Nitrate, nitrite, and nitrous oxide transformations in sediments along a salinity gradient in the Weser Estuary

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ABSTRACT: The dynamics of the nitrogen intermediates N₂O and NO₂⁻ in estuarine sediments were studied along a salinity gradient in the eutrophied Weser Estuary (Germany) using microsensors for N₂O, NO₂⁻, NO₃⁻, and O₂. During dark incubations in the laboratory of sediment samples from a freshwater site, a brackish water site with fluctuating salinity, and a marine site, the effects of environmental changes in nitrogen availability and salinity on microscale sediment dynamics were examined. Generally, sediment levels of intermediates were low: 1 to 25 µM NO₂⁻ and 0 to 8 µM N₂O. However, significant variation was found in accumulation patterns and in the potential of the residing microbial community to control sediment releases of the intermediates. At fresh- and brackish water sites, NO₂⁻ production was found in the anoxic denitrification zone, and release from the sediments was effectively prevented by activity of nitrite oxidisers in oxic surface layers. In contrast, high rates of NO₂⁻ release occurred in marine sediment, where NO₂⁻ production was predominantly associated with incomplete nitrification in oxic layers. Similarly, stimulated partial nitrification due to NH₄⁺ addition led to NO₂⁻ liberation from brackish water sediment. Production of N₂O was never observed in sediment from the brackish water site, which is naturally exposed to a daily regime of water column variations, but transient N₂O accumulation was observed in the other sediments. The production of N₂O could be induced by an abrupt change in either NO₃⁻ or salinity, and was found in anoxic or micro-oxic sediment layers. Because oxic sediment layers showed little or no potential for N₂O consumption (in contrast to NO₂⁻) the accumulation of N₂O always resulted in release from the sediment surface. Results demonstrate that changes in environmental parameters such as salinity and NO₃⁻ can trigger sediment production and release of NO₂⁻ and N₂O, but further suggest that microbial sediment communities are highly adaptive and can become resistant towards intermediate release when regularly exposed to fluctuating conditions.

KEY WORDS: Nitrite · Nitrous oxide · Sediment · Denitrification · Nitrification · Emission

INTRODUCTION

Estuaries are active sites for reduction of the inorganic nitrogen pool in river waters and play an important role in limiting the nitrogen input to marine environments (Ogilvie et al. 1997). However, associated with high nitrogen turnover rates is the increased risk of production of the nitrogen intermediates nitrite (NO₂⁻) and nitrous oxide (N₂O), which is a concern due to their potentially harmful effects on the environment.

NO₂⁻ is toxic and adversely affects aquatic organisms at relatively low concentrations. For example, certain groups of sediment-dwelling invertebrates are harmed by NO₂⁻ concentrations between 10 and 20 µM (Neumann et al. 2001, Kelso et al. 1999b), and European Union guidelines recommend NO₂⁻ concentrations below 0.25 µM in waters that support salmonid fishes (European Economic Community 1978). N₂O oxide, on the other hand, is an aggressive greenhouse gas with a warming potential 200 to 300 times greater than CO₂.
(Kramlich & Linak 1994) and, furthermore, is the dominant source for stratospheric NO radicals that are causing ozone layer destruction (Conrad 1996). Estuarine environments have been estimated to contribute about 60% of global marine N$_2$O production (Bange et al. 1996), and for NO$_2^-$, water-phase concentrations up to 140 µM have been reported (Dong et al. 2002).

The dynamics of NO$_2^-$ and N$_2$O in sediments are complex, and the set of factors that control their accumulation and subsequent release to the water phase or to the atmosphere (N$_2$O) are not fully resolved. There are several aerobic and anaerobic microbial processes that produce nitrogen intermediates, and a range of environmental parameters affect their accumulation, while microbial interactions may further regulate their release. NO$_2^-$ is an intermediate of nitrification, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA), while N$_2$O is an intermediate of denitrification and nitrifier-denitrification, and is formed as a by-product during nitrification and DNRA (Wrage et al. 2001, de Bie et al. 2002, Philips et al. 2002). Despite several potential sources, NO$_2^-$ and N$_2$O are usually found in low concentrations, illustrating that microbial sediment communities are usually finely tuned to prevent significant accumulation and release. Nitrification and denitrification are recognized as the primary processes responsible for the generation of intermediates in sediments, although reports also exist on the contribution from DNRA (Kelso et al. 1997). In the case of nitrification, NO$_2^-$ will accumulate if NO$_2^-$ oxidation is slower than ammonia oxidation, which can be related to existing population numbers, availability of ammonium (NH$_4^+$) and NO$_2^-$ as electron-donors, oxygen status, and inhibitory compounds (Venterea & Rolston 2000). Both NO$_2^-$ and N$_2$O can accumulate by incomplete denitrification because of kinetic limitations on a community (Holtan-Hartwig et al. 2000) or enzyme level (Betlach & Tiedje 1981).

Elevated production of NO$_2^-$ and N$_2$O appear strongly linked to nitrogen availability, which may reflect why build-ups are usually observed in eutrophic environments. Oxygen is equally considered to have a strong regulating effect (i.e. de Bie et al. 2002). Low oxygen levels may trigger production of N$_2$O and NO$_2^-$ from nitrification as well as N$_2$O from denitrification, and can stimulate sediment release of NO$_2^-$ produced in anoxic layers (Stief et al. 2002). Other known regulatory abiotic factors include temperature, pH, organics, NH$_4^+$ (for NO$_2^-$), and NO$_2^-$ (for N$_2$O). Further, it has been proposed that temporal variation in environmental parameters (i.e. oxygen and nitrogen) can markedly enhance the production of intermediates (Robinson et al. 1998, Naqvi et al. 2000, Laursen & Seitzinger 2004). Therefore, tidally induced fluctuations in physicochemical parameters in estuarine sediments may result in temporally increased NO$_2^-$ and N$_2$O concentrations and subsequent release to the water column or atmosphere. In a recent microsensor study of nitrogen dynamics in subtropical estuarine sediment, occurrence of enhanced production and release of NO$_2^-$ and N$_2$O was thus demonstrated in response to excessive loading with either NO$_3^-$ or NH$_4^+$ (Meyer et al. 2008).

We hypothesize that change in environmental parameters such as increased availability of NO$_3^-$ and NH$_4^+$ or rapid changes in salinity may cause imbalances in the sequential nitrogen conversions leading to enhanced production of NO$_2^-$ and N$_2$O in estuarine sediment. The Weser estuary was chosen to study the effect of such environmental disturbances, as tidal cycles in this system expose the sediments to highly variable salinity and nutrient regimes. We further expect that a site regularly exposed to tidally induced salinity variations will be the most resistant to release of nitrogen intermediates due to the adaptive capacity of present microbial populations. Our work was facilitated by the use of microsensors that allow O$_2$, N$_2$O, NO$_2^-$, and NO$_3^-$ concentrations to be measured at high spatial resolution within the sediment. The use of microscale biosensors for NO$_2^-$, and NO$_3^-$ made it possible to analyse these ions in a saline environment. It was not our intention to replicate in detail the in situ conditions but to illustrate aspects of nitrogen intermediate dynamics at a microscale when estuarine sediments are exposed to a change in salinity or nitrogen loading.

**MATERIALS AND METHODS**

**Study area.** The Weser estuary (Germany) stretches more than 80 km from the city of Bremen to beyond Bremerhaven where it merges into the German Bight (see Fig. 1). The estuary receives water from the river Weser and its tributaries and has a mean water discharge of 317 m$^3$ s$^{-1}$. The Weser system is nutrient-rich with high water concentrations of inorganic nitrogen, primarily found as NO$_3^-$. A surveillance program for the Weser river and estuary dates back to 1979 (data available at http://tgg-weser.de/download_nue.html). At Brake, about 40 km downstream of Bremen, the water NO$_3^-$ concentration ranged between 115 and 460 µM in 2004 to 2006, with minimum levels in late summer and maximum levels during winter. During the same period, water concentrations of NO$_2^-$ and NH$_4^+$ were always below 3 and 10 µM, respectively. The estuary has a mean tidal range of about 3.5 m with the intrusion of high salinity, lower nutrient water from the German Bight (Beddig et al. 1997). Areas that stretch about 50 to 70 km downstream of Bremen are
exposed to strong diurnal variation in salinity of about 2 to 15‰ (Grabemann et al. 1997).

**Sampling and experimental setup.** Sediment samples were collected during early fall (September) from 3 locations along the Weser Estuary: a freshwater site, a brackish water site with diurnal fluctuations in salinity, and a marine site (Fig. 1). Prior to sampling, the salinity regime was surveyed at the brackish water location during a full tidal cycle, and observed to vary between 2 and 12‰. On the day of sediment collection, water samples were taken to determine \( \text{in situ} \) \( \text{NO}_3^- \) concentrations. Sediment sampling was performed at low tide on exposed tidal flats with fine particulate sediment material, and the ~10 cm deep samples were collected with a rectangular Plexiglas sampler that fit the flow cells (12 × 8 cm) used for sediment incubations in the laboratory. At the sampling locations, the sediment samples were carefully transferred to flow cells without destroying the layered architecture. They were then brought back to the laboratory, where they were connected to 60 l water reservoirs. Recirculating water (flow rate ~20 l h\(^{-1}\)) was prepared from artificial sea salt (Preis-Meersalz, Preis Aquaristik) and deionized water, and adjusted to salinity levels of 0.5, 6.5, and 25 g l\(^{-1}\). NaNO\(_3\) and NH\(_4\)Cl were supplemented from stock solutions to obtain various \( \text{NO}_3^- \) and NH\(_4^+\) concentrations in the water phase. During the 10 d experimental period, the flow systems were kept in the dark to prevent photosynthetic activity. Temperature was kept at 22.5 ± 1°C, corresponding to mid-summer \textit{in situ} temperature. The salinity was measured routinely and adjusted through additions of deionized water to compensate for evaporative losses. Every day, 10 ml filtered water samples were taken from each system to determine \( \text{water phase} \) concentrations of \( \text{NO}_3^- \) and \( \text{NH}_4^+\). Concentrations of \( \text{NO}_3^- \) (+\( \text{NO}_2^-\)) were measured with a macroscale version of a \( \text{NO}_x^-\) biosensor (Unisense A/S) whereas \( \text{NH}_4^+\) concentrations were determined using a conventional spectrophotometric method (Keeney & Nelson 1982).

Microprofiles of each parameter were measured separately with microsensors mounted on a computer-controlled micro-manipulator for automatic profiling. Through a computer interface (ADC-216USB and Profix software, Unisense A/S) it was possible to program important profiling settings including step size, sensor resting time at each profile depth, and maximum profile depth. A step size of 200 µm was used for \( \text{O}_2\) profiles, whereas 400 or 800 µm was used for \( \text{NO}_2^-\), \( \text{NO}_x^-\), and \( \text{N}_2\text{O}\) profiles. Larger steps for the latter profiles were chosen because of the relatively long sensor response times that necessitated similar long resting times (45 to 75 s) at each depth. A dissection microscope was used to determine the position of the sediment–water interface before each profile measurement.

**Microsensors.** Microsensors for \( \text{NO}_2^-\), \( \text{NO}_x^-\), \( \text{N}_2\text{O}\), and \( \text{O}_2\) were prepared and calibrated as described previously (Revsbech 1989, Larsen et al. 1997, Andersen et al. 2001, Nielsen et al. 2004). The \( \text{NO}_2^-\) and \( \text{NO}_x^-\) micro-biosensors had tip diameters of about 50 to 80 µm and had 90% response times of between 45 and 75 s. Electrophoretic sensitivity control (ESC) was used to control sensitivity of the biosensors (Kjær et al. 1999), which made it possible to measure \( \text{NO}_2^-\) and \( \text{NO}_x^-\) concentrations down to about 0.2 µM. As the \( \text{NO}_x^-\) and \( \text{NO}_2^-\) biosensors are also sensitive to \( \text{N}_2\text{O}\), the response to \( \text{N}_2\text{O}\) was quantified so that it was possible to correct for \( \text{N}_2\text{O}\) interference on the signal. The \( \text{N}_2\text{O}\) microsensors had detection limits of about 0.3 µM \( \text{N}_2\text{O}\). The 90% response time of 40 to 50 µm thick sensors was about 30 s.

A modified version of the diffusivity sensor described by Revsbech et al. (1998), based on outflux of \( \text{N}_2\text{O}\) instead of \( \text{H}_2\), was used to determine the effective diffusion coefficient (\( \phi D_e\)). The sediment samples for diffusivity determinations were incubated overnight with 20% acetylene in the \( \text{NO}_x^-\)-free water phase to inhibit \( \text{N}_2\text{O}\) transformations during the measurements. To integrate over a representative volume, diffusivity sensors should have diameters of at least 3 times the average sediment grain size. The diffusivity sensor was therefore manufactured with a tip diameter of 0.8 mm,

![Fig. 1. Weser estuary sampling locations. Freshwater (1*), brackish water (2*), and marine (3*) sites](image-url)
sediment samples from the 3 sites along the salinity gradient. (Table 1. Nitrogen intermediate concentrations and salinities used in steady-state and perturbation experiments conducted with 
N2O was calculated using measured mean local N2O
ions in the sediments relative to the values in water
the flux calculations, where diffusion coefficients for
ions in the sediments relative to the values in water
were assumed to be reduced with the same percentage
concentration values. At conditions where N2O was detected in the sediment, NO3– and NO2– profiles were further corrected. The part of NO2– and NO3– biosensor signals that was related to N2O was calculated using measured mean local N2O concentrations and biosensor N2O calibration values.

Flux rates across the sediment–water interface were calculated from concentration gradients in the uppermost sediment layer using Fick’s first law of diffusion, J = –φ DdC/dx, where J is the flux, φ the porosity, D the diffusion coefficient, and dC/dx the concentration gradient. Directly measured values for φD were used for the flux calculations, where diffusion coefficients for ions in the sediments relative to the values in water were assumed to be reduced with the same percentage as determined for gas (N2O). The diffusivities for NO2– and NO3– were set to 79% of the value for O2 (Li & Gregory 1974), and the diffusivity for N2O was identical (within 0.5%) to that of O2 (Broecker & Peng 1974). Activity profiles in the sediment were calculated from the profile simulation program ‘Profile’ (Berg et al. 1998). Mean concentration values of 3 different profiles were used for activity calculations. By modeling NOx– (i.e. NO2– + NO3–) profiles it is possible to estimate depth profiles of production (i.e. oxidation of NH4+) and consumption of NO3–.

Experiments. In steady-state experiments, the sediment distributions of NO3–, NO2–, N2O, and O2 were examined for all samples with approximately 10 or 200 µM NO3– in the water phase (Table 1). The low concentration of 10 µM allowed precise modelling of nitrification activities where modelling at high NO3– may be less accurate. In sediment from the brackish water site, concentration profiles were also measured in the presence of 50 µM NH4+ plus 200 µM NO3–. For each experimental condition, a minimum incubation time of 16 h was used following a change in water phase concentration to ensure that gradients in the sediment had approached equilibrium when the measurements were performed. For all analysed conditions, 3 replicate profiles of each parameter were recorded. Additionally, during the transition in water phase concentration from 10 to 200 µM NO3– in sediment from the brackish water site, single profiles of NO3– and N2O were measured at 0, 1, 3, 5, and 17 h.

In perturbation experiments, the different samples were exposed to abrupt changes in salinity and NO3– concentration in the overlying water phase (Table 1), corresponding to changes that might happen between low and high tide. In 2 independent perturbation events, sediment from the brackish water site, incubated with 200 µM NO3–, was exposed to 0.5‰ water with 200 µM NO3– and to 25‰ water with 10 µM NO3–. Similarly, sediment incubated with 200 µM NO3– and marine sediment incubated with 10 µM NO3– were exposed to 6.5‰ water with 200 µM NO3–. During each perturbation event, single profiles of N2O and O2 were measured at 0, 1, 2, 4, 6, and 9 h. In freshwater sediment in which N2O was found beyond 9 h, the duration of the perturbation experiment was extended to 31 h. NO2– and NOx– profiles were only measured at 0 and 9 h during salinity perturbations (data not shown) as the biosensor signals are salinity-dependent (Nielsen et al. 2004) and thus do not allow accurate analysis in the presence of a steep salinity gradient.

Sediment core samples were taken from the flow cells for characterisation of porosities, organic C content, and C:N ratios in the upper 5 mm. Porosities were determined by drying 5 mm thick sediment slices at 105°C for 24 h with subsequent determination of water loss. Organic C contents and C:N ratios were determined with a Carlo-Erba CN analyser.

| Table 1. Nitrogen intermediate concentrations and salinities used in steady-state and perturbation experiments conducted with sediment samples from the 3 sites along the salinity gradient. (→): Change in salinity and NO3– concentration. nd: not determined

<table>
<thead>
<tr>
<th></th>
<th>Freshwater</th>
<th>Brackish water</th>
<th>Marine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steady-state</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO3– (µM)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>NO2– (µM)</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>NO3– (µM) + NH4+ (µM)</td>
<td>nd</td>
<td>200 + 50</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Perturbation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity (ppt)/NO3– (µM)</td>
<td>0.5/200 → 6.5/200</td>
<td>6.5/200 → 6.5/200</td>
<td>25/10 → 6.5/200</td>
</tr>
</tbody>
</table>
RESULTS

Sediment characteristics

At all sampling locations, the sediments were silty with porosities in the upper 5 mm of 0.60, 0.73, and 0.72 for freshwater, brackish water and marine sediments, respectively. The organic C contents were determined to be 11, 21 and 23 mg g⁻¹ dry weight, respectively, and the organic C:N ratios were about 25 (21, 24 and 27, respectively). The φDₚ values (for O₂ or N₂O diffusion in the matrix) at 22.5°C were 1.01, 1.15, and 0.95 × 10⁻⁵ cm² s⁻¹, respectively. Using the conversion factor of 0.79 between gas and the ions in question, this corresponds to φDₚ values for NO₃⁻ or NO₂⁻ in the samples of 0.80, 0.91, and 0.75 × 10⁻⁵ cm² s⁻¹, respectively.

Water phase NO₃⁻ and NH₄⁺ concentrations

Analyses of water samples taken at the day of sediment collection showed in situ NO₃⁻ concentrations for freshwater, brackish water and marine sediments of about 160, 150, and 50 µM, respectively. Sampling at low tide explained the similar in situ water NO₃⁻ concentrations at the first 2 sampling locations and the relatively high concentration at the marine site. Water phase NO₃⁻ concentrations in the incubation systems during steady-state experiments varied between 9 and 14 µM for low NO₃⁻ incubations and between 170 and 190 µM for high NO₃⁻ incubations. The NH₄⁺ concentration in the water phase remained low (<4 µM) in sediment samples from fresh- and brackish water sites during the experimental period, except when the water above the sediment from the brackish water site was supplemented with NH₄⁺ (47 µM). In the water column above the marine sediment, there was a continuous increase in NH₄⁺ concentrations, reaching a maximum level of 28 µM corresponding to an average sediment NH₄⁺ efflux of about 24 pmol cm⁻² s⁻¹.

NO₂⁻ and NO₃⁻ at steady state conditions

Concentration profiles of NO₂⁻, NO₃⁻, N₂O, and O₂ at conditions with either 10 µM NO₃⁻, 200 µM NO₃⁻, or 200 µM NO₃⁻ plus 50 µM NH₄⁺ (brackish water sediment) in the water phase are presented as means of 3 replicates (see Figs. 2 to 5). As the error bars indicate, distributions of the different nitrogen forms were characterized by some degree of heterogeneity between replicate measurements.

At low NO₃⁻ concentrations (~10 µM) in the overlying water phase, distributions of NO₂⁻, NO₃⁻, and O₂ were similar in fresh- and brackish water sediments (Figs. 2a,b & 3a,b). In both sediment types, O₂ penetrated to depths of about 3 mm. Distinct NO₃⁻ production zones were found in oxic layers, with concentration peaks of 21 µM in the freshwater sediment and 19 µM in sediment from the brackish water site. The highest NO₃⁻ production rates were observed just above the oxic–anoxic interface, whereas NO₃⁻ reduction occurred in deeper anoxic layers. In both sediment types, there was an efflux of NO₃⁻ to the water phase. Sediment NO₂⁻ concentrations were low, with maximum peaks of about 1 µM (freshwater) and 2.5 µM brackish water found within anoxic NO₃⁻ reduction zones. Anaerobically produced NO₂⁻ was consumed in deeper anoxic layers and in oxic surface layers just above the oxic–anoxic interface. In marine sediment, the O₂ penetration depth was less than 2 mm (Fig. 4a,b). Aerobic NO₃⁻ production resulted in a concentration peak of about 20 µM within the oxic layer. In contrast to the other samples, the NO₂⁻ distribution profile in marine sediment was characterized by a distinct aerobic production zone that resulted in NO₂⁻ accumulation to about 10 µM. Aerobically produced NO₂⁻ (and NO₃⁻) was partly consumed within the marine sediment, and there was a NO₂⁻ efflux of about 0.71 pmol cm⁻² s⁻¹ (Table 2).

Increasing the NO₃⁻ concentration in the water from 10 to 200 µM did not affect the O₂ penetration depth in any of the samples, but resulted in significant changes in sediment distributions of NO₂⁻ and NO₃⁻. The flux of NO₂⁻ from the water to the sediment was stimulated by the elevated NO₃⁻ concentration, and the increased supply to anoxic layers resulted in higher rates of anaerobic NO₃⁻ reduction (Table 3). However, the effect on NO₃⁻ penetration depth was highly variable. In freshwater sediment (Fig. 2c,d), NO₃⁻ penetration was largely unaltered (~4 mm), whereas the penetration depth in brackish water (Fig. 3c,d) and marine (Fig. 4c,d) sediments increased from 5 to 11.5 mm and from 2.6 to 4 mm, respectively. From the microprofiles, we calculated for all samples a pronounced stimulation of nitrification with increased rates of aerobic NH₄⁺ oxidation and NO₃⁻ production when comparing activities during incubations with 10 and 200 µM (Table 3, Figs. 2 to 4). Increased NO₃⁻ supply to the sediment also stimulated net anaerobic NO₂⁻ formation. In freshwater sediment, the anoxic NO₂⁻ peak increased from 1 to 3 µM, whereas the deep NO₃⁻ penetration in sediment from the brackish water site resulted in a 5 mm broad NO₂⁻ production zone and increased the anoxic NO₂⁻ peak from 2.5 to 15 µM. The increased rates of net anaerobic NO₂⁻ production in fresh- and brackish water sediments did not lead to an elevated NO₂⁻ efflux from the sediments, as the produced NO₂⁻ in both cases was
consumed in the oxic sediment layers before it could reach the water phase. In marine sediment, there was a large increase in aerobic NO$_2^-$ production, although no apparent stimulation of anaerobic NO$_2^-$ formation occurred; the NO$_2^-$ peak in the sediment increased from 10 to 26 µM with a concomitant increase in the efflux of NO$_2^-$ from 0.71 to 2.26 pmol cm$^{-2}$ s$^{-1}$ (Table 2). The calculated net areal NO$_2^-$ production rates in anoxic layers (at 200 µM NO$_3^-$) varied by a factor of 10 and were 0.00054, 0.0012, and 0.00012 nmol cm$^{-2}$ s$^{-1}$ for freshwater, brackish water, and marine sediments, respectively.

Supplementation with NH$_4^+$

Increasing the NH$_4^+$ concentration in the water phase overlying sediment from the brackish water site from 3 to 47 µM was followed by significant changes in NO$_2^-$ and NO$_3^-$ profiles due to marked stimulation of the nitrification process (Table 3, Fig. 5). The increased rates of NH$_4^+$ and NO$_2^-$ oxidations resulted in a sub-surface NO$_3^-$ peak and turned the sediment into a source of NO$_2^-$ to the water phase, with an efflux rate of 0.49 pmol cm$^{-2}$ s$^{-1}$ (Table 2). Anaerobic NO$_3^-$ reduction and NO$_2^-$ production rates were similar with and without NH$_4^+$ addition (Table 3, Figs. 3 & 5), but the change in overall gradients led to increased concentrations of NO$_2^-$ with a concentration peak of 20 µM.

N$_2$O formation at constant salinity

Sediment N$_2$O concentrations in the different samples during steady-state incubations were generally below the sensor detection limit of about 0.3 µM. N$_2$O could, however, be detected in freshwater sediment when incubated with 200 µM NO$_3^-$ in the water phase (Fig. 2). Under this condition, net N$_2$O production was...
observed in anoxic sediment layers just below the oxic–anoxic interface, but N$_2$O production was highly variable, with concentration peaks between 3 and 12 µM N$_2$O among the 3 replicates. Net N$_2$O production in the anoxic sediment layer was followed by release of N$_2$O from the sediment to the overlying water with an average efflux of 0.16 pmol N$_2$O cm$^{-2}$ s$^{-1}$ (Table 2). The accumulation of N$_2$O was a transient phenomenon; N$_2$O was undetectable in the sediment 48 h after the profiles were measured.

Table 2. Efflux rates of NO$_2^-$ and N$_2$O (pmol cm$^{-2}$ s$^{-1}$) from the sediment surface under different experimental conditions, calculated from concentration gradients in the uppermost sediment layers. Unless indicated otherwise, values are mean ± SD of 3 replicates. nd: no data, no significant efflux rates were observed.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Freshwater</th>
<th>Intermediate</th>
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<tr>
<td><strong>Steady-state</strong></td>
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<tr>
<td>10 µM NO$_3^-$</td>
<td>nd</td>
<td>nd</td>
<td>0.71 ± 0.04 (NO$_2^-$)</td>
</tr>
<tr>
<td>200 µM NO$_3^-$</td>
<td>0.16 ± 0.08 (N$_2$O)</td>
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<td>2.26 ± 0.20 (NO$_2^-$)</td>
</tr>
<tr>
<td>200 µM NO$_3^-$ / 50µM NH$_4^+$</td>
<td>nd</td>
<td>0.49 ± 0.05 (NO$_2^-$)</td>
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<td><strong>Perturbation</strong></td>
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<td></td>
</tr>
<tr>
<td>N$_2$O, max. rate</td>
<td>0.072</td>
<td>nd</td>
<td>0.075</td>
</tr>
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</table>
NO$_3^-$ perturbation at constant salinity

The sediment NO$_2^-$ and N$_2$O dynamics from the brackish water site were also monitored during a transition phase, when the water NO$_3^-$ concentration was elevated from 12 to 190 µM (Fig. 6). While N$_2$O remained undetectable throughout, there was a gradual build-up of NO$_2^-$ in the anoxic sediment layer, with the peak concentration rising from 3 to 16 µM within the 17 h experimental period. At the same time, gradual changes in depth and width of the NO$_2^-$ production zone took place, which resulted in a distinct displacement of the NO$_2^-$ peak in the sediment from about 3.5 to 6 mm depth.

<table>
<thead>
<tr>
<th>Reduction rates (pmol cm$^{-2}$ s$^{-1}$)</th>
<th>Freshwater</th>
<th>Brackish water</th>
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<tr>
<td>NH$_4^+$-oxidation</td>
<td>1.9</td>
<td>1.2</td>
<td>4.2</td>
</tr>
<tr>
<td>10 µM NO$_3^-$</td>
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<td></td>
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<tr>
<td>200 µM NO$_3^-$</td>
<td>5.2</td>
<td>2.8</td>
<td>7.5</td>
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<tr>
<td>200 µM NO$_3^-$/50 µM NH$_4^+$</td>
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<td>5.1</td>
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<tr>
<td>NO$_3^-$-reduction</td>
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<td>1.0</td>
<td>1.4</td>
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<tr>
<td>200 µM NO$_3^-$</td>
<td>6.2</td>
<td>3.4</td>
<td>6.1</td>
</tr>
<tr>
<td>200 µM NO$_3^-$/50 µM NH$_4^+$</td>
<td></td>
<td>3.1</td>
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</table>

Table 3. Calculated net NH$_4^+$ oxidation and NO$_3^-$ reduction rates in sediment samples under different incubation conditions during steady-state experiments

Fig. 4. Average concentration profiles of NO$_2^-$, NO$_3^-$ and O$_2$, and reaction rates of NO$_2^-$ and NO$_3^-$ in marine sediment (mean ± SD). Incubations of (a, b) 10 µM and (c, d) 200 µM NO$_3^-$. The dotted lines indicate the sediment surface. N$_2$O concentrations were always below detection limit.
Perturbation experiments with an abrupt change in water phase salinity (Table 1) caused N\textsubscript{2}O to accumulate in freshwater and marine sediments (Fig. 7a,c), but never in sediment from the brackish water site (data not shown). During salinity perturbations, no distinct change in O\textsubscript{2} penetration depths could be identified (Fig. 7b,d). The N\textsubscript{2}O production zone in freshwater sediment was found below the oxic–anoxic interface, and the highest N\textsubscript{2}O concentration (4 µM) was encountered 9 h after the onset of the salinity increase (Fig. 7a). N\textsubscript{2}O was still detectable (2 µM) after 31 h. In marine sediment, the N\textsubscript{2}O production zone was located at or just below the oxic–anoxic interface (Fig. 7c). The N\textsubscript{2}O dynamics in marine sediment were faster, and the maximum N\textsubscript{2}O concentration encountered was about 2 µM. Build-up to the highest level took place within the first 2 h after the decrease in salinity, and after 9 h the N\textsubscript{2}O peak had almost disappeared again. In both freshwater and marine samples, the transient build-up resulted in N\textsubscript{2}O release from the sediment surface with almost identical maximum rates of 0.072 and 0.075 pmol N\textsubscript{2}O cm\textsuperscript{-2} s\textsuperscript{-1}, respectively (Table 2).
knowledge about nitrogen cycling in less accessible environments.

The concentration profiles were initially measured at conditions of 10 µM NO$_3^-$ in the water phase, which is significantly lower than in situ concentrations, especially at the fresh- and brackish water sites. However, analyses at low NO$_3^-$ concentrations allowed precise identification of nitrification zones in the sediment without the inaccuracy caused by a high NO$_3^-$ background. Subsequently, NO$_3^-$ concentrations were increased to 200 µM to study effects of NO$_3^-$ supply to anoxic regions on anaerobic NO$_2^-$ and N$_2$O dynamics. Finally, the metabolic potential of the nitrifying population in sediment from the brackish water site was examined by addition of NH$_4^+$. The rationale behind the perturbation experiments was to study the effects of naturally occurring fluctuations in salinity and NO$_3^-$ on sediment N$_2$O dynamics at the brackish water site during tidal cycles and at the freshwater and marine sites during extreme weather events. If N$_2$O production is stimulated by salinity changes, sediments from such areas may represent a significant atmospheric N$_2$O source. By investigating sediments along a gradient in tidal fluctuation, it was possible to elucidate whether the microbial communities can adapt to such a continuously changing chemical environment. It has previously been shown how changes in oxygen concentration in the water phase (Larsen et al. 1997) or changes in oxygen caused by benthic photosynthesis (Lorenzen et al. 1998) affect microprofiles of nitrification and NO$_3^-$ respiration. However, due to the limitations imposed by the complexity of the microsensor array, we limited this investigation of nitrogen intermediate accumulation to the effects of changes in nitrogen availability and salinity. None of the investigated sites were subject to intense activity of burrowing infauna, but such fauna may have profound impacts on both nitrification (Kristensen et al. 1985) and denitrification (Binnerup et al. 1992), and thus on the formation of nitrogen intermediates, when they create a 3-dimensional network of oxic and anoxic sites.

Fig. 7. Concentration profiles of N$_2$O and O$_2$ in (a, b) freshwater and (c, d) marine sediments during salinity perturbation experiments. The freshwater sample was subject to a change from 0.5‰ salinity, 200 µM NO$_3^-$ water to 6.5‰ salinity, 200 µM NO$_3^-$ water. The marine sample was subject to a change from 25‰ salinity, 10 µM NO$_3^-$ water to 6.5‰ salinity, 200 µM NO$_3^-$ water. The dotted lines indicate the sediment surface.
Generally, intermediates can accumulate if subsequent steps in a sequence of conversions are limited by kinetics or by limited supply of a reactant. Microbial communities can respond quickly to kinetic limitations by gene induction, whereas cell growth is a slower process, especially for the NH$_4^+$ and NO$_2^-$ oxidising populations. With our microsensor experiments, we aimed to elucidate whether and when either nitrification or denitrification can be responsible for nitrogen intermediate release and consumption.

**NO$_2^-$ dynamics**

Significant variation in sediment NO$_2^-$ dynamics was observed along the estuary, in terms of both accumulation and release patterns. The concentration profiles revealed NO$_2^-$ production in both oxic and anoxic sediment layers, and hence showed examples of NO$_2^-$ accumulation related to either aerobic or anaerobic processes. Although nitrification activity was high at all sites, the potential of the nitrifying community to further oxidise the produced NO$_2^-$ to NO$_3^-$ differed. In oxic layers of fresh- and brackish water sediments, the accumulation of NO$_2^-$ was nearly absent, which contrasts the high level of NO$_3^-$ accumulation in the marine sediment. The different scenarios may be partially explained by differences in NH$_4^+$ availability. In marine sediment, nitrification was evenly distributed throughout the oxic layer (low NO$_3^-$ incubation), indicating that NH$_4^+$ availability was not process limiting. Indeed, the measured sediment NH$_4^+$ efflux exceeded the estimated NH$_4^+$ oxidation rates 3- to 6-fold. In contrast, the nitrification activity at fresh- and brackish water sediments was primarily associated with the oxic–anoxic boundary, where NH$_4^+$ was likely supplied from the deep anoxic layers and fully oxidised. The presence of ammonium limitation in oxic surface layers was further confirmed for the brackish water site by the NH$_4^+$ addition experiment, which showed a greatly stimulated nitrifying activity that was distributed evenly throughout the oxic layer (Fig. 5). Accumulation of NO$_2^-$ in the nitrification zone indicates an imbalance between the ammonia and nitrite oxidising communities, with a higher ammonia than NO$_2^-$ oxidation rate. There may be historic reasons for the incomplete nitrification, such as a recent disturbance removing most of the oxic microbial community that, subsequently, must re-establish. The time period for a disturbed nitrifying community to re-establish itself may be >1 wk (Nielsen & Revsbech 1998), and the relative biomass increase of the 2 bacterial groups may depend on a multitude of factors, such as O$_2$, NH$_4^+$, and NO$_2^-$ concentrations, pH, and temperature (Hellinga et al. 1998). According to the observed dynamics at our 3 sites, the anaerobic formation of NO$_3^-$ in sediments appears to be highly variable. The variation along the estuary is probably related to differences in resident microbial populations in the sediment that are adapted to NO$_3^-$ respiration. A positive correlation between NO$_3^-$ availability and anaerobic NO$_2^-$ production was observed in fresh- and brackish water sediments, as has also been reported for other systems (e.g. Stief et al. 2002).

The calculated rates of ammonia oxidation were 1.8 to 2.7× higher in incubations with 200 µM than with 10 µM NO$_3^-$ (Table 3). Such a stimulating effect of increased NO$_3^-$ availability on nitrification is rather surprising. It can be reasoned that NO$_3^-$ addition to the water column results in enhanced DNRA in anoxic sediment zones, followed by increased nitrification activity in the overlying oxic zone due to upward NH$_4^+$ diffusion. However, such an explanation seems unlikely for the marine sediment, which, in contrast to fresh- and brackish water sediments with suspected NH$_4^+$ limitation, released significant amounts of NH$_4^+$.

Determination of nitrifying activity in sediments by use of $^{15}$N stable isotope techniques generally do not show increased rates of nitrification when the NO$_3^-$ concentration is elevated (L. P. Nielsen pers. comm.). Hence, the consistent finding of higher ammonia oxidation rates after NO$_3^-$ addition is likely an artefact of our calculation, with larger errors for the estimates at high water phase NO$_3^-$ concentration. We were aware of this potential source of error while planning the experiment, as surface determinations and exact diffusivity determinations become critical with almost vertical surface NO$_3^-$ profiles at high concentrations, and we therefore conducted additional experiments at artificially low NO$_3^-$ concentrations. The inaccuracy at high concentrations is essentially the same problem as usually encountered when a small value is obtained by subtracting 2 large values. Ideally, the data would have been supplemented with microprofiles of NH$_4^+$, which would have helped in resolving this issue, but NH$_4^+$ microsensors do not function in saline environments.

Sediment release of NO$_2^-$ to the water phase, as demonstrated in the present study, is regulated through interactions between aerobic and anaerobic transformation processes. From an environmental perspective, the production of NO$_2^-$ in oxic sediment layers appears particularly critical, because it could give rise to high efflux rates (as observed in marine sediment) due to a short diffusion distance from the production zone to the sediment–water interface. In comparison, the effect of increased anaerobic NO$_2^-$ formation on water column efflux is less predictable. Anoxic NO$_2^-$ accumulation will lead to diffusion of NO$_2^-$ into oxic sediment layers, which may act as a
NO$_3^-$ sink, thus preventing the escape of anaerobically formed NO$_2^-$ from the sediment surface. This occurred in fresh- and brackish water sediments, where all NO$_2^-$ was consumed, most likely by NO$_2^-$ oxidation. When NH$_4^+$ was supplied to the overlying water, the oxic zone of sediments from the brackish water site could no longer oxidise all NO$_2^-$ produced, simply because the NO$_2^-$-oxidising potential was then saturated by the combined oxic and anoxic NO$_2^-$ supply. Because ammonium oxidisers in general cope better with low oxygen concentrations than nitrite oxidisers (Hanaki et al. 1990), reduced oxygen availability for sediment processes will proportionally reduce the effectiveness of the oxic layer as a NO$_2^-$ sink, leading to increased sediment efflux of NO$_2^-$ originating from the anoxic layers (Stief et al. 2002). The effect of microphytobenthos on NO$_2^-$ release was not studied here, but it is likely that a cover of microphytobenthos would reduce the release. Microphytobenthos has been shown to reduce overall nitrification and thus NO$_2^-$ production (Risgaard-Petersen 2003), likely due to competition for substrate.

**N$_2$O dynamics**

The production of N$_2$O varied along the Weser estuary but, characteristically, only transient sediment N$_2$O accumulation was observed. At the freshwater site, either increased NO$_3^-$ concentration or elevated salinity caused N$_2$O accumulation. In subtropical Australian mangrove sediment, NO$_3^-$ loading also induced N$_2$O accumulation; however, the increased production was not a transient phenomenon in this system, as high concentrations of N$_2$O (>12 µM) were detected over a period of 10 d (Meyer et al. 2008). The observed difference in temporal N$_2$O dynamics at the 2 locations may reflect that the mangrove sediment was exposed to a more dramatic perturbation, originating from a lower nutrient environment (*in situ* NO$_3^-$ < 100 µM), and experimentally incubated with 500 µM NO$_3^-$.

However, the stimulated formation of N$_2$O by NO$_3^-$ occurred in anoxic layers in both sediments and was therefore related to anaerobic processes. In the respiratory pathway of denitrification, the induction of nitrous oxide reductase synthesis is often found to lag behind the induction of the other enzymes, which leads to a peak in N$_2$O formation at the onset of anoxic conditions or the addition of nitrate (Firestone & Tiedje 1979). The stimulated production of N$_2$O during an abrupt change in salinity likely reflects that either N$_2$O-reductases or populations specialized on N$_2$O reduction are salinity sensitive. In the marine sediment, transient accumulation of N$_2$O was only observed when the sediment was exposed to low salinity water with high NO$_3^-$, and it is therefore difficult (even with high resolution microprofiles) to conclude whether accumulation in this situation was related to activity of nitrifiers, denitrifiers, or both. This is in contrast to the microsensor work by Meyer et al. (2008), where a distinct oxic N$_2$O production zone (at high NH$_4^+$ conditions) was clearly identified and hence could be attributed to nitrification with higher certainty. It should be noted that it is difficult to isolate the causal factor for N$_2$O accumulation in the marine sediment in the present study. Because the transient dynamics were fast, it cannot be ruled out that a similar scenario also occurred when the NO$_3^-$ concentration was increased from 10 to 200 µM (at constant salinity). Therefore, the accumulation of N$_2$O could be a response to the increased NO$_3^-$ supply, rather than being a salinity effect.

N$_2$O oxide accumulation was, in all cases, observed to induce sediment release of N$_2$O to the overlying water phase. Thus, in contrast to NO$_3^-$ dynamics, where NO$_3^-$ oxidation in the aerobic layer might prevent release of anaerobically produced NO$_2^-$ to the water phase, aerobic transformation of N$_2$O did not take place. Indeed, the only microbial process that consumes N$_2$O under oxidic conditions is aerobic denitrification, which is rarely reported in natural samples (Robertson et al. 1995). Interestingly, almost equal N$_2$O efflux rates were found in the marine and freshwater samples during salinity perturbations (Table 2), although N$_2$O accumulated to an almost 2-fold higher concentration in the freshwater sediment (Fig. 7). The deeper location of the anoxic N$_2$O production zone and, consequently, longer diffusion path for N$_2$O to the sediment surface in the freshwater sample explains this situation, elegantly demonstrating the importance of diffusion distance on sediment release rate.

The significant contribution of estuarine environments to atmospheric N$_2$O is well recognized, but mechanistic insight is limited. Low salinity regions, rather than marine sites, have been identified as important areas for N$_2$O release (Robinson et al. 1998, Barnes & Owens 1999, De Bie et al. 2002). This correlates well with the results from the present study, where the highest N$_2$O efflux was observed from freshwater sediment. In the hypernutrified Colne Estuary, high N$_2$O release rates were found for tidal sediments that experience diurnal fluctuations in NO$_3^-$ availability (Robinson et al. 1998). The transient N$_2$O release dynamics at our freshwater site (and possibly at the marine site) upon transition from low to high NO$_3^-$ water indicate that fluctuation in NO$_3^-$ availability represents an important mechanism of N$_2$O formation in the Weser estuary, as such variation is often encountered during the tidal cycles. Further, the demonstration of salinity-induced N$_2$O release from
Weser sediments suggests salinity as a potential causal factor of N₂O production, but the environmental significance of the observed dynamics remains to be addressed. Clearly, the naturally occurring tidally induced changes in salinity are usually slower and less extreme than the applied perturbations; therefore, our findings may not reflect in situ dynamics. As opposed to freshwater and marine sites that only experience fluctuations in salinity under more extreme weather conditions, the brackish water site experiences diurnal variations in salinity, hence representing the area of the estuary where salinity-induced N₂O production is most likely to constitute a major source. For this reason, we especially focused on N₂O dynamics at the brackish water site, exposing the sediment to a range of perturbations; however, the sediment proved highly resistant to N₂O accumulation. The absence of N₂O accumulation at this site may have been caused by adaptation of the resident microbial communities to the continuously changing ambient conditions; only the sites that were not regularly exposed to diurnal variations were sensitive to salinity perturbations.

CONCLUSIONS

The fluxes of NO₃⁻ and N₂O from estuarine sediments to the overlying water or atmosphere are governed by complex interactions between resident microbial populations and overlying water chemistry. In general, the processes that convert large amounts of NO₃⁻ and N₂O to their end-products are surprisingly well geared and, as such, perturbations in environmental factors usually only result in transient accumulations.

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