Denitrification and nitrous oxide effluxes in boreal, eutrophic river sediments under increasing nitrate load: a laboratory microcosm study

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Abstract Intact sediment cores from rivers of the Bothnian Bay (Baltic Sea) were studied for denitrification based on benthic fluxes of molecular nitrogen (N_2) and nitrous oxide (N_2O) in a temperature controlled continuous water flow laboratory microcosm under 10, 30, 100, and 300 μM of ^{15}N enriched nitrate (NO_3^-, ~98 at. %). Effluxes of both N_2 and N_2O from sediment to the overlying water increased with increasing NO_3^- load. Although the ratio of N_2O to N_2 increased with increasing NO_3^- load, it remained below 0.04, N_2 always being the main product. At the NO_3^- concentrations most frequently found in the studied river water (10–100 μM), up to 8% of the NO_3^- was removed in denitrification, whereas with the highest concentration (300 μM), the removal by denitrification was less than 2%. However, overall up to 42% of the NO_3^- was removed by mechanisms other than denitrification. As the microbial activity was simultaneously enhanced by the NO_3^- load, shown as increased oxygen consumption and dissolved inorganic carbom efflux, it is likely that a majority of the NO_3^- was assimilated by microbes during their growth. The ^{15}N content in ammonium (NH_4^+) in the efflux was low, suggesting that reduction of NO_3^- to NH_4^+ was not the reason for the NO_3^- removal. This study provides the first published information on denitrification and N_2O fluxes and their regulation by NO_3^- load in eutrophic high latitude rivers.

Keywords Stable isotopes · Eutrophication · NO_3^- removal · N_2O/N_2 ratio · Bothnian Bay · River sediment
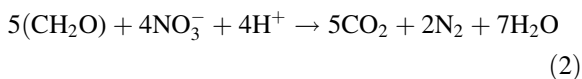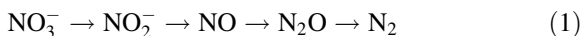
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Introduction

The Bothnian Bay is the northernmost part of the Baltic Sea, which is receiving an increasing nutrient and organic carbon load primarily via Finnish rivers. During the years 1995–2000, the largest 24 rivers brought an annual average of 47,200 tons of N to the Bothnian Bay, which accounts for >90% of the total annual N load (Kronholm et al. 2005). The Bothnian Bay differs substantially from other parts of the Baltic Sea: (a) The area is less saline due to physical separation from the Baltic proper by shallow sills at the Archipelago Sea and a large freshwater inflow, (b) the open sea area of the Bothnian Bay is in a near-pristine state and harmful cyanobacterial blooms are rarely observed and (c) primary production in the

open sea area is limited by phosphorus (P) instead of nitrogen (N).

Microbial processes in estuarine sediments have been estimated to remove up to 90% of the external N input and can thus have importance in controlling anthropogenic N loading to seas (Seitzinger 1988). N removal capacity of watersheds and sediments has received growing interest during recent decades due to increased anthropogenic N loading and its effects on the recipient waterbodies (Jenkins and Kemp 1985; Bange et al. 1996; Middelburg et al. 1996). In non-vegetated sediments, NO_3^- can be removed from overlying water by four different microbiological processes: denitrification, anaerobic ammonium oxidation, dissimilatory nitrate reduction to ammonium and assimilation to microbial biomass.

The most studied of the four processes, and the one that has been found to be most important, is denitrification, which is reduction of NO_3^- via nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) to dinitrogen (N_2) (Eq. 1). In denitrification, two moles of NO_3^- are reduced to one mole of N_2 (Eq. 2).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \qquad (2)$$

Denitrifying bacteria are facultative aerobes using NO_3^- as an electron acceptor when oxygen is limited. As a form of heterotrophic metabolism, denitrification is dependent on the supply of labile organic carbon. In freshwater, estuarine and coastal sediments, denitrification occurs in the suboxic layer a few millimeters to centimeters below the sediment-water interface, and directly below the oxic layer where nitrification (i.e., the aerobic oxidation of NH_4^+ to NO_3^-) occurs. Denitrification is thus regulated by the transport of NO_3^- and O_2, principally by molecular diffusion along concentration gradients to the site of biological reaction within the sediments. In sediments, denitrification is fuelled by NO_3^- diffusing from the overlying water (Dw, uncoupled denitrification) or by NO_3^- derived from mineralization and nitrification processes in the oxic sediment layers (Dn, coupled nitrification denitrification). High denitrification rates have been measured in eutrophic aquatic ecosystems and denitrification has also been found to be highly efficient in reducing NO_3^- loading in temperate

rivers (15–30%, Pfenning and McMahon 1996) and coastal ecosystems (70–100%, Kaspar 1983; Kaspar et al. 1985). Few data are available from high latitude aquatic ecosystems, but denitrification was found to remove 23% of the annual N load in the open sea area of the Bothnian Bay (Stockenberg and Johnstone 1997). N_2O, an effective greenhouse gas, is produced as an intermediate of denitrification. In water-saturated ecosystems the ratio of N_2O to N_2 has been found to vary from 0.002 to 0.05 (Seitzinger 1988). High saturations of N_2O have been measured from estuarine and open sea waters (e.g., Kroeze and Seitzinger 1998). N_2O/N_2 has been reported to exhibit a high positive correlation with NO_3^- concentrations (Oren and Blackburn 1979; Oremland et al. 1984; Koch et al. 1992). Therefore, especially in watercourses affected by agricultural runoff, there is a risk for high N_2O emissions as N loads increase.

Anaerobic ammonium oxidation, i.e. oxidation of NH_4^+ by nitrite (NO_2^-) to N_2, anammox (Kuypers et al. 2003) (Eq. 3) has been reported to contribute to N_2 fluxes along with denitrification. Anammox has not been extensively described in fresh waters, but has been reported to bias measured denitrification rates in marine ecosystems (e.g., Hulth et al. 2005; Hietanen et al. 2007).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (3)$$
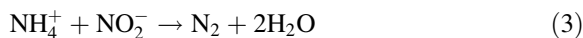
The remaining two processes, DNRA and assimilation to microbial biomass, do not exhaust NO_3^- from water, but convert it into forms of N that are available to primary producers directly (NH_4^+) or after mineralization processes (microbial N). The regulation and magnitude of DNRA (dissimilatory NO_3^- reduction to NH_4^+) is still poorly understood, but co-occurrence with denitrification has been reported in aquatic ecosystems with high NO_3^- concentrations. High NO_3^- reduction rates via DNRA have been reported in lower latitude rivers (30%, Brunet and Garcia-Gil 1996) and coastal marine ecosystems (18–100%, Bonin et al. 1998). Few studies report signs of NO_3^- uptake and assimilation into microbial biomass in aquatic ecosystems. It has been reported from a riparian wetland (22% of NO_3^-, Matheson et al. 2002) and at low levels from estuarine and coastal ecosystems (<5% of NO_3^- Goyens et al. 1987; Jørgensen 1989).

The processes involved in N cycling in river and estuarine ecosystems are driven by a range of environmental factors, with availability of NO_3^-, carbon and oxygen together with temperature being the most important ones. As a result of the changes in these driving factors, reported seasonal patterns vary remarkably (e.g., Christensen and Sørensen 1986; Jørgensen and Sørensen 1988; Koch et al. 1992; Nielsen et al. 1995; Ogilvie et al. 1997; Pind et al. 1997; Trimmer et al. 1998). In addition to temperature, the availability of NO_3^- fluctuates during the year. During summer, when the temperature is highest, the availability of NO_3^- is low due to low discharges and high uptake by primary producers in river water, thus limiting denitrification. NO_3^- concentration is an important factor regulating the N cycle in sediments. The nitrogen leaching from fertilized agricultural soils into rivers is mainly in the form of NO_3^- (Kronholm et al. 2005). Additionally, NO_3^- has a dual role in sediment as a source of N for growth and as an electron acceptor in organic carbon oxidation.

There are few studies on denitrification and N_2O effluxes from high latitude rivers. In the eutrophic rivers discharging into the Baltic Sea and receiving increasing amounts of NO_3^- from terrestrial ecosystems, denitrification could be important in diminishing NO_3^- concentrations. Furthermore, high NO_3^- loading can lead to enhanced N_2O production during denitrification. We report here our determinations of denitrification rates in the laboratory as benthic fluxes of N_2 and N_2O from intact riverine sediments from a boreal eutrophic river, under different external NO_3^- concentrations. We use these results to evaluate the potential for denitrification to regulate the riverine NO_3^- load.

Material and methods

Site description

The sediment samples were collected 17 May 2003 from the mouth of the Temmesjoki River (64° 84′ N, 25° 37′ E) (Fig. 1a). The Temmesjoki River is characterized as a eupolytrophic river for total N concentrations and a polytrophic river for total P. N generally limits primary production in the Temmesjoki River, but occasionally there is a shortage of P.

The drainage basin of the river consists of different and clearly separate catchments including forested areas, wetlands and agriculture. Dissolved inorganic nitrogen (DIN) leaches from catchments, where diffuse anthropogenic sources (e.g., agriculture and forestry) have a great importance to the total N load, nitrate (NO_3^-) being the major fraction of DIN. The Temmesjoki River has a drainage basin which, in comparison to most rivers of the Bothnian Bay, is small in size (1,190 km^2) and has a high coverage with agricultural fields (15%). The river has a low annual mean flow (11 m^3 s^{-1}). Thus, the annual N load to the Bothnian Bay from the Temmesjoki River is rather low (520 tons), despite the high NO_3^- concentrations in the river water. The NO_3^- concentration in the main channel of the river varies greatly (<1 to 100 μM), being generally highest during winter and lowest in summer (Fig. 1b) (Data from the Environmental Information System, HERTTA). The NO_3^- concentration range investigated in this study varied from 10 to 300 μM NO_3^-, which covers well the current concentration range in the main channel. The highest concentration also allows estimation of the impact of greatly increased NO_3^- concentrations on denitrification.

Sampling and experimental set-up

Sediments were collected directly into transparent acrylic tubes (ø 94 mm, height 650 mm). The height of the collected intact sediment was ≤200 mm. The sediments were placed in a laboratory microcosm equipped with continuous water flow (Liikanen et al. 2002a). The microcosm was situated in a dark, temperature controlled room (15°C). Water was pumped from an 80-l water reservoir over the cores by a peristaltic pump (IPC-24, Ismatec, Glattbrugg-Zürich, Switzerland) at a rate of 50 ml h^{-1}. Water overlying the cores was gently stirred with a rotating magnet to prevent stratification (Liikanen et al. 2002a).

The water reservoir was flushed continuously with a gas mixture consisting of Ar/O_2 80/20 (v/v) (AGA, Finland) to allow the use of the isotope pairing technique in distinguishing between coupled and uncoupled denitrification. The sediments (five replicate intact sediment samples) were incubated under 10, 30, 100, and 300 μM $^{15}NO_3^-$ (98 at. %) for the
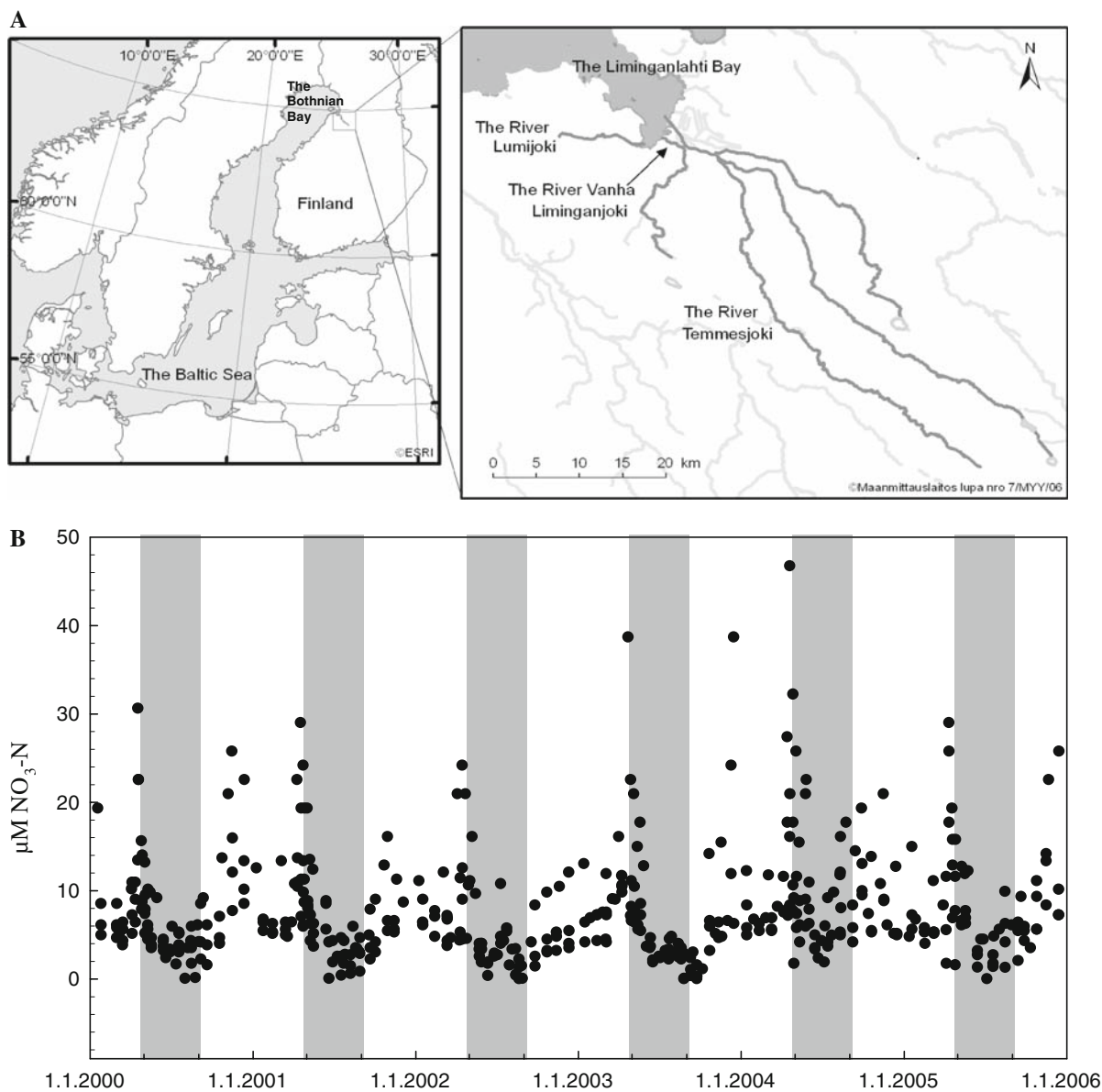
Fig. 1 **a** The study site. **b** Annual variation in the NO_3^- concentrations (μM) in surface waters (depth 0.5 m) of the Temmesjoki River in years 1999–2005 (Data from the Environmental Information System, HERTTA). Summers (May–August) are shaded grey

1st, 2nd, 3rd and 4th incubation weeks, respectively. Incubation for each NO_3^- concentration lasted 1 week starting with the lowest concentration. The concentrations of N_2, N_2O, NO_3^-, and NH_4^+ in water were measured and the effluxes were calculated from the difference between concentrations in the in- and outflowing waters and by taking into account the flow rates and sediment surface area (69 cm²).

Analyses of N_2 and N_2O

For determining the N_2, N_2O, and DIC effluxes, the effluent water samples were preserved with sulfuric acid (1 ml H_2SO_4 20% v/v) and equilibrated for 1 day with Ar headspace, which was then measured for gas concentrations and isotopic composition of N_2. The gas concentrations in the original water sample were calculated according to Henry's law

(McAuliffe 1971). Due to sulfuric acid preservation, all the inorganic carbon was liberated to the syringe headspace as CO_2 and therefore the values presented here represent the dissolved inorganic carbon (DIC) fluxes. Nitrous oxide and DIC concentrations were analyzed with a Gas Chromatograph (GC) (Hewlett Packard Series II, Palo Alto, US) equipped with two two-meter long packed columns [Hayesep Q (80/100 mesh), Porapak S (80/100 mesh)] and an electron capture detector for N_2O analyses (see Nykänen et al. 1995 for details). N_2 concentrations and isotopic compositions were measured by Gas Chromatography–Quadrupole Mass Spectrometer coupling (GC–QMS) (QP 2000, Shimadzu Corp., Japan) (see Russow and Förstel 1993 for details). The masses 28, 29, and 30 were measured and the peaks were calibrated against normal air (78% N_2) for concentration measurements. A detailed description of the precision and accuracy of measurement is presented in Russow and Förstel (1993). 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 helium 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 (Hauck et al. 1958; Siegel et al. 1982).

NO_3^- and NH_4^+ analyses

NO_3^- and NH_4^+ concentrations and isotopic composition ($^{15}N/^{14}N$, at. %) of in- and outflowing waters were determined three times for each NO_3^- concentration (4th, 5th and 6th incubation days). Both water and sediment samples were stored at −20°C prior to analyses. Nitrate concentrations were measured with an ion chromatograph (Dionex DX-130, Sunnyvale, US, with an anion column A59-HC, 12 mM Na_2CO_3 as an eluent). Ammonium was determined photometrically according to the standard SFS 3032 (SFS standardization 1976). The isotopic compositions (at. %) of NO_3^- and NH_4^+ were determined with a R/CF-QMS (Reaction/Continuous Flow–Quadrupole Mass Spectrometer) (Russow 1999; Stange et al. 2007).

Oxygen and pH measurements

At the end of each incubation week, the oxygen (O_2) concentrations and pH of overlying water (1 cm above the sediment surface) were measured. Oxygen concentrations were measured with an oxygen electrode (dissolved oxygen meter Oxi 330 with dissolved oxygen probe CellOx 325, WTW, Germany) and pH was measured with a pH electrode (Microprocessor pH meter pH 320, WTW, Germany, with Hamilton pH electrode).

Data processing

Denitrification (Dtot) in the system can be divided into Dn—coupled nitrification denitrification, and Dw—denitrification from the added NO_3^- in the overlying water (and carried by diffusion into the sediment pore water) e.g., uncoupled denitrification. Total denitrification (Dtot) was calculated as the sum of measured N_2O and N_2. Dn was calculated from the difference between the ^{15}N label (at. %) of the output NO_3^- and the output N_2 (and N_2O). The isotopic dilution gives a value for the formation of N_2 (and N_2O) from sediment-derived nitrogen. Dw (with the substrate of denitrification being the NO_3^- in the overlying water) was calculated as the remaining part of the total denitrification (Dtot–Dn).

The response of total denitrification to NO_3^- addition was fitted to a Michaelis-Menten type function (the Lineweaver-Burk transformation). In this function the reciprocal of the reaction (denitrification) rate is plotted against the reciprocal of the substrate (NO_3^-) concentration. Extrapolating the linear regression line of the function to its intercept on the abscissa gives the negative reciprocal of the half-saturation concentration (K_m). This parameter gives an estimate of the NO_3^- concentration at which the denitrification rate is half maximal (Eq. 4).

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (4)$$

where v = Reaction rate, i.e., the rate of total denitrification (μmol N m^{-2} day^{-1}), V_{max} = Maximal reaction rate, i.e. maximal rate of total denitrification (μmol N m^{-2} day^{-1}), [S] = Concentration of substrate (i.e. NO_3)(μM), K_m = Substrate (i.e., NO_3^-) concentration, at which the reaction rate is half maximal (μM).

Statistical analyses

Statistical analyses were done using the SPSS statistical package (SPSS Inc. US). The normal

distribution of the variables was tested with the Kolmogorov-Smirnov Test. As the responses of the parameters to NO_3^- addition were not linear, non-parametric Spearman correlation coefficients were applied to study the interactions between various parameters.

Results

Denitrification and N_2O effluxes

N_2 and N_2O effluxes and uncoupled denitrification (Dw) increased with increasing NO_3^- load (Table 1; Fig. 2). The greatest N_2 effluxes ($1,650 \pm 210$ µmol N_2 m^{-2} day^{-1}) were detected with the highest NO_3^- load, as were the greatest N_2O effluxes (68 ± 12 µmol N_2O m^{-2} day^{-1}). Both N_2 (0.587, $P < 0.01$) and N_2O (0.865, $P < 0.01$) effluxes calculated from the non-averaged data show a positive correlation with the NO_3^- load (Table 1). The treatment averages of N_2 and N_2O effluxes and Dw exhibited a logarithmic response to NO_3^- load (P of the regression <0.01 for all three parameters) (Fig. 2) With lower concentrations (10–100 µM NO_3^-) the effluxes increased linearly, but as the input NO_3^- increased to 300 µM NO_3^-, the response levelled off (Fig. 2). Uncoupled denitrification (Dw) always accounted for a greater part of denitrification than denitrification coupled with nitrification (Dn). The proportion of Dw/Dtot showed a significant positive correlation with NO_3^- load (0.550, $P < 0.01$, Table 1). The response of total denitrification to NO_3^- addition fitted to a Michaelis–Menten type curve ($R^2 = 0.90$) exhibited an apparent K_m value of 20 µM NO_3^--N (Fig. 3). The K_m value obtained is an apparent rather than actual K_m as it includes the limitation of diffusion of NO_3^- to the denitrifiers created by the undisturbed sediment cores.

The ratio of N_2O to N_2 was always low (<0.04). The ratio increased with increase in NO_3^- load up to 100 µM NO_3^- but decreased at 300 µM. The maximum proportion of N_2O in the nitrogenous gases was 3.9%.

The amount of NO_3^- reduced to nitrogenous gases increased with increasing NO_3^- load (Table 1), but at the same time the proportion of NO_3^- reduced in denitrification decreased as the NO_3^- load increased. With a 30 µM NO_3^- load, 7.6% of the NO_3^- was

Table 1 Measured parameters of denitrification and NO_3^- removal under different NO_3^- treatments

NO3-N loading to CF-Microcosm		Denitrification					NO3− removal		Background parameters		
Treatment NO3-N Load (µM)	NO3-N Load (mmol m^{-2} day^{-1})	N	N2 (µmol m^{-2} day^{-1})	N2O-N (µmol m^{-2} day^{-1})	N2O/N2 (%)	Dw/Dtot (%)	Total (%)	Via denitrification (%)	O2 consumption (mmol m^{-2} day^{-1})	DIC (mmol m^{-2} day^{-1})	NH4-N (µmol m^{-2} day^{-1})
10	3.3 (0.03)	5	440 (89)	4.1 (0.5)	1.0 (0.01)	52 (1.3)	9.3 (2.0)	5.3 (1.3)	ND	22 (3.3)	730 (180)
30	5.2 (0.01)	5	610 (97)	19 (3.0)	2.8 (0.01)	59 (2.0)	13 (1.0)	7.6 (1.0)	3.8 (0.1)	22 (5.2)	850 (240)
100	11 (0.9)	5	960 (200)	39 (5.2)	3.9 (0.01)	67 (0.2)	17 (3.0)	5.6 (1.2)	4.8 (1.4)	23 (6.3)	1,120 (320)
300	49 (6.5)	5	1,650 (210)	68 (12)	3.4 (0.01)	69 (0.01)	42 (11)	1.8 (0.5)	2.5 (0.8)	35 (2.5)	1,590 (540)
Spearman correlations of parameters with NO3− treatments (µM)			0.587 **	0.865 **	0.736 **	0.884 **	0.633 **	−0.390 *	−0,327	0.71	0.146

Averages and standard deviations in parenthesis

* $P < 0.05$, ** $P < 0.01$
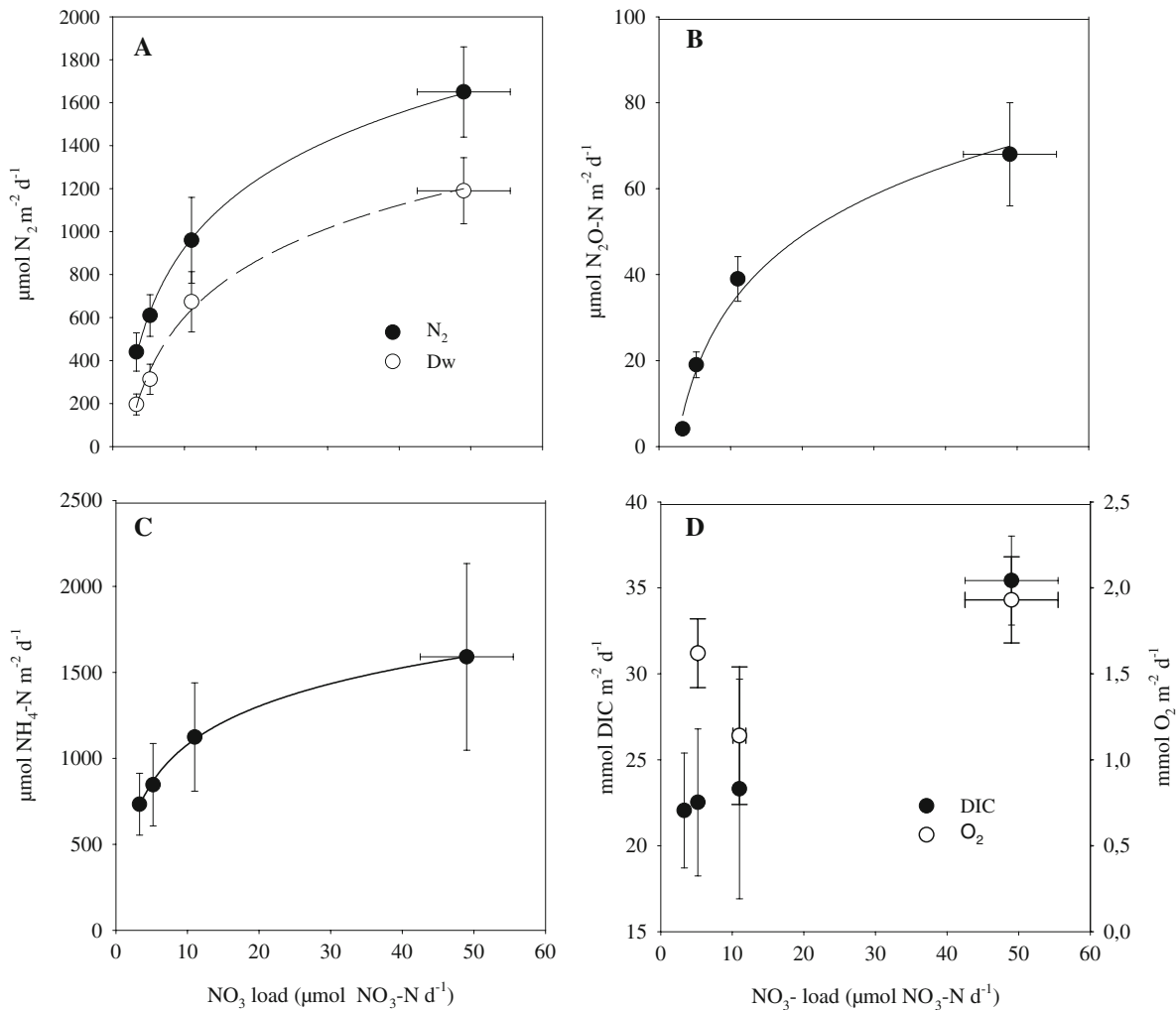
Fig. 2 **a** N_2 effluxes and Dw as a function of NO_3^- load. Logarithmic curve fit for N_2, $y = 435 \ln (x) - 119$, $R^2 = 0.99$, $P < 0.01$; and for Dw, $y = 377 \ln (x) - 268$, $R^2 = 0.99$, $P < 0.01$. **b** N_2O effluxes as a function of NO_3^- load. Logarithmic curve fit $y = 23 \ln (x) - 20$, $R^2 = 0.98$,

$P < 0.01$ **c** NH_4^+ fluxes as function of NO_3^- load. Logarithmic curve fit $y = 322 \ln (x) + 336$, $R^2 = 0.99$, $P < 0.01$. **d** DIC effluxes and O_2 consumption as a function of NO_3^- load. In all figures NO_3^- load is the NO_3^- input (μmol/day) into the sediment

denitrified, but at 300 μM NO_3^-, only 1.8% was denitrified (Table 1). The sediments were always a sink for NO_3^- from overlying water (Table 1). The total amount of NO_3^- removed at the sediment-water interface increased with increasing NO_3^- load. The proportions of $^{15}NO_3^-$ removed from the overlying water were 9.3 ± 2.0, 13 ± 1.0, 17 ± 3.0 and $42 \pm 11\%$ with treatments of 10, 30, 100, and 300 μM NO_3^-, respectively (Table 1).

Ammonium effluxes were scattered, and due to the high variation only a low, statistically insignificant positive correlation with NO_3^- concentration was

observed (Table 1). However, the treatment averages show a significant logarithmic increase as a function of NO_3^- load ($R^2 = 0.99$, $P < 0.01$, Fig. 2). The O_2 concentrations in the input water were 8.0, 8.5, and 8.0 mg O_2 l^{-1} at the 30, 100, and 300 μM NO_3^-, respectively. The oxygen was consumed efficiently, especially at the highest NO_3^- concentration. 30 and 100 μmol NO_3^- treatments exhibited oxygen concentrations of 4.1 and 5.2 mg O_2 l^{-1} at the sediment surface. At the highest NO_3^- concentration, the O_2 concentration was 3.4 mg O_2 l^{-1}. Both DIC fluxes and O_2 consumption exhibited a high variation but
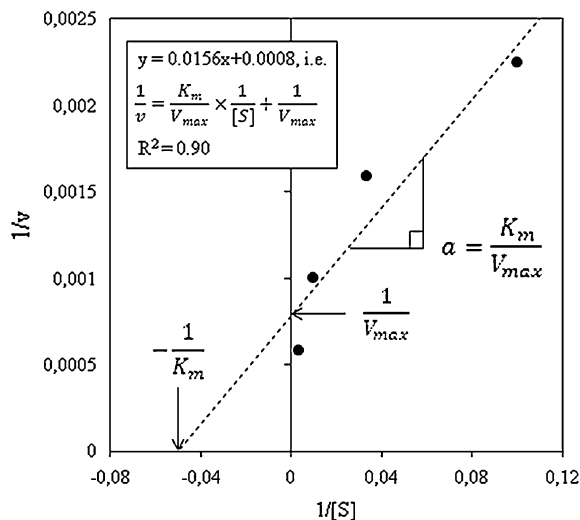
Fig. 3 Lineweaver–Burk transformation of the Michaelis–Menten type curve for total denitrification. V is the measured total denitrification rate (mmol N_2 m^{-2} day^{-1}) and [S] is the NO_3^- concentration (μM NO_3^-). The intercept of the linear regression estimate on the abscissa gives a value of -0.05 μM NO_3^-, which is a negative reciprocal of the K_m value (20 μM NO_3^-)

increased with the highest NO_3^- treatment (Table 1; Fig. 2).

The pH increased slightly, from 6.1 to 6.3, with increasing NO_3^-, but the observed positive correlation was low (0.151) and statistically insignificant.

Discussion

Denitrification rates as affected by NO_3^- concentration

The denitrification rates measured in this study exhibited a positive correlation (0.617, $P < 0.01$, Table 1) with NO_3^- load. This result is consistent with many site-specific studies from different ecosystems which have demonstrated a positive relationship between denitrification rates and NO_3^- concentration in lake (Anderssen 1977), estuarine and marine sediments (Oren and Blackburn 1979; Oremland et al. 1984; Nielsen et al. 1995; Kana et al. 1998) and in sediments of rivers in temperate regions (Royer et al. 2004; García-Ruiz et al. 1998b). The N_2 fluxes, Dw and N_2O fluxes that represent denitrification scaled over the entire studied NO_3^- addition range exhibit a logarithmic response to NO_3^-

(Fig. 2). At the lowest three concentrations the response is linear, but the response plateaus at the highest concentration. At the lowest NO_3^- concentrations, denitrification is probably limited by the availability of NO_3^- but as the concentration increases, denitrification reaches its maximum rate (K_m was 20 μM NO_3^-). A similar logarithmic response was found from intertidal mudflats of San Francisco Bay, where NO_3^- addition increased denitrification rates linearly only up to ~ 100 μM NO_3^- (Oremland et al. 1984).

Denitrification rates measured in this study were similar to or higher than the rates measured from open sea sediments of the Bothnian Bay (0–940 μmol N $m^{-2}day^{-1}$, Stockenberg and Johnstone 1997) and the Gulf of Finland (150–650 μmol N m^{-2} day^{-1}; Tuominen et al. 1998), and they are considerably higher than the rates found for estuary sediments of the Gulf of Finland (30–50 μmol N m^{-2} day^{-1}, Gran and Pitkänen 1999). Nitrate concentrations of near-bottom waters in both the Bothnian Bay and Gulf Finland [8–14 μM in the Neva Estuary, <14 μM in the central Gulf of Finland, <10 μM in the Bothnian Bay (Stockenberg and Johnstone 1997; Tuominen et al. 1998; Gran and Pitkänen 1999)], were similar to our lowest NO_3^- treatment (10 μM), which exhibited a denitrification rate of 440 μmol N $m^{-2}day^{-1}$.

The denitrification rates measured in this study (440–1,718 μmol N m^{-2} d^{-1}, Table 1) were closest to denitrification rates found in marine sediments (up to 1,440 μmol N m^{-2} d^{-1}) (Piña-Ochoa and Álvares-Cobelas 2006 and references therein). River sediments generally exhibit higher denitrification rates (up to 79,000 μmol N m^{-2} d^{-1}) than either lake sediments (up to 7,500 μmol N m^{-2} d^{-1}) or estuary sediments (up to 14,200 μmol N m^{-2} d^{-1}). The higher denitrification rates measured in rivers and estuaries than in coastal areas and oceans could be due to higher anthropogenic loading. The rates measured here were an order of magnitude lower than the rates measured from very eutrophic rivers at lower latitudes (García-Ruiz et al. 1998a), presumably as the denitrifiers have adjusted to the lower NO_3^- availability. Piña-Ochoa and Álvares-Cobelas (2006) plotted a data set of denitrification rates from all the main aquatic environments around the world in a multiple regression model with the main factors controlling denitrification, and they found that only dissolved oxygen and NO_3^- concentrations

significantly explained the denitrification rates, the latter being responsible for 70% of the variation in the rates.

Denitrification rates obtained in this study were always primarily based on added NO_3^- (Dw/Dtot 52–69%, Table 1). The proportion of Dw/Dtot showed a significant positive correlation with NO_3^- load. In contrast to our study, denitrification in open sea sediments of the Bothnian Bay and Gulf of Finland has been found to be mostly due to denitrification coupled with nitrification (Dn) (Stockenberg and Johnstone 1997), which can be explained by the lower external NO_3^- availability in open sea sediments than in the sediments in our laboratory experiments. Although the NO_3^- concentrations in those studies were similar to our lowest treatment, the continuous loading with NO_3^- in the river sediments increased the penetration of NO_3^- into sediments and consequently the availability of NO_3^- to denitrifiers (e.g., Law and Owens 1990; Kana et al 1998). Several studies from coastal and marine environments report high proportions of coupled denitrification, suggesting that the low availability of NO_3^- from the overlying water enhances the role of nitrification in sediment as the provider of the substrate for denitrification. For example, Rysgaard et al. (1993) showed that when NO_3^- concentrations in the water phase were low (~ 5 μM), coupled denitrification accounted for a larger fraction of the total denitrification than when the NO_3^- concentration in the water column was higher. The sediments studied here under higher NO_3^- concentrations (10–300 μM) always exhibited a predominance of uncoupled denitrification, and thus support the conclusions by Rysgaard et al. (1993) presented above.

The apparent half-saturation concentrations (K_m) measured in this study (20 μM NO_3^-) fall well within the lower end of the range measured in previous studies, indicating that the bacteria are well adjusted to living under low NO_3^- availability, i.e., they have a high affinity for NO_3^-. K_m values for marine sediments using the slurry technique generally range from 27 to 53 μM (Seitzinger 1988), with a value of 344 μM reported in one study. Results obtained from the Swale-Ouse river continuum in NE England varied between 13.1 and 90.4 μM NO_3^- (García-Ruiz et al. 1998b). Since the apparent K_m value in this study was measured with intact sediment samples, it reflects the actual conditions in these sediments and thus offers a tool for integrated denitrification models for similar sediments. However, spatial and temporal variation of denitrification is well known (McClain et al. 2003), and therefore caution must be practiced when the denitrification rates obtained in the laboratory are extrapolated to the river or landscape level.

In this experimental set-up, neither the possibility of an increase in the measured N_2 pool by annamox from non-labeled NH_4^+ and NO_2^- in the sediment nor the contamination of the N_2 pool by airborne nitrogen contamination can be eliminated. Mathematical approaches for separating anammox, denitrification and airborne nitrogen contamination have been presented recently (Thamdrup and Dalsgaard 2002; Risgaard-Petersen et al. 2003; Trimmer et al. 2006; Spott and Stange 2007). However, all of these approaches require an accurate time-dependent quantification of NO_2^- and are therefore not suitable for this data. Both of these problems decrease the proportion of Dw from Dtot in favor of Dn. In the case of airborne contamination, estimated NO_3^- removal via denitrification would be even lower.

N_2O effluxes as affected by increasing the NO_3^- load

In our study, N_2O effluxes showed an increasing trend with increasing NO_3^- concentrations (Table 1; Fig. 2), but the fraction of the N_2O from the end products was, at most, only 3.9%. Thus, the contribution of N_2O production via denitrification is consistently a small fraction of the total denitrification and NO_3^- consumption rates throughout the studied range of NO_3^- concentrations in this study.

Several studies in terrestrial (Blackmer and Bremner 1978; Weier et al. 1993) and aquatic (Oren and Blackburn 1979; Oremland et al. 1984; Koch et al. 1992) ecosystems have shown that the presence of high NO_3^- concentrations limits the conversion of N_2O to N_2 and results in higher N_2O/N_2 ratios. Thus, it is possible that increased local N_2O emissions due to denitrification activity in boreal eutrophic rivers and estuaries have resulted from the increase in the N_2O/N_2 ratios (Seitzinger and Kroeze 1998; García-Ruiz et al. 1999). Seitzinger (1988) reported that in eutrophic water ecosystems, up to 5% of the gases produced in denitrification were released as N_2O. Ratios as high as 80% have been measured from very

eutrophic rivers in NE England (García-Ruiz et al. 1998b).

The N_2O production rates measured in this study (4–68 μmol N_2O-N m^{-2}day^{-1}) from boreal river sediments were lower than the rates reported for rivers in general (Elkins et al. 1978; García-Ruiz et al. 1999; de Bie et al. 2002; Laursen and Seitzinger 2004). N_2O production rates have not been measured in the rivers of the northern Baltic Sea before, but in shallow profundal sediments of a freshwater lake of the same latitude, the production rates in aerobic conditions were of the same magnitude (up to 17 μmol N_2O-N m^{-2} day^{-1}) (Liikanen et al. 2002b) as those measured from the rivers in this study.

Nitrate removal and sediment metabolism

Our main goal was to study the effect of increasing NO_3^- loads on denitrification and the N_2O/N_2 ratio. The experimental set-up was designed for studying those processes and therefore has a limited ability to detect or study other potential NO_3^- removing processes. However, the results of this study show that processes other than denitrification are important in the N cycling of the boreal river sediments studied. Although denitrification rates increased with increasing NO_3^- load, only a small fraction (<10%) of the added NO_3^- was removed by denitrification, an effect that was particularly strong at the highest NO_3^- load, where only 1.8% of the added NO_3^- was denitrified and 96% of the removed NO_3^- remains unaccounted for. There are two processes that could be responsible for the unaccounted for removal: dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and assimilation of NO_3^- to microbial biomass. In contrast to denitrification, the end product of DNRA (NH_4^+), is immediately available to primary producers, and can be assimilated into microbial biomass as is NO_3^-, being therefore only temporarily removed. Similarly, the assimilated N can be released to the water during degradation of biomass. In prior studies, denitrification has been recognized as the most important process in removing NO_3^-, but several studies have reported the importance of DNRA, especially in marine sediments (e.g., Bonin 1996; An and Gardner 2002). Brunet and Garcia-Gil (1996) reported as high as 30% NO_3^- removal via DNRA in temperate river sediments. Only a few studies exist on assimilation into microbial biomass in estuaries

(Jørgensen 1989) and riparian wetlands (Matheson et al. 2002), showing a great variation (<5–22%) in N removal. To our knowledge, no studies on N assimilation exist from boreal river sediments.

Microbial activity in the sediment seems to be generally enhanced as a function of increased NO_3^- loading, which can be seen as increased DIC effluxes and enhanced O_2 consumption. The DIC produced in denitrification contributes only a small fraction of the total DIC efflux (according to Eq. 2), indicating that in this sediment NO_3^- has a more profound function as a source of N than as an electron acceptor. Therefore, it seems that the sediment metabolism is generally limited by N availability, and a great part of the added NO_3^- was probably due to microbial growth (biomass production). There was evidence that the addition of NO_3^- to this system enhanced both assimilation and mineralization. The enhanced mineralization was seen as an increase in the DIC and NH_4^+ effluxes. As the output NH_4^+, although well correlated to NO_3^-, exhibited only a low level of ^{15}N labeling (<4 excess at. %) at the highest NO_3^- treatments, direct reduction of NO_3^- to NH_4^+ (DNRA) does not explain the observed NO_3^- removal. DNRA would have produced NH_4^+ with levels of ^{15}N labeling more similar to those of the added NO_3^- (98 at. %). Therefore, the NH_4^+ efflux most likely increases as the mineralization of the top sediment layers is enhanced. The low ^{15}N enrichment in NH_4^+ probably originates from the labeled NO_3^- that was assimilated during earlier treatments and further remineralized.

Conclusions

This study provides the first information on denitrification and N_2O fluxes, and their regulation by NO_3^- load, in eutrophic high latitude rivers. Increased NO_3^- loading in boreal rivers enhances denitrification. However, denitrification has a limited capacity to remove the NO_3^- from rivers (1.2–7.9% of the added NO_3^-), especially at very high NO_3^- concentrations. An increased availability of NO_3^- also stimulates N_2O production, but the N_2O/N_2 ratio in riverine denitrification remains low even with very high NO_3^- concentrations. Therefore, NO_3^- removal during denitrification in rivers will not lead to large emissions of N_2O, an efficient greenhouse gas, to the

atmosphere. Additionally, the results of this study show the potential of other processes, especially assimilation to microbial biomass, for removing NO_3^-, and the impact of increased N loads on overall metabolism in sediments.

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