

Available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/watres

Potential nitrate removal in a coastal freshwater sediment (Haringvliet Lake, The Netherlands) and response to salinization

Anniet M. Laverman*, Richard W. Canavan, Caroline P. Slomp, Philippe Van Cappellen

Department of Earth Sciences—Geochemistry, Faculty of Geosciences, Utrecht University, P.O. Box 80021, 3508 TA Utrecht, The Netherlands

ARTICLE INFO

Article history:

Received 9 June 2006

Received in revised form

5 April 2007

Accepted 6 April 2007

Available online 4 June 2007

Keywords:

Nitrate reduction

Denitrification

Dissimilatory nitrate reduction to

ammonia

Salinization

Sediment

ABSTRACT

Nitrogen transformations and their response to salinization were studied in bottom sediment of a coastal freshwater lake (Haringvliet Lake, The Netherlands). The lake was formed as the result of a river impoundment along the south-western coast of the Netherlands, and is currently targeted for restoration of estuarine conditions. Nitrate porewater profiles indicate complete removal of NO_3^- within the upper few millimeters of sediment. Rapid NO_3^- consumption is consistent with the high potential rates of nitrate reduction (up to $200 \text{ nmol N cm}^{-3} \text{ h}^{-1}$) measured with flow-through reactors (FTRs) on intact sediment slices. Acetylene-block FTR experiments indicate that complete denitrification accounts for approximately half of the nitrate reducing activity. The remaining NO_3^- reduction is due to incomplete denitrification and alternative reaction pathways, most likely dissimilatory nitrate reduction to NH_4^+ (DNRA). Results of FTR experiments further indicate that increasing bottom water salinity may lead to a transient release of NH_4^+ and dissolved organic carbon from the sediment, and enhance the rates of nitrate reduction and nitrite production. Increased salinity may thus, at least temporarily, increase the efflux of NH_4^+ from the sediment to the surface water. This work shows that salinity affects the relative importance of denitrification compared to alternative nitrate reduction pathways, limiting the ability of denitrification to remove bioavailable nitrogen from aquatic ecosystems.

© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Nearshore sediments often exhibit high rates of organic carbon (C_{org}) mineralization and intense cycling of nitrogen (N). Up to 40% of C_{org} mineralization in coastal sediments may be coupled to denitrification (Soetaert and Herman, 1995), which converts dissolved nitrate to gaseous N products, principally N_2 , that are released to the atmosphere (Seitzinger, 1988). This makes denitrification a crucial removal pathway of bioavailable N from aquatic ecosystems.

Denitrification is widely viewed as the dominant process of nitrate reduction in coastal sediments (Herbert, 1999), although alternative pathways such as dissimilatory reduction of nitrate (NO_3^-) to ammonium (NH_4^+), or anaerobic NH_4^+ oxidation coupled to nitrite (NO_2^-) reduction without organic matter oxidation (De Graaf et al., 1996) can also occur. Dissimilatory nitrate reduction to NH_4^+ (DNRA) may be an important process in sediments with high inputs of labile organic substrates and limited nitrate availability (Megonigal et al., 2003). Unlike denitrification and

*Corresponding author. Current address: UMR 7619 SISYPHE, Université Pierre et Marie CURIE, Tour 56—couloir 56/46, 4 Place Jussieu—75252 Paris Cedex 05, France. Tel.: +33 1 44 27 62 56; fax: +33 1 44 27 51 25.

E-mail address: Anniet.Laverman@ccr.jussieu.fr (A.M. Laverman).
0043-1354/\$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2007.04.002

anammox, DNRA does not remove bioavailable N from the ecosystem.

The pathways and rates of nitrate reduction depend on a series of environmental factors; the concentrations of nitrate and organic carbon being most important (Herbert, 1999). In addition, temperature and salinity also play a role; increased salinity displaces NH_4^+ from cation exchange sites of the sediment, hence reducing the pool of NH_4^+ available for nitrifying bacteria, which, in turn, decreases denitrifying activity fueled by nitrification (Seitzinger et al., 1991). Although salinization may stress freshwater nitrifiers and denitrifiers and lower their activities (Rysgaard et al., 1999), denitrification in the Douro River estuary (Portugal) was not affected by changes in salinity (Magalhães et al., 2005). Thus, previous studies do not reveal a consistent response of nitrate reduction to changes in salinity, likely due to differences in environmental conditions and microbial populations between the sites studied.

The aim of this study was to quantify potential NO_3^- reduction activities and rates of production of dissolved inorganic nitrogen species (NO_3^- , NO_2^- , NH_4^+) in a coastal freshwater sediment. The Haringvliet Lake (The Netherlands) was chosen as a study site because this freshwater lake is currently targeted for restoration of estuarine conditions. The accompanying rise in bottom water is expected to affect the biogeochemical dynamics in the underlying sediments, thereby modifying benthic exchange fluxes of inorganic nutrients. In particular, we hypothesized that an increase in salinity would lower NO_3^- reduction activity due to physiological stress of the freshwater NO_3^- reducing community inhabiting the freshwater lake sediment.

2. Materials and methods

2.1. Field sampling

Haringvliet Lake is a coastal freshwater lake located in a heavily populated area in the south-west of the Netherlands (Canavan et al., 2006; Smit et al., 1997). To increase the ecological diversity of the Haringvliet, a partial restoration of estuarine conditions has been proposed. This would be accomplished by changing the management of the sluices of the storm-surge dam, which separates the lake from the North Sea. The sampling site is located near the dam at a water depth of approximately 7.5 m, within the area that would be affected by salinization. Partial opening of the Haringvliet sluices during a five day period in 1997 led to maximum chloride concentrations of 2.7 mg l^{-1} in the bottom water at this site (Jacobs et al., 2003).

Bottom sediment was sampled in fall (November 2001), late-summer (September 2002), and spring (April 2003), using a cylindrical box corer at $\sim 7.5 \text{ m}$ depth. Each box core contained approximately 40 cm of surface sediment and 30 cm of overlying water. Box cores were immediately sub-sampled with polycarbonate tubes (10 cm i.d.) for pore water analysis, and with perspex cores (4.2 cm i.d.) for microprofiling. The sub-cores for pore water analysis were processed under N_2 atmosphere, inside a shipboard, temperature-controlled laboratory, as described in Canavan et al. (2006).

Filtered pore water sub-samples for nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) were stored frozen until analysis. Oxygen (O_2) microprofiles were measured within 30 min using a miniaturized Clark-type O_2 sensor mounted on a micromanipulator (Unisense, Århus, Denmark, see Gundersen et al., 1998; Revsbech, 1989). Calibration of the oxygen electrode was performed with the bottom water from the sampling site.

Sediment for flow-through reactor (FTR) experiments was obtained from the box cores by sub-sampling with a shuttle corer. The latter consisted of vertically stacked Plexiglas[®] reactor cells of 10 mm length and 42 mm inside diameter enclosed in a stainless steel sleeve. After retrieval of the shuttle corer, each reactor cell contained an undisturbed sediment slice from a well-defined depth interval. The reactor cells were carefully separated from one another with a Teflon[®] knife and immediately covered with $0.2 \mu\text{m}$ pore size nitrocellulose filters and glass fiber filters (1.2 mm thick, 47 mm diameter), and then closed by POM (poly-oxy methylene) Delrin[®] caps kept in place by four screws. Rubber O-rings provided a seal between the reactor and caps. Input and output channels open at the center of the caps, in direct contact with the glass fiber filters, to ensure radially homogeneous flow through the reactors. FTR experiments were started within 2 h of sampling. Detailed descriptions of the shuttle corer and the FTR approach are given in Laverman et al. (2006) and Pallud and Van Cappellen (2006).

2.2. Rate measurements

Nitrogen transformation kinetics were measured in FTR experiments (Fig. 1) on 1 cm thick, undisturbed sediment slices subjected to various conditions (see overview Table 1). Nitrate was the only external electron acceptor supplied to the reactors via the inflow (0.5–5 mM KNO_3). Aerobic (e.g. nitrification) and light-sensitive processes were inhibited by vigorously bubbling the inflow solutions with N_2 and keeping the reactors in the dark. Outflow samples were collected at

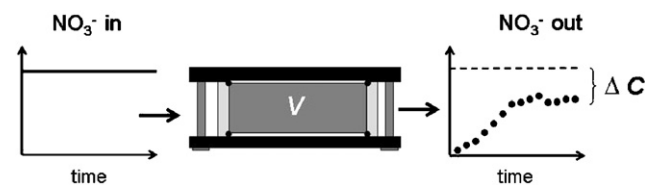


Fig. 1 – Potential nitrate reduction rate determinations with sediment flow-through reactors (FTRs). The reactor contains an intact slice of sediment of known volume ($V = 13.85 \text{ cm}^3$). It is supplied with an inflow solution containing a constant concentration of nitrate ($\text{NO}_3^- \text{ in}$), at a known flow-rate (Q). The outflow is collected and analyzed for the concentrations of nitrate ($\text{NO}_3^- \text{ out}$) and potential metabolites (NH_4^+ and NO_2^-). The net rate of nitrate consumption in the FTR is obtained from the measured concentration difference between inflow and outflow nitrate (ΔC), once the outflow concentration of NO_3^- has stabilized. (See text for further details.)

Table 1 – Overview of potential rate measurements in flow through reactor (FTR) experiments

	Experimental conditions				Sampling		
	Depth intervals (cm)	# Reactors	[NO ₃ ⁻] input solution (mM)	Treatments	Fall	Late summer	Spring
NRR	0–1, 1–2, 2–3, 3–4	4	2.5	—	—	×	×
NRR and DNR	0–1	1	2.5	C ₂ H ₂	8, 12, 18 °C	—	×
NRR and DNR	0–1	8	0.5–5	C ₂ H ₂	0, 10‰ NaCl	—	×

DNR stands for denitrification rate, NRR for nitrate reduction rate.

Table 2 – Bottom water O₂ and NO₃⁻ concentrations and sediment properties

Sampling time		Bottom water		Sediment (0–4 cm)				
		O ₂ (μM)	NO ₃ ⁻ (μM)	Temperature (°C)	Porosity (vol%)	C _{org} (%)	N (%)	C _{org} :N (mol/mol)
Fall	November 2001	225	160	8	82–90	4.29–4.66	0.25–0.32	19.1
Late summer	September 2002	218	120	18	86–92	4.78–4.94	0.29–0.32	18.3
Spring	April 2003	274	180	12	88–92	4.85–4.60	0.31–0.37	18.1

The range of the porosities, total organic C (C_{org}) and N are given for the four depth intervals.

regular (0.5, 1 or 2 h) intervals for chemical analyses. The flow rate (Q) through the reactors was determined by weighing the outflow samples. At the end of each experiment, the water content and the concentrations of C_{org} and N of the sediment in the reactor were measured.

Potential rates of NO₃⁻ reduction, NH₄⁺ production and NO₂⁻ production were calculated from measured concentration differences between inflow and outflow (ΔC in nmol cm⁻³), the flow rate (Q in cm h⁻¹) and the volume of sediment inside the reactor (V in cm³):

$$\text{rate} = \frac{\Delta C \times Q}{V}, \quad (1)$$

where the rate (nmol cm⁻³ h⁻¹) is expressed per unit total (or wet) sediment volume, and C is the concentration of NO₃⁻, NH₄⁺ or NO₂⁻. The rates were determined under steady-state conditions, that is, when the outflow concentration of the solute of interest no longer changed with time. For the FTR experiments reported here, steady-state was always reached within 12 h.

For each of the three sampling times, potential rates were measured at in situ sediment temperature (Table 1) on four depth intervals: 0–1, 1–2, 2–3 and 3–4 cm. The FTR experiments ran for 24 h. A more extensive set of experiments was carried out during the April 2003 (spring) campaign using 0–1 cm depth interval sediment slices retrieved from a single box core. The inflow solutions in these experiments were amended with acetylene (10% v/v). Acetylene inhibits the final

step of denitrification, that is, the conversion of N₂O to N₂ (Sørensen, 1978). Potential rates of denitrification were then obtained by monitoring the nitrous oxide concentration in the outflow using a Clark-type N₂O microsensor, with a detection limit of 1 μM (Unisense, Århus, Denmark).

One FTR with the 0–1 cm spring sediment was subjected successively to three temperatures (8, 12 and 18 °C), for at least 18 h at each temperature, thereby covering the annual temperature range of the bottom waters at the sampling site. Eight separate reactors were supplied with inflow solutions with either no NaCl (–NaCl) or 10‰ NaCl (+NaCl) added. These experiments ran for 50 h. NaCl solutions, rather than filtered seawater, were used, in order to avoid potential interference from sulfate reduction sustained by seawater sulfate. In addition to NO₃⁻, NO₂⁻ and NH₄⁺, the concentration of dissolved organic carbon (DOC) was measured in the outflow solutions.

2.3. Analyses

Concentrations of NO₃⁻, NO₂⁻ and NH₄⁺ were determined colorimetrically with a Nutrient Autoanalyzer 3 (Bran and Luebbe). Porosity was derived from the weight loss of a known volume of wet sediment upon oven-drying at 60 °C. Sediment C_{org} and total N contents were determined on a Carlo Erba CN analyzer. DOC was measured with a Shimadzu TOC-5050A analyzer. The most probable numbers (MPN) of denitrifying organisms were obtained as described in Laverman et al.

(2006). The significance of rate differences in FTR experiments with or without NaCl amendment was evaluated by the t-test or the Mann–Whitney Rank Sum Test, using a statistical software package (SigmaStat 3.5).

3. Results

3.1. Sediment properties and pore water profiles

The sediment is highly porous and organic-rich (Table 2). Bulk sediment C:N ratios (~ 16) exceeded the Redfield values (6.6). The overlying water remains fully oxygenated throughout the year, with a somewhat lower O_2 concentration in late summer. Bottom water NO_3^- concentrations varied between 120 and 180 μM . Oxygen penetration depths were shallower in late summer and spring (3.5 mm) than in fall (6.5 mm; Fig. 2). Pore water NO_3^- concentrations were always below the detection limit ($1 \mu M$), even in the 0–0.5 cm depth interval (Fig. 2). Pore water NH_4^+ concentrations increased with depth, with the highest values recorded in spring (Fig. 2).

3.2. Rates

Potential NO_3^- reduction rates measured in FTRs at in situ temperatures were highest in late summer, with maximum values up to $200 \text{ nmol cm}^{-3} \text{ h}^{-1}$ (Fig. 3). Potential nitrate reduction activity was detected at all depths sampled, but the rates showed no obvious trend with depth. Denitrifiers were abundant in all sediment intervals studied (Fig. 3); MPNs, however, exhibited no significant trends with depth, sampling time or potential NO_3^- reduction activity. Net rates of NH_4^+ production in FTRs fed with NO_3^- -containing inflow solutions were also highest in late summer (Fig. 3). For both late summer and fall, the rates increased slightly with depth.

At in situ temperature (12°C), the average N_2O production rate in C_2H_2 -amended FTR experiments with the 0–1 cm depth interval of the spring sediment was $35 \text{ nmol cm}^{-3} \text{ h}^{-1}$. Denitrification therefore only explained 47% of the total NO_3^- consumption measured during the same experiments (Fig. 4). Note that N_2O was undetectable in the outflow of control reactors fed with acetylene-free inflow solutions. Given the detection limit of the N_2O sensor ($1 \mu M$), this means that N_2O production was less than $1 \text{ nmol cm}^{-3} \text{ h}^{-1}$ in the absence of acetylene. Nitrite was also detected in the outflow solutions. Assuming that all NO_2^- resulted from NO_3^- reduction, nitrite production accounted for 17% of the total NO_3^- consumption at 12°C .

Both potential NO_3^- reduction and denitrification rates increased near linearly from 8 to 18°C (results not shown), yielding Q_{10} temperature coefficients (Winkler et al., 1996) of 2.5 and 3.6 for NO_3^- consumption and N_2O production, respectively. In the C_2H_2 -amended FTR experiments run at 8 and 18°C , 54% and 34% of total NO_3^- reduction was recovered as N_2O , respectively.

3.3. Effect of salinity

Addition of 10‰ NaCl to the FTR inflow significantly enhanced potential rates of NO_3^- reduction and NO_2^- production in the 0–1 cm spring sediment (Fig. 4, $p < 0.001$). The

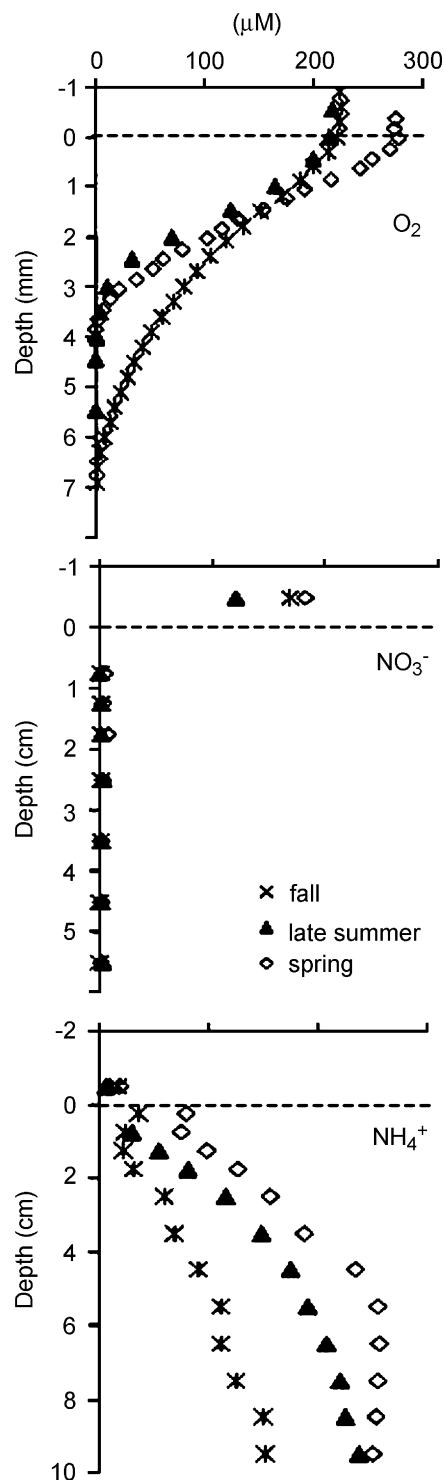


Fig. 2 – Pore water profiles of O_2 , NO_3^- and NH_4^+ : fall (asterisk) late summer (closed triangles) and spring (open diamonds). Note the differences in depth scales between the panels. The broken horizontal line corresponds to the sediment–water interface.

average, steady-state NO_3^- reduction rate was $115 \text{ nmol cm}^{-3} \text{ h}^{-1}$ in NaCl-amended FTRs, compared to $80 \text{ nmol cm}^{-3} \text{ h}^{-1}$ without added NaCl. Nitrite production rates increased by a factor of three in the NaCl-amended

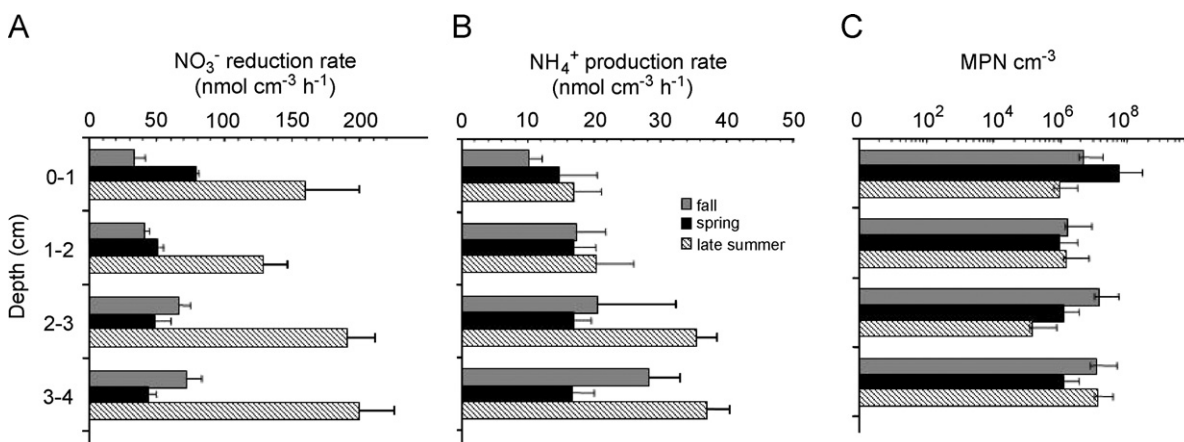


Fig. 3 – Potential rates of nitrate reduction (A), and NH_4^+ production (B), plus the most probable numbers (MPNs) of denitrifiers (C) in four depth intervals collected in fall, late summer and spring. The rates were measured in FTR experiments incubated at the in situ temperatures (see Table 1). For the rates, error bars indicate the standard deviations of rate measurements between 14 and 24 h of the experiments. For the MPNs, error bars indicate 95% confidence intervals.

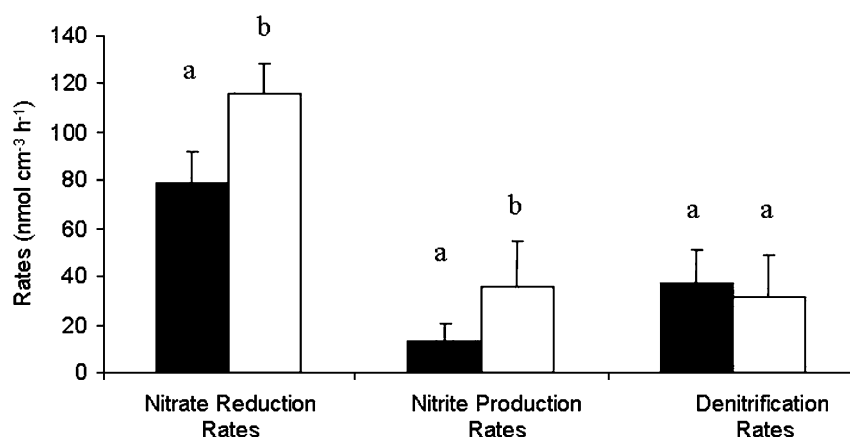


Fig. 4 – Effects of NaCl amendments on nitrate consumption and the rates of NO_2^- and N_2O production, in the 0–1 cm depth interval of sediment collected in April 2003 (spring). FTRs were supplied with NO_3^- -containing inflow solutions without (filled bars) or with added NaCl (10‰, open bars). Values plotted are averages from four parallel reactor experiments supplied with different nitrate concentrations (0.5–5 mM). The error bars correspond to standard deviations. The experiments were run at in situ temperature (12 °C) and with a flow rate of 6 ml h⁻¹. Different letter assignments (a, b) indicate a significant difference ($p < 0.001$) in pairwise comparison.

FTR experiments, accounting for 31% of total NO_3^- consumption (Fig. 4). In contrast, N_2O production in acetylene-amended FTRs did not change significantly upon addition of NaCl (Fig. 4, $p = 0.366$).

Addition of NaCl to the inflow also had pronounced effects on the NH_4^+ and DOC in the FTRs (Fig. 5). The average rate of NH_4^+ release during the first 30 h of the experiments more than doubled when NaCl was added (Fig. 5). Although the net rates of NH_4^+ and DOC release decreased with time, they remained significantly higher in the NaCl-amended experiments ($p < 0.001$ for NH_4^+ and $p < 0.05$ for DOC).

4. Discussion

Intense sediment metabolism, which is reflected in the limited penetration depths of O_2 and NO_3^- in the sediment

(Fig. 3), implies a close link between benthic and water column processes in the shallow, eutrophic Haringvliet Lake. In particular, the sediments represent an important sink for NO_3^- . Based on reactive transport calculations, on the order of 20% of the depth-integrated rate of C_{org} mineralization in the sediment studied may be coupled to nitrate reduction (Canavan et al., 2006).

Temperature variations only explain part of the observed differences in potential NO_3^- reducing activity in sediments sampled during the different seasons. Based on the measured Q_{10} value, the 10 °C difference between late summer and fall sampling times accounts for about half (55%) of the four-fold difference in potential NO_3^- reduction rates. Most likely, the rates also reflect seasonal fluctuations in the deposition of labile organic substrates from the water column (Canavan et al., 2006). The presence of denitrifying organisms and high potential NO_3^- reduction rates well below the depth of NO_3^-

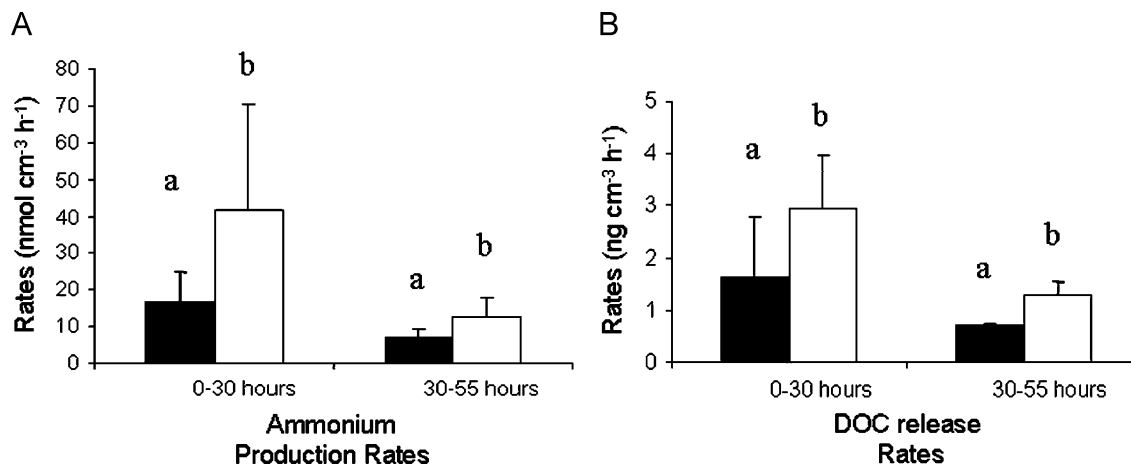


Fig. 5 – Effects of NaCl amendment on net rates of NH_4^+ (A) and dissolved organic carbon (DOC) (B) release, from the 0–1 cm depth interval of sediment collected in April 2003 (spring). Flow-through reactors were supplied with NO_3^- -containing inflow solutions without (filled bars) or with added NaCl (10%, open bars). Values plotted are averages from four parallel reactor experiments supplied with different nitrate concentrations (0.5–5 mM). The error bars correspond to standard deviations. Rates were higher during the first 30 h and are therefore plotted separately from the subsequent, steady-state production rates (from 30 to 55 h). The experiments were run at in situ temperature (12 °C) and with a flow rate of 6 ml h⁻¹. Different letter assignments (a, b) indicate a significant difference ($p < 0.001$) in pairwise comparison.

penetration (Figs. 3 and 4) indicate that benthic NO_3^- reduction is limited by the supply of NO_3^- from the overlying bottom water. In addition to molecular diffusion, pore water irrigation by benthic infauna is a major mechanism for transferring NO_3^- from the water column to the sediment (Canavan et al., 2006).

Two immediate effects of a future salinization of the Haringvliet would be the release of sediment-bound NH_4^+ and organic matter (Fig. 5). While the mobilization of NH_4^+ due to cation exchange by Na^+ is well established (Rysgaard et al., 1999; Seitzinger et al., 1991), salinity-dependent DOC release is poorly understood. It probably involves changes in the macromolecular structure of the organic matter with increased ionic strength (Tombacz and Meleg, 1990), which may enhance its bioavailability (Nyvang, 2003; Weston et al., 2006). DOC mobilization following saltwater intrusion has been invoked to explain increased microbial respiration in a Danish coastal aquifer (Nyvang, 2003). The increase in potential nitrate reduction rates when NaCl-amended inflow solutions are supplied to the reactors (Fig. 4) may thus reflect increased C_{org} availability.

Complete denitrification to N_2 only accounts for about 50% of NO_3^- reduction in the FTR experiments with the 0–1 cm sediment interval collected in spring. In addition, denitrification exhibits a higher Q_{10} than nitrate reduction, indicating that denitrification is more sensitive to temperature changes than alternative nitrate consuming processes. Experimental artifacts due to incomplete blockage by acetylene of N_2O reductase as a result of NO_3^- limitation (Oremland et al., 1984) is unlikely, given the high NO_3^- concentrations supplied by the inflow solutions. Similarly, an interference by sulfide (Dalsgaard and Bak, 1992) is not expected, as no sulfate is supplied to the reactors. Furthermore, for other freshwater sediments the exact same experimental approach yields N_2O recoveries approaching 100% (Laverman et al., 2006).

Denitrification is often assumed to be the main NO_3^- reducing process in aquatic sediments (Herbert, 1999). However our results and those of other studies indicate that alternative pathways of NO_3^- reduction such as DNRA and anammox may be important (Kelly-Gerreyn et al., 2001; Trimmer et al., 1998). The occurrence of nitrate reduction processes other than denitrification in Haringvliet sediment helps explain why denitrification and total NO_3^- consumption exhibit distinct temperature dependencies and respond differently to NaCl amendments.

For sediment collected in spring, denitrification (47%) and incomplete denitrification to NO_2^- (17%) are estimated to account for 64% of total NO_3^- reduction. The two most likely processes responsible for the remaining 36% of NO_3^- consumption are DNRA and anammox. A major distinction between these pathways is that DNRA produces NH_4^+ while anammox consumes NH_4^+ . It is therefore instructive to compare the net ammonium production rates (APR) and the net NO_3^- removal rates (NRR) in the FTR experiments.

The theoretical molar APR:NRR ratio for denitrification ranges between 0.09 and 0.19, depending on whether the measured bulk $\text{C}_{\text{org}}:\text{N}$ ratio (16; Table 2) or the Redfield C:N ratio of phytoplankton (6.6) is considered to be representative of the organic matter undergoing decomposition in the sediment (for details, see Laverman et al., 2006). The measured late summer APR:NRR values fall within this range (Fig. 6). Most FTR experiments run with sediments collected in fall and spring, however, yield higher values, indicating excess NH_4^+ production, relative to denitrification. The latter is consistent with DNRA as a pathway for NO_3^- reduction in sediments of the Haringvliet Lake sediments.

According to the APR:NRR ratios, the relative contribution of DNRA would be expected to be highest during fall. Possibly, the relatively high influx of degradable organic matter to the sediments coupled to the limited supply of NO_3^- from the

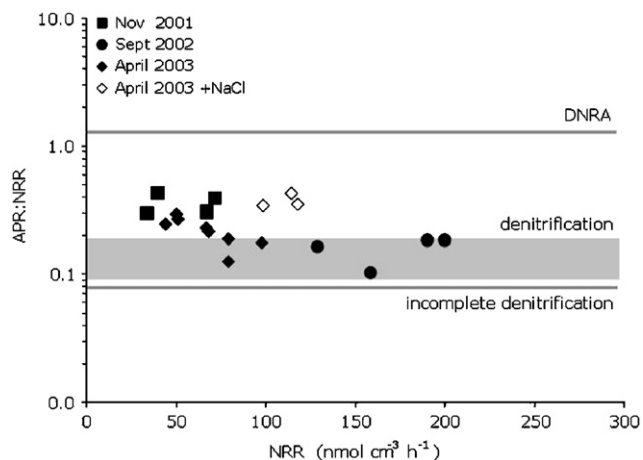


Fig. 6 – Ratio of the net NH_4^+ production rate (APR) and nitrate reduction rate (NRR) in FTR experiments with Haringvliet sediments, plotted as a function of NRR. The shaded area corresponds to the APR:NRR ratios expected for denitrification to N_2 , assuming an idealized stoichiometry of the decomposing organic matter of $(\text{CH}_2\text{O})_x (\text{NH}_3)_y$ and end-member molar C:N ratios ranging from the measured bulk $\text{C}_{\text{org}}:\text{N}$ ratio in the sediments (16, Table 2) to the Redfield ratio for fresh phytoplankton (6.6). Also shown as horizontal lines are the theoretical APR:NRR ratios for DNRA and incomplete denitrification to NO_2^- , assuming the Redfield C:N ratio for the decomposing organic matter.

water column creates conditions favorable for DNRA in the sediments (Herbert, 1999). Based on the available information, however, the occurrence of other NO_3^- consumption processes cannot be excluded.

The increased production of NO_2^- in NaCl-amended FTR experiments (Fig. 4), suggest that salinity stress affects the reduction of NO_2^- . A negative impact of increased salt concentrations on NO_2^- reductase has also been observed in nitrate-rich wastewater (Glass and Silverstein, 1999). Both NO_2^- and N_2O reductases are periplasmic enzymes and, hence, more sensitive to environmental stress than NO_3^- reductase, which is membrane-bound (Zumft, 1997). In principle, an increase in both NO_2^- and NH_4^+ release from the sediments could also reflect inhibition of anammox, although a significant role of the latter process is not supported by the excess NH_4^+ production in the FTR experiments with fall and spring sediments (Fig. 6).

5. Conclusions

Sediments of Haringvliet Lake exhibit a high potential for NO_3^- removal. In addition to denitrification to N_2 , incomplete denitrification and DNRA represent significant pathways of NO_3^- reduction. As the latter processes generate non-gaseous N species (NO_2^- , NH_4^+), they limit the permanent elimination of nutrient N from the lake ecosystem. Contrary to our initial expectation, increasing salinity enhanced NO_3^- consumption, possibly as a result of the salinity-induced mobilization of sediment-bound organic matter. The higher NO_3^- reducing

activity, however, was not due to an increase in denitrification, but rather due to that of alternative NO_3^- reduction pathways. Combined with the displacement of NH_4^+ from cation exchange sites, the immediate impact of bottom water salinization is therefore expected to be an increased efflux of ammonium and nitrite from the sediments. The long-term response of benthic N cycling to the restoration of an estuarine salinity gradient in Haringvliet Lake will depend on changes of a variety of coupled environmental forcings and variables, including circulation patterns in the water column, benthic infaunal activity, organic matter deposition and microbial community structure. Further research will be needed to fully delineate the range of consequences of estuarine restoration on benthic biogeochemistry and the chemical exchanges between sediments and the water column.

Acknowledgments

We thank the crew of the R.V. Navicula for their expert help during the cruises. Debby Los and Dineke van de Meent assisted with the experiments and analyses. Financial support was provided by the Netherlands Organisation for Scientific Research, NWO (Veni Grant to AML, Pioneer Grant to PVC), the Royal Netherlands Academy of Arts and Sciences, KNAW (Fellowship to CPS) and the Netherlands Institute for Inland Water Management and Waste Water Treatment, RIZA (Fellowship to RWC).

REFERENCES

- Canavan, R.W., Slomp, C.P., Jourabchi, P., Van Cappellen, P., Laverman, A.M., Van den Berg, G.A., 2006. Organic matter mineralization in sediment of a coastal freshwater lake and response to salinization. *Geochim. Cosmochim. Acta* 70 (11), 2836–2855.
- Dalsgaard, T., Bak, F., 1992. Effect of acetylene on nitrous-oxide reduction and sulfide oxidation in batch and gradient cultures of *Thiobacillus denitrificans*. *Appl. Environ. Microbiol.* 58 (5), 1601–1608.
- De Graaf, A.A.V., De Bruijn, P., Robertson, L.A., Jetten, M.S.M., Kuenen, J.G., 1996. Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology—UK* 142, 2187–2196.
- Glass, C., Silverstein, J., 1999. Denitrification of high-nitrate, high-salinity wastewater. *Water Res.* 33 (1), 223–229.
- Gundersen, J.K., Ramsing, N.B., Glud, R.N., 1998. Predicting the signal of O_2 microsensors from physical dimensions, temperature, salinity and O_2 concentration. *Limnol. Oceanogr.* 43 (8), 1932–1937.
- Herbert, R.A., 1999. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiol. Rev.* 23 (5), 563–590.
- Jacobs, P., Steenkamp, B.P.C., de Goederen, S., 2003. Analyse zoutmetingen inlaatproef Haringvliet in maart 1997. RIZA Report 2003.001.
- Kelly-Gerrey, B.A., Trimmer, M., Hydes, D.J., 2001. A diagenetic model discriminating denitrification and dissimilatory nitrate reduction to ammonium in a temperate estuarine sediment. *Mar. Ecol. Prog. Ser.* 220, 33–46.
- Laverman, A.M., Van Cappellen, P., van Rotterdam-Los, D., Pallud, C., Abell, J., 2006. Potential rates and pathways of microbial nitrate

- reduction in coastal sediments. *FEMS Microbiol. Ecol.* 58 (2), 179–192.
- Magalhães, C.M., Joye, S.B., Moreira, R.M., Wiebe, W.J., Bordalo, A.A., 2005. Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. *Water Res.* 39 (9), 1783–1794.
- Megonigal, J.P., Hines, M.E., Visscher, P.T., 2003. Anaerobic Metabolism: linkages to trace gases and aerobic processes. *Treatise on Geochemistry*. In: Schlesinger, W.H., Hollands, H.D., Turekian, K.K. (Eds.), *Biogeochemistry*, vol. 8. Elsevier-Per-gamon, Oxford, pp. 317–424.
- Nyvang, V., 2003. Redox processes at the salt-/freshwater interface in an anaerobic aquifer. Ph.D. Thesis, Technical University of Denmark.
- Oremland, R.S., Umberger, C., Culbertsen, C.W., Smith, R.L., 1984. Denitrification in San Francisco Bay intertidal sediments. *Appl. Environ. Microbiol.* 47 (5), 1106–1112.
- Pallud, C., Van Cappellen, P., 2006. Kinetics of microbial sulfate reduction in estuarine sediments. *Geochim. Cosmochim. Acta* 70 (5), 1148–1162.
- Revsbech, N.P., 1989. Diffusion characteristics of microbial communities determined by use of oxygen microsensors. *J. Microbiol. Methods* 9, 111–122.
- Rysgaard, S., Thastum, P., Dalsgaard, T., Christensen, P.B., Sloth, N.P., 1999. Effects of salinity on NH_4^+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments. *Estuaries* 22 (1), 21–30.
- Seitzinger, S.P., 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* 33 (4, part 2), 702–724.
- Seitzinger, S.P., Gardner, W.S., Spratt, A.K., 1991. The effect of salinity on ammonium sorption in aquatic sediments—Implications for benthic nutrient recycling. *Estuaries* 14 (2), 167–174.
- Smit, H., Van der Velde, G., Smits, R., Coops, H., 1997. Ecosystem responses in the Rhine-Meuse delta during two decades after enclosure and steps toward estuary restoration. *Estuaries* 20 (3), 504–520.
- Soetaert, K., Herman, P.M.J., 1995. Nitrogen dynamics in the Westerschelde Estuary (Sw Netherlands) estimated by means of the ecosystem model Moses. *Hydrobiologia* 311 (1–3), 225–246.
- Sørensen, J., 1978. Denitrification rates in a marine sediment as measured by the acetylene inhibition technique. *Appl. Environ. Microbiol.* 36 (1), 139–143.
- Tombacz, E., Meleg, E., 1990. A theoretical explanation of the aggregation of humic substances as a function of pH and electrolyte concentration. *Org. Geochem.* 15 (4), 375–381.
- Trimmer, M., Nedwell, D.B., Sivyler, D.B., Malcolm, S.J., 1998. Nitrogen fluxes through the lower estuary of the river Great Ouse, England: the role of the bottom sediments. *Mar. Ecol. Prog. Ser.* 163, 109–124.
- Weston, N.B., Dixon, R.E., Joye, S.B., 2006. Ramifications of increased salinity in tidal freshwater sediments: geochemistry and microbial pathways of organic matter mineralization. *J. Geophys. Res.* 111 (G1).
- Winkler, J.P., Cherry, R.S., Schlesinger, W.H., 1996. The Q_{10} relationship of microbial respiration in a temperate forest soil. *Soil Biol. Biochem.* 28 (8), 1067–1072.
- Zumft, W.G., 1997. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* 61 (4), 533–616.