F41 in the eastern end of the Gulf of Finland (Fig. 5d). The NO_3^- concentration in the near-bottom water was lowest at Stn SR5 in the Bothnian Sea (ca 5 µM; Fig. 5f). In the Baltic Proper (Stns LL17 and LL23), the concentration was slightly above 10 µM, while in the Gulf of Finland it fluctuated around 10 µM (Fig. 5). The NO_2^- concentration was always very low (0 to 0.2 µM, except for 0.7 µM at LL17 in August 1996 and 1.0 µM at F41 in July 1995).

Denitrification

The highest denitrification activity was found in the central Gulf of Finland, at Stns GF1, LL5, GF2 and GF3, where denitrification varied between 150 and 650 µmol N m^{-2} d^{-1} (Fig. 6b, c, f). JML, the station at the

entrance to the Gulf of Finland and with a water depth of about 80 m, showed lower denitrification activity (100 to 400 µmol N m^{-2} d^{-1}) than the other stations in the Gulf of Finland (Fig. 6a). Denitrification rate was statistically significantly higher (2-tailed t-test, p = 0.039) at GF2 than at JML (the 2 stations with most intense sampling). At the eastern end of the Gulf of Finland, at Stn F41, denitrification was 100 to 300 µmol N m^{-2} d^{-1} (Fig. 6d). At the deep stations in the northern Baltic Proper, denitrification rate was 15 ± 9 µmol N m^{-2} d^{-1} (± SD) at LL23 (446 m) and 125 to 300 µmol N m^{-2} d^{-1} at LL17 (172 m; Fig. 6e). The result from Stn LL23 is not shown in Fig. 6 due to a different order of magnitude. In addition, the denitrification at Stn LL23 was measured from the top centimeter of the sediment since, due to the loose structure of the sediment, subsampling with the incubation cores was not possible at this station.

At Stn SR5 in the Bothnian Sea, denitrification was between 250 and 300 µmol N m^{-2} d^{-1} (Fig 6f). However, at that station negative values were sometimes observed and very high standard deviation was found between replicates (see legend for Fig. 6f). On 1 occasion (August 1996), variation was enormous, with an average of −2200 µmol N m^{-2} d^{-1} and SD 6400 µmol N m^{-2} d^{-1}

Denitrication was usually at its highest in late summer and early autumn, from July to September (Fig. 6a, b) The lowest activity was found in spring during April and May. The only measurement carried out during winter, at Stn JML in January 1996, showed very high denitrification activity (400 µmol N m^{-2} d^{-1}), actually the highest measured at JML (Fig. 6a).

At all the other stations except LL17, the bulk of the denitrification was based on NO_3^- produced in the sediment by nitrification, i.e. coupled nitrification-denitrification (D_n; Fig. 6). At LL17, D_n was less than half of the total denitrification (Fig. 6e). The highest D_n per-
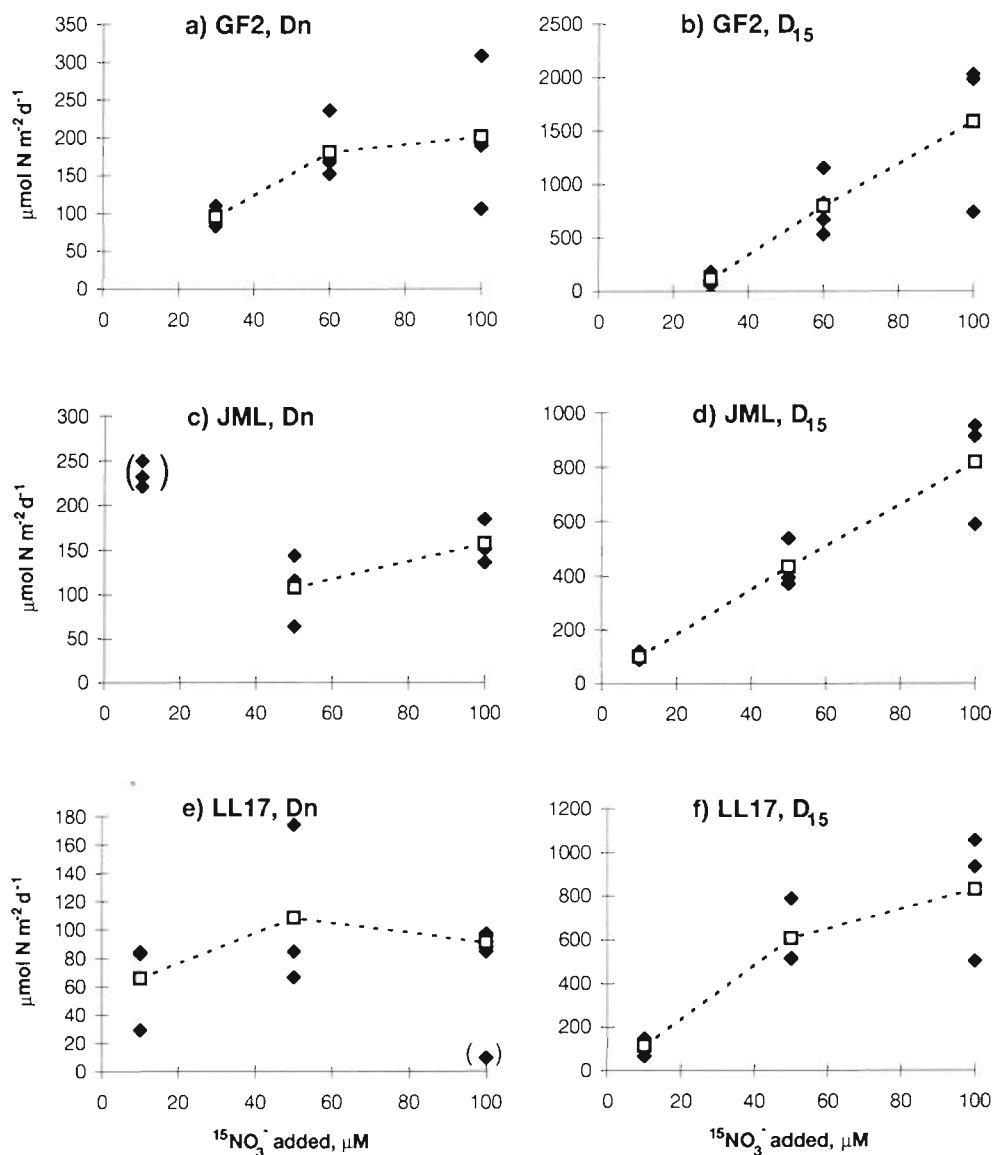
Fig. 4. Saturation kinetic incubations for Stns GF2, JML and LL17. Coupled nitrification-denitrification (D_n; left panel) and denitrification based on added $^{15}NO_3^-$ (D_{15}; right panel) as a function of the concentration of added $^{15}NO_3^-$ (♦) Replicates; (--□--) averages of replicates

centages were found at Stn SR5 in the Bothnian Sea (97 to 100%; Fig. 6f) and at F41 at the eastern end of the Gulf of Finland (86 to 94%; Fig. 6d). No clear differences were found in the D_n percentages between seasons.

Total denitrification correlated positively with wet mass of benthic fauna and negatively with depth (Table 1). The positive correlation with bacterial production was almost significant at the $p \leq 0.05$ level as well. D_w correlated positively with NO_3^- concentration, salinity and temperature in the overlying water, and negatively with O_2 concentration (Table 1). These 4 variables correlated significantly with each other, too

(data not shown). D_n correlated negatively with depth and salinity, and positively with O_2 concentration (Table 1). Benthic fauna had a positive correlation both with D_w and with D_n as well.

DISCUSSION

Methodological questions

One precondition in the isotope pairing technique is a uniform mixing of added $^{15}NO_3^-$ and natural $^{14}NO_3^-$ in the denitrification sites (Nielsen 1992). If uniform
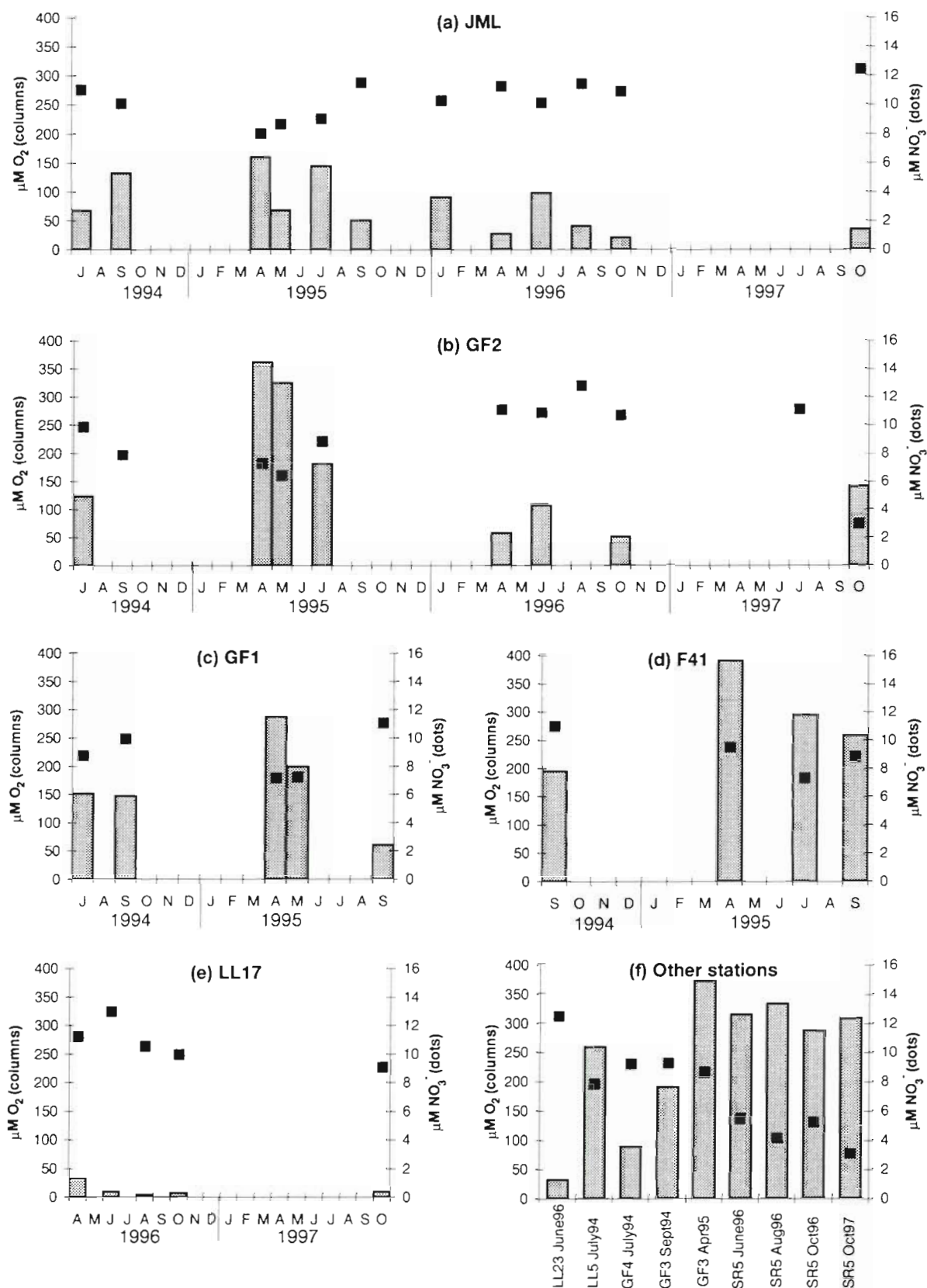
Fig. 5. O_2 concentration (columns, left axis) and NO_3^- concentration (■, right axis) at stations (a) JML, (b) GF2, (c) GF1, (d) F41, (e) LL17 and (f) other stations. No column: O_2 concentration not analysed
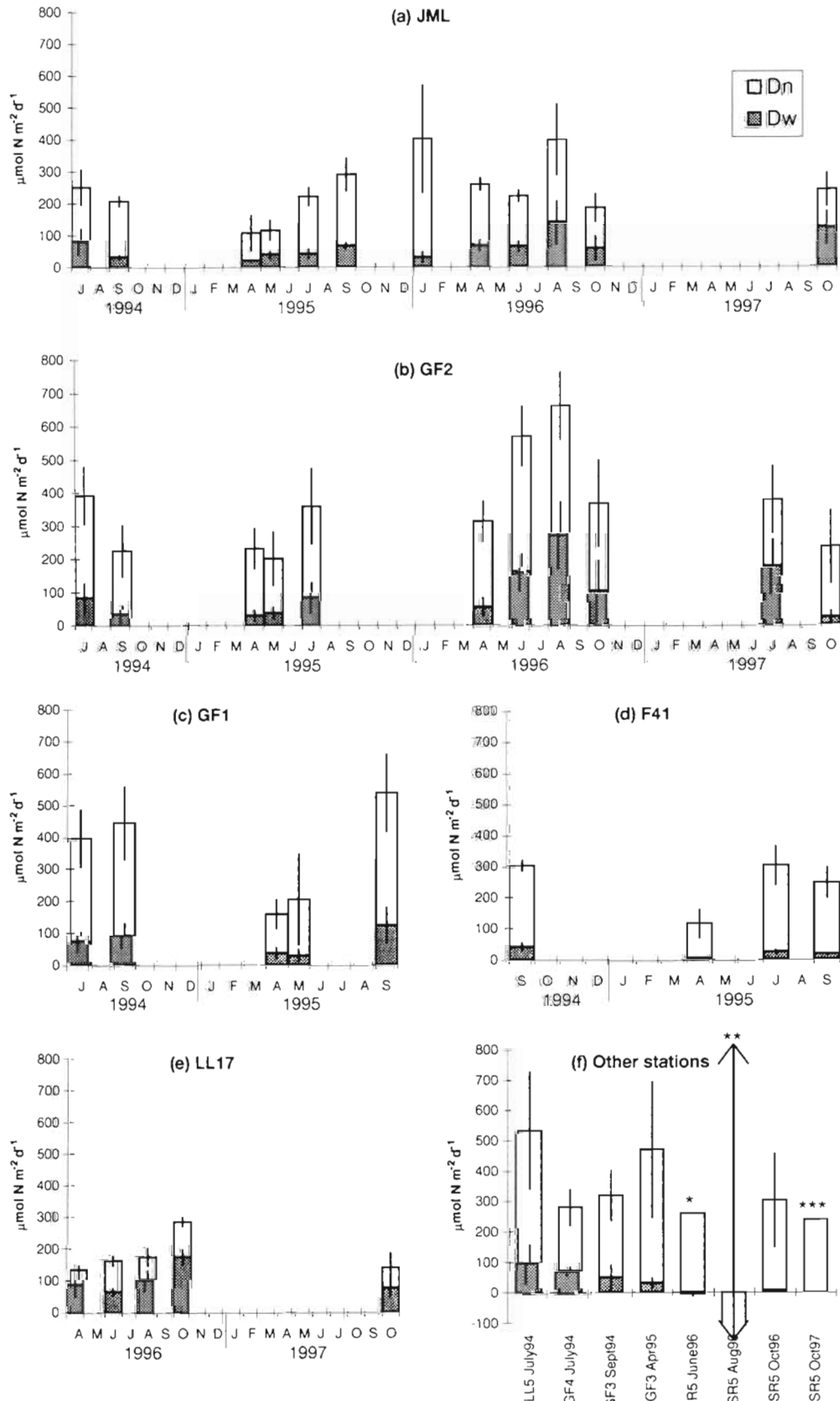
Fig. 6. Denitrification based on coupled nitrification-denitrification (D_n) and denitrification based on overlying water NO_3^- (D_w) at Stns (a) JML, (b) GF2, (c) GF1, (d) F41, (e) LL17 and (f) other stations. Error Bars denote ± SD. Total denitrification = $D_n + D_w$. (*) SD for D_n 1170 µmol N m^{-2} d^{-1}; (**) total denitrification −2220 ± 6447 µmol N m^{-2} d^{-1}, D_w 0.31%; (***) SD for D_n 530 µmol N m^{-2} d^{-1}

Table 1. Correlation between total denitrification, denitrification based on overlying water NO_3^- (D_w) and coupled nitrification-denitrification (D_n) with various environmental parameters. Number of observations in parentheses. •Significant at $p \leq 0.05$ level

	Denitrification	D_w	D_n
Denitrification	1		
D_w	0.52• (48)	1	
D_n	0.92• (48)	0.15 (48)	1
Depth	−0.34• (48)	−0.05 (48)	−0.37• (48)
NO_3^-	0.01 (48)	0.55• (48)	−0.24 (48)
NO_2^-	−0.10 (48)	0.02 (48)	−0.12 (48)
Salinity	−0.27 (38)	0.41• (38)	−0.51• (38)
Temperature	−0.01 (38)	0.35• (38)	−0.17 (38)
O_2	0.15 (45)	−0.58• (45)	0.36• (45)
O_2 consumption[a]	−0.18 (30)	−0.35 (30)	−0.05 (30)
Bacterial production[a]	0.45 (14)	0.27 (14)	0.33 (14)
Benthic fauna[a]	0.54• (21)	0.46• (21)	0.51• (21)
Pontoporeia/ Monoporeia[a]	0.30 (21)	0.22 (21)	0.32 (21)
Meiofauna[a]	−0.08 (13)	−0.05 (13)	−0.08 (13)

[a]Data on O_2 consumption by the sediment, bacterial production in the sediment (as leucine uptake), benthic fauna (wet mass), Pontoporeia/Monoporeia (abundance) and meiofauna (abundance) kindly provided by the scientists at the Finnish Institute of Marine Research

mixing is not achieved. denitrification rate will be underestimated, since unmeasurable $^{14}N^{14}N$ pairs will be formed. This leads to an underestimation in the calculations. Correct results can be achieved by using a sufficiently high concentration of $^{15}NO_3^-$ after testing the saturation of D_{14}. However, it has to be ensured that the high concentration added will not affect the assumed first-order kinetics of denitrification. This is achieved by analysing the potential denitrification (D_{15}) as a function of the added concentration. In the present study, an optimal concentration was found to be 100 µM, which is in accordance with results obtained by Pelegrí et al. (1994) and Jensen et al. (1996). Middelburg et al. (1996) concluded after model simulation that the D_{14} estimate is reliable even at high $^{15}NO_3^-$ concentrations in the water and with a

substantial overlap of nitrification and denitrification zones.

SR5, the station in the Bothnian Sea, sometimes showed negative denitrification rates and an enormous variation between replicates (see legend for Fig. 6). At that station, the $^{15}N^{15}N$ production was at the detection limit with the present incubation time and set up. Therefore, minor variations in the $^{15}N^{15}N$ ratio had tremendous effects on the calculated D_n. The incubation time of 3 h might have been too short at this well oxygenated station for the $^{15}NO_3^-$ to diffuse in and for enough $^{15}N_2$ to accumulate.

Spatial and temporal variability of denitrification

The rates of denitrification measured from the northern Baltic Proper, the Gulf of Finland and the Bothian Sea were comparable with the rates measured for other estuarine and marine environments (Koike & Sørensen 1988, Seitzinger 1988, and Table 2). However, the rates measured from the northern Baltic Proper (Stns LL17 and LL23) were lower than e.g. rates measured from the eastern North Pacific continental shelf at comparable depths (Table 2). Evidently, the deep areas of the Baltic Sea have lower denitrification rates than areas in the oceans with the same depths, presumably due to anoxic conditions. Koop et al. (1990) estimated denitrification rates from sediment-water nutrient fluxes and O:N flux ratios in the northern Baltic Proper for 3 stations with depths of 47, 82 and 130 m. According to the results, no denitrification was calculated to occur at the deepest station, whereas at the 82 m station the relative N loss was largest. The mean rate of denitrification at the 2 shallowest stations (1.2 mmol N m^{-2} d^{-1}; Koop et al. 1990) is higher than the results obtained in the present study for stations at the same depths.

The mean denitrification rate in the Gulf of Finland, ca 300 µmol N m^{-2} d^{-1} = 4.2 mg N m^{-2} d^{-1}, is about 1/6 of the denitrification rate as calculated for the sediment of the Baltic Proper (25 mg N m^{-2} d^{-1}) by Shaffer & Rönner (1984). The rates measured at Stns LL17 and LL23 in the Baltic Proper (present study) are even much lower. Rönner (1985) found in his calculations for the Baltic Proper an unexplained N loss of 247 kt N yr^{-1}, which he assumed to be denitrification in the sediments of the Gulf of Finland and the shallow Baltic Proper. The mean denitrification rate found in the present study for the Gulf of Finland multiplied by the area of the Gulf of Finland (29.6×10^3 km^2) gives denitrification of 45 kt N yr^{-1} Similarly, this is about 1/6 of the unexplained loss of Rönner (1985).

The highest denitrification activity found in the central Gulf of Finland is most obviously attributable to

Table 2. Recent estimates of denitrification and coupled nitrification-denitrification (D_n) in estuarine and marine environments

Location	Denitrification (μmol N m^{-2} d^{-1})	D_n	Method	Source
Gulf of Finland, Baltic Sea	100–650	54–95%	Isotope pairing method	This study
Northern Baltic Proper	15–300	39–67%	Isotope pairing method	This study
Northern Baltic Proper	1200		Non-stoichiometric loss of nitrogen + NO$_3^-$ flux to sediment	Koop et al. (1990)
Gulf of Bothnia. Baltic Sea	0–940		Acetylene blockage method	Stockenberg & Johnstone (1997)
Norsminde Fjord, Denmark	3900–5300	16–31%	Isotope pairing method	Rysgaard et al. (1993)
Norsminde Fjord, Denmark	2100–5200	8–27%	Isotope pairing method	Risgaard-Petersen et al. (1994)
North Sea	200–300	95%	Isotope pairing method	Lohse et al. (1996)
Wadden Sea, Germany	0–400	25–31%	Isotope pairing method	Jensen et al. (1996)
River Colne estuary, England	80–11000	0–81%	Isotope pairing method	Ogilvie et al. (1997)
River Great Ouse estuary, England	520–2300		C$_2$H$_2$ blockage	Trimmer et al. (1998)
Nueces and Guadalupe Estuaries, Texas	200–3400	'Tight coupling between nitrification and denitrification'	N$_2$ prod. in gas-tight chambers	Yoon & Benner (1992)
Gulf of Maine	700–1000		Non-stoichiometric loss of nitrogen	Christensen et al. (1996)
Boston Harbor	2600		N$_2$ prod. in gas-tight chambers	Nowicki et al. (1997)
Massachusetts Bay	1100	'Most of the N loss was apparently from coupled nitrification-denitrification'	N$_2$ prod. in gas-tight chambers	Nowicki et al. (1997)
Chesapeake Bay	0–2000	'Denitrification generally controlled by nitrification'	C$_2$H$_2$ blockage, NO$_3^-$ balance	Kemp et al. (1990)
North Atlantic continental shelf	700		Model (denitrif. = 0.019 × phytoplankton prod.)	Seitzinger & Giblin (1996)
Eastern North Pacific continental shelf	3200	'Most of the N$_2$ production is coupled to nitrification'	In situ benthic flux chamber	Devol (1991)
Eastern North Pacific continental shelf	800–4800	'A significant within-sediment source'	In situ benthic flux chamber	Devol & Christensen (1993)
Western Arctic shelf	1000–2000		In situ benthic flux chamber	Devol et al. (1997)

higher mass of benthic fauna in that region (data not shown). This is supported by the significant correlation found between benthic fauna and denitrification (Table 1), and was also found previously (Henriksen & Kemp 1988, Kristensen 1988, Seitzinger 1988, Pelegrí et al. 1994). Since the abundance of benthic fauna fluctuates strongly in the Baltic Sea, depending on the hydrographical regime (Laine et al. 1997), the rate of denitrification may have large temporal variations as well. The years 1994 to 1996 during the present study represented a time-span of very high abundance of benthic fauna, after which the abundance collapsed to about 1/10 (Laine pers. comm.).

Since the bulk of the denitrification was coupled to the NO$_3^-$ production by nitrification, other factors which regulate the rate of nitrification, e.g. supply of NH$_4^+$ or O$_2$ conditions, may also be important. A close coupling of these 2 processes has been found in many marine systems (Seitzinger 1988, Kemp et al. 1990, Devol 1991, Yoon & Benner 1992, Devol & Christensen 1993, Lohse et al. 1996). Since nitrification is an oxic process and denitrification mainly anoxic or suboxic, the most efficient coupling of nitrification and denitrification can be assumed to occur under moderate O$_2$ concentrations where the 2 processes can be situated close to each other. At the eastern end of the Gulf of

Finland at Stn F41, the O_2 concentrations were probably too high for efficient coupling. This is also supported by the very low rate of D_w, indicating that denitrification may have been restricted to deeper sediment layers (Fig. 6d; cf. Christensen et al. 1990).

The very low O_2 concentrations (4.5 to 31 µM 1 m above the bottom) at the deep Stns LL17 and LL23 in the northern Baltic Proper obviously limited nitrification and therefore denitrification. The lower percentage of D_n compared to the other stations (Fig. 6e) further supports this. In addition, HS^- has been found to inhibit denitrification (Sørensen et al. 1987) and nitrification (e.g. Joye & Hollibaugh 1995). Although HS^- was not found in our samples 1 m above the bottom (data not shown), the sediment may well have contained it.

The effect of benthic fauna in the activation of denitrification has 2 routes. Directly, D_w is activated by the pumping of NO_3^- from the overlying water into the burrows in the sediment. Indirectly, nitrification is high in the burrow walls (e.g. Kristensen 1988), which therefore activates D_n. Other factors significant in the correlation analysis for D_w and D_n, i.e. NO_3^- concentration, salinity, temperature and O_2 concentration, all correlated significantly with each other. They reflect the presence of old, deep water from below the halocline with higher salinity and nutrient concentration, and lower O_2 concentration. The presence of old, deep water was found to activate D_w but suppress D_n. The non-periodical inflows of water to the Baltic Sea from the North Sea fill the basins in the Baltic Proper with highly saline water containing high temperature and O_2 concentration (Matthäus & Lass 1995). During stagnation the salinity, temperature and O_2 concentration of this deep water decrease until the old water is replaced by the next inflow. The old water mass then continues its way towards the Gulf of Finland along the bottom. If long stagnation periods occur, the basins become filled with H_2S and no denitrification can therefore be expected to happen since NO_3^- cannot be produced by nitrification.

The denitrification rate was highest in late summer and early autumn. It has been found in many studies that denitrification was high in spring after the spring phytoplankton bloom with possibly another peak in the autumn (Jørgensen & Sørensen 1988, Jørgensen 1989, Kemp et al. 1990, Jensen et al. 1996). However, Smith et al. (1985) and Yoon & Benner (1992) found highest denitrification in late summer It must be noted that all these studies were made in relatively shallow areas where sedimentation of algae may have a more direct effect on the benthic processes. At Stns GF2 and JML (depths 84 and 80 m, respectively), other factors are probably of greater importance since the algal material is largely decomposed when reaching the sediment (cf. Wassmann 1990). However, the reason for the seasonal rhythm remained unclear.

The peak of denitrification observed in January 1996 at Stn JML is surprising since the input of settling material is scarce at the end of the year. As there was only 1 measurement carried out during winter, it must be viewed with caution. However, Devol et al. (1997) have likewise found that the denitrification rate in the western Arctic shelf sediment did not differ between August-September and March although the sediment in March had not received fresh autochthonous settling material for months due to ice cover.

Importance of denitrification in N cycling

In estuaries, an average of 20 to 50% of external N input has been found to be released by denitrification (Smith et al. 1985, Jørgensen & Sørensen 1988, Seitzinger 1988, Yoon & Benner 1992, Christensen et al. 1996, Stockenberg & Johnstone 1997). Calculated as an average for the area of the Gulf of Finland, the denitrification rate obtained in the present study is 45 kt N yr^{-1}. The loading of N to the Gulf of Finland via rivers and by direct discharges from coastal municipalities and industries was estimated to be 140 kt in 1990, and the annual total (wet + dry) atmospheric deposition ca 13 kt in the late 1980s (HELCOM 1996), together giving a yearly N input of ca 153 kt. This gives release of ca 30% of the external N input by denitrification. However, this is only an estimate since denitrification was calculated from results obtained only from the open area of the Gulf of Finland. Nevertheless, the calculation probably gives a fairly correct estimate since the area of the open waters is by far larger than the area of the shallow and littoral waters. In addition, the area of the erosion bottoms where no or very little denitrification can be assumed to occur may be compensated for by the relatively more intense denitrification in the littoral areas. The value obtained is in good accordance with the 23 and 31% obtained for the Bothnian Bay and the Bothnian Sea, respectively, by Stockenberg & Johnstone (1997).

The release of N_2O during denitrification may also remove N from the ecosystem. However, the formation of N_2O during denitrification has been found to be minor (0 to 10%) in marine systems (Smith & DeLaune 1983, Seitzinger 1988, Capone 1991); N_2O may even be consumed during denitrification (Capone 1991). N_2O, although advantageous for the N removal from eutrophicated marine systems together with N_2, is harmful as a greenhouse gas (Dickenson & Cicerone 1986) and a participant in the destruction of stratospheric ozone (Cicerone 1987).

Although release of N by denitrification was quite efficient in the Gulf of Finland, it cannot be counted on that denitrification will compensate for increased N loading. Seitzinger & Nixon (1985) have found that under experimental nutrient addition, although denitrification rate increased, the amount of N removed was a constant or progressively smaller fraction of the N input. In an extreme situation, observed in the Chesapeake Bay (Kemp et al. 1990), denitrification could be totally eliminated during midsummer since anoxic conditions led to the elimination of nitrification and subsequent coupled denitrification. This leads to a vicious circle where eutrophication catalyzes itself through repression of denitrification. In the Baltic Sea, this possibility is further enabled since the non-periodical inflows of highly saline water cause anoxic periods which can be prolonged and extended by eutrophication.

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