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ORIGINAL ARTICLE

Aerobic denitrification in permeable Wadden Sea sediments

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Permeable or sandy sediments cover the majority of the seafloor on continental shelves worldwide, but little is known about their role in the coastal nitrogen cycle. We investigated the rates and controls of nitrogen loss at a sand flat (Janssand) in the central German Wadden Sea using multiple experimental approaches, including the nitrogen isotope pairing technique in intact core incubations, slurry incubations, a flow-through stirred retention reactor and microsensor measurements. Results indicate that permeable Janssand sediments are characterized by some of the highest potential denitrification rates ($\geqslant$0.19 mmol N m^{-2} h^{-1}) in the marine environment. Moreover, several lines of evidence showed that denitrification occurred under oxic conditions. In intact cores, microsensor measurements showed that the zones of nitrate/nitrite and O_2 consumption overlapped. In slurry incubations conducted with $^{15}NO_3^-$ enrichment in gas-impermeable bags, denitrification assays revealed that N_2 production occurred at initial O_2 concentrations of up to $\sim$90 μM. Initial denitrification rates were not substantially affected by O_2 in surficial (0–4 cm) sediments, whereas rates increased by twofold with O_2 depletion in the at 4–6 cm depth interval. In a well mixed, flow-through stirred retention reactor (FTSRR), $^{29}N_2$ and $^{30}N_2$ were produced and O_2 was consumed simultaneously, as measured online using membrane inlet mass spectrometry. We hypothesize that the observed high denitrification rates in the presence of O_2 may result from the adaptation of denitrifying bacteria to recurrent tidally induced redox oscillations in permeable sediments at Janssand.

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Introduction

Nitrogen (N) is primarily removed from coastal ecosystems by microbially mediated denitrification that occurs on the seafloor (Hulth *et al.*, 2005). Continental shelf sediments are important sites of N removal, which may account for 50–70% of oceanic N loss (Codispoti *et al.*, 2001). Although the majority of continental margins is covered by coarse-grained relict sediments (Emery, 1968; Johnson and Baldwin, 1986), most previous biogeochemical research has focused on muddy or fine-grained sediments. Pore-water advection, driven mainly by pressure gradients from wave action and bottom currents interacting with surface topography, causes rapid solute exchange and allows direct transfer of suspended particles into permeable sediment strata. Recent studies indicate that advective transport leads to an acceleration of organic matter mineralization and a stimulation of biogeochemical cycling proportional to the extent of pore-water exchange (Huettel and Rusch, 2000; de Beer *et al.*, 2005; Werner *et al.*, 2006). Up- and downward flow of pore-water associated with migrating sandy sediment ripples generates vertical oscillations in oxic and anoxic conditions as redox zones move horizontally through the surface layer of the bed. The dynamic redox conditions found in permeable marine sediments resemble those found in wastewater treatment plants (Gray, 1990). In other words, high

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transport rates of organic matter and electron acceptors from the water column into the seafloor and the presence of oscillating oxic/anoxic conditions allow marine sands to act as an efficient nutrient filter that may facilitate N removal. However, few studies have investigated N-loss by denitrification in coastal permeable sediments; of these studies, fewer still have considered the effects of advective pore water flows on the rates of denitrification (Cook *et al.*, 2006; Hunter *et al.*, 2006; Rao *et al.*, 2007, 2008; Gihring *et al.*, 2010). Further research is needed to determine the rates and controls of N removal from permeable marine sediments.

The current paradigm is that denitrification is an anaerobic process in marine sediments, and oxygen is believed to act as a major control of the process (Brandes *et al.*, 2007). Denitrification is considered to require completely anoxic conditions due to the fact that O_2 acts as a competing electron acceptor for NO_3^- respiration and key enzymes of the denitrification pathways are inhibited by relatively small amounts of O_2 (Tiedje *et al.*, 1982; Zumft, 1997; Shapleigh, 2006). However, in contrast to the observations made in natural environments, a large number of laboratory studies have reported that denitrification occurs under aerobic conditions in pure cultures of bacteria (Robertson and Kuenen, 1984; Ronner and Sorensson, 1985; Trevors and Starodub, 1987; Robertson *et al.*, 1995). Such findings suggest that denitrification may not always be so effectively inhibited by O_2. Microbiologists have defined aerobic denitrification as the co-respiration or co-metabolism of O_2 and NO_3^-. Physiological studies show that microorganisms are able to use branches of their electron transport chain to direct electron flow simultaneously to denitrifying enzymes as well as to O_2 (Robertson and Kuenen, 1988; Huang and Tseng, 2001; Chen *et al.*, 2003). Although some environmental studies suggest that denitrification can occur in the presence of O_2 (Carter *et al.*, 1995; Bateman and Baggs, 2005; Rao *et al.*, 2007), substantial rates of aerobic denitrification have not yet been verified in the natural marine environment. Through a combination of new techniques using stable N isotopes for the direct determination of denitrification rates as well as the rapid quantification of aqueous gases over short time scales, the study of aerobic denitrification becomes more feasible.

The main objective of this study was to investigate the impact of O_2 dynamics on N loss by denitrification in permeable marine sediments of the Wadden Sea. Taking advective transport into account, we investigated denitrification rates in permeable sediments under near *in situ* conditions using a variety of experimental approaches. Surprisingly, multiple lines of evidence indicated that denitrification was not inhibited in the presence of substantial oxygen concentrations but rather the co-respiration of O_2 and NO_x^- occurred. Therefore, we hypothesized that where NO_x^- and O_2 co-occur, O_2 may not act as the primary or exclusive control of N_2 production in permeable sediment environments.

Materials and methods

Site description

The Janssand sand flat (13 km^2) is in the back barrier area of Spiekeroog Island in the German Wadden Sea. The western edge of the flat faces the 17-m-deep tidal channel separating the barrier islands Spiekeroog and Langeoog. The entire Janssand flat is inundated with $\sim$2 m of seawater for 6–8 h during each semi-diurnal tidal cycle, becoming exposed to air for 6–8 h during low tide, depending on the tidal range.

The central region of Janssand comprises the main area of the sand flat and is termed the upper flat due to the sloping margin downwards to the low water line. The upper flat is itself almost level and the physical appearance is homogeneous, mainly consisting of well-sorted silica sand with a mean grain size of 176 μm (Billerbeck *et al.*, 2006a). The mean permeability of $7.2–9.5 \times 10^{-12}$ m^2 (upper 15 cm of sediment; Billerbeck *et al.*, 2006a) permits advective pore water flows (Huettel and Gust, 1992). Detailed descriptions of the Janssand flat are available in Billerbeck *et al.* (2006a, b), Røy *et al.* (2008) and Jansen *et al.* (2009).

The sampling site (53°44.11′N, 7°41.95′E) is situated on the northeastern margin of the upper flat, about 80 m upslope from the mean low water line. Flat-bottom ships, the Spes Mea and Doris von Ochtum, were used to investigate the site in March 2007 and April 2008, respectively. All sediment core and seawater sampling was conducted at the upper Janssand tidal flat in March 2007, unless otherwise indicated.

Dissolved inorganic nitrogen in sediment pore water

Rhizon samplers (Seeberg-Elverfeldt *et al.*, 2005) were used to extract pore water directly from sediment cores on the deck of the ship. Cores were sampled by pushing Plexiglas core liners (inner diameter (ID), 9.5 cm) with side ports into the sediment, and Rhizons were then inserted horizontally into the ports at 1 cm intervals to 10 cm depth. Site seawater was also collected during low tide and filtered through a 0.2-μm syringe filter. All samples were immediately frozen onboard ship at −20 °C for later analysis. Dissolved ammonium (NH_4^+) concentrations were determined using a flow injection analyzer (Hall and Aller, 1992). Nitrate + nitrite (NO_x^-) was determined by chemiluminescence after reduction to NO with acidic vanadium(II) chloride (Braman and Hendrix, 1989).

Intact core incubations

Denitrification rates were determined by the isotope pairing technique (Nielsen, 1992) in intact core

incubations modified according to De Beer *et al*. (2005) to simulate *in situ* pore water advection in the permeable sediments. A set of 15 sediment cores was collected in parallel to obtain 10 cm of sediment and 15 cm of overlying water each from a 1 m^2 area using Plexiglas push-cores (ID, 3.5 cm; height, 28 cm). Seawater was also collected in parallel from the site. Water overlying the sediment was removed and replaced with $^{15}NO_3^-$-amended site seawater (final concentration of 50 μM). Rubber bottom stoppers were fitted with valves to allow for pore water perfusion over the upper 5 cm depth, and each core was percolated by 20 ml $^{15}NO_3^-$-amended aerated seawater at the perfusion speed of 12 ml min^{-1}. Cores were immediately sealed without any headspace by rubber stoppers after percolation, incubated at *in situ* temperature (8–10 °C) and were destructively sampled in triplicate at regular intervals between 0 and 6 h. The overlying water of all cores was mixed continuously at approximately 60 r.p.m. during the incubations by externally driven magnetic stirring bars. Cores were killed in reverse order of percolation. Briefly, 1 ml of zinc chloride (50% w/v) was added to the sediment surface. The cores were resealed with no headspace before mixing by inversion. After allowing the sediment particles to settle, an aliquot of water was removed from each core and transferred to a 12 ml Exetainer (Labco, High Wycombe, UK) prefilled with 200 μl saturated $HgCl_2$ for $^{29}N_2$ and $^{30}N_2$ determinations. The concentrations of excess $^{29}N_2$ and $^{30}N_2$ were calculated from $^{29}N_2$:$^{28}N_2$ and $^{30}N_2$:$^{28}N_2$ ratios of He-equilibrated headspace in Exetainers determined by gas chromatography-isotope ratio mass spectrometry (GC-IRMS; VG Optima, Manchester, UK). Denitrification rates were calculated based on the linear production of excess $^{29}N_2$ and $^{30}N_2$ according to Nielsen (1992).

Another set of intact core incubations was conducted to provide direct evidence for the co-respiration of O_2 and NO_x^- using multiple microsensors. An O_2 microsensor and a NO_x^- biosensor were simultaneously applied to freshly sampled sediment cores. Advective transport in sediments cores was simulated by percolation as described above.

Oxygen microsensors were constructed as described previously (Revsbech, 1989). A two-point calibration method for the O_2 sensor was performed using the signal in saturated overlying site seawater and anoxic sediments, and the O_2 solubility was corrected for the ambient water temperature (18 °C) and salinity (32‰) using the spreadsheet supplied by Unisense (www.unisense.dk). The NO_x biosensor was constructed, according to Larsen *et al*. (1997), with a tip diameter of 100 μm and was calibrated in seawater with additions of increasing amounts of NO_3^- to confirm linearity of the response (0–500 μM). For the calculation of pore water concentrations, the slope and offset were corrected for NO_3^- concentrations in the overlying water determined as described above.

For simultaneous measurements of vertical concentration profiles, the O_2 and NO_x sensors were mounted on a three-axis micromanipulator (MM 33; Märzhäuser, Wetzlar, Germany). The vertical axis was motorized for μ-positioning and controlled by μ-Profiler software described in Polerecky *et al*. (2005). The microsensor tips were aligned carefully to the same horizontal axis. The even sediment topography allowed alignment of both sensor profiles to the sediment surface with a precision of 1 mm, using the initial decrease of O_2 in the diffuse boundary layer. Microsensor measurements were made at 0.5 and 1 cm below the sediment surface during each percolation. Data were recorded over a time series to determine rates of potential O_2 uptake and NO_x^- consumption under oxic and anoxic conditions.

Slurry incubations in gas-tight bags

The depth-specific response of denitrification to O_2 was initially examined in bag incubations using the ^{15}N tracer isotope pairing technique according to Thamdrup and Dalsgaard (2002). Sediments were sampled using Plexiglas push-cores (ID, 9.5 cm; height, 60 cm) and sectioned into 2-cm-depth intervals to a depth of 6 cm. Afterwards, sediment and air-saturated site seawater were mixed at a ratio of ~1:1 in the gas-tight bag while expelling all air bubbles. The slurries were amended with $^{15}NO_3^-$ to a final concentration of 200 μM, and the bags were incubated at *in situ* temperature (same as for intact core incubations). During the incubation, the bags were periodically shaken to ensure that the labeled N species were homogenously distributed. Subsamples of the interstitial water were withdrawn from the bags at regular intervals and preserved in 6 ml Exetainer vials (Labco) prefilled with 100 μl saturated $HgCl_2$. An initial subsample was taken immediately after the addition of the tracer and at regular intervals to 16 h.

The aqueous O_2 concentration of the subsamples was determined using an O_2 microelectrode (MPI, Bremen, Germany) as described above. The 6 ml Exetainer vials were opened only briefly during the measurement and were afterwards stored with no headspace for further analysis of dissolved N_2 by GC-IRMS as described above.

FTSRR incubation experiment

To provide further corroboration for the co-respiration of O_2 and NO_3^- in a sediment slurry, we directly determined aqueous gases (O_2 and N_2) in line by membrane inlet mass spectrometry (MIMS; GAM200, IPI) in a FTSRR system. Surface sediments (0–3 cm) and site seawater were collected from the upper flat in April 2008, and stored at 4 °C during transport to the laboratory. Sediments and site seawater were mixed at a ratio of 1:3 in the gas-tight FTSRR without headspace. The slurry

was vigorously mixed at 200 r.p.m. by a magnetic stir bar, and the incubation was carried out in the dark at room temperature. The FTSRR system consisted of a sealed cylinder chamber (Plexiglas, inner diameter 9 cm, height 6 cm) fitted with three ports for the input and output of water. The effluent pumped through a filter by one port from the chamber was injected directly into the membrane inlet using a peristaltic pump with the pumping speed of 0.5 ml min^{-1}. Gastight syringes connected to the chamber by the other two ports, each filled with 50 ml of air-saturated site seawater, provided replacement water during pumping.

Simultaneous online measurements of mass 28 ($^{14}N^{14}N$), 29 ($^{14}N^{15}N$), 30 ($^{15}N^{15}N$), 40 (Ar), 31 (^{15}NO), 32 (O_2), 44 ($^{14+14}N_2O/CO_2$), 45 ($^{14}N^{15}NO$) and 46 ($^{15}N^{15}NO$) were obtained by MIMS. A standard calibration curve was constructed, based on measurements obtained under both air-saturated and anoxic conditions using a two-point calibration for each. The slurry in the incubator was amended with $^{15}NO_3^-$ to a final concentration of ~150 μM. Mass abundance signals were recorded by MIMS at 1 s time intervals and the flow-through samples were collected in 2 ml vials and stored at −20 °C for dissolved inorganic nitrogen (DIN) analysis as described above (see section Dissolved inorganic nitrogen in sediment pore water).

Results

DIN and O_2 penetration in permeable sediments

Zones of O_2 penetration and NO_x^- depletion largely overlapped in the upper 2–3 cm of tidal flat sediments. During the winter/spring, NO_x^- concentrations with a mean value of ~67 μM were observed in the overlying seawater, whereas NH_4^+ concentrations were comparably 10 times lower (<7 μM) (Figure 1a). In surficial sediments, pore water NO_x^- decreased rapidly with depth to ~40 μM at 1 cm depth, and a minimum concentration was observed below 3 cm depth. Concomitantly, pore water NH_4^+ increased to ~70 μM from the surface to 3 cm depth and remained consistently high (70–105 μM) below that depth (Figure 1a). *In situ* O_2 measurements in the upper flat from March 2006 showed that O_2 penetrated to ~3 cm during tidal inundation (Figure 1b) and O_2 penetration depths of up to 5 cm were observed at other locations on Janssand tidal flat (Billerbeck *et al.*, 2006b; Jansen *et al.*, 2009). The decrease in NO_x^- was equivalent to approximately half of the observed increase of NH_4^+ with depth (Figure 1a).

Denitrification potential in intact cores and gastight bag incubations

Following with the overlap in O_2 penetration and NO_x depletion, we observed the immediate and rapid production of ^{15}N-labeled N_2 in both incubations amended with $^{15}NO_3^-$ throughout the first 4 h of incubation under oxic conditions (Figure 2). Our study of the intact core incubations was motivated by a previous study of O_2 consumption using the same pore water percolation method that observed substantial O_2 was present during the first 1–2 h of intact core incubations in March (Polerecky *et al.*, 2005; Billerbeck *et al.*, 2006b). In this study, $^{29}N_2$ and $^{30}N_2$ were produced linearly ($r^2_{29N_2}=0.93$ and $r^2_{30N_2}=0.91$, respectively) without any lag during the first 2 h of incubation in the presence of O_2 (Figure 2a). In bag incubation experiments conducted in parallel, high potential denitrification rates were observed in sediment slurries from the 0 to 2, 2 to 4 and 4 to 6 cm depth intervals in which

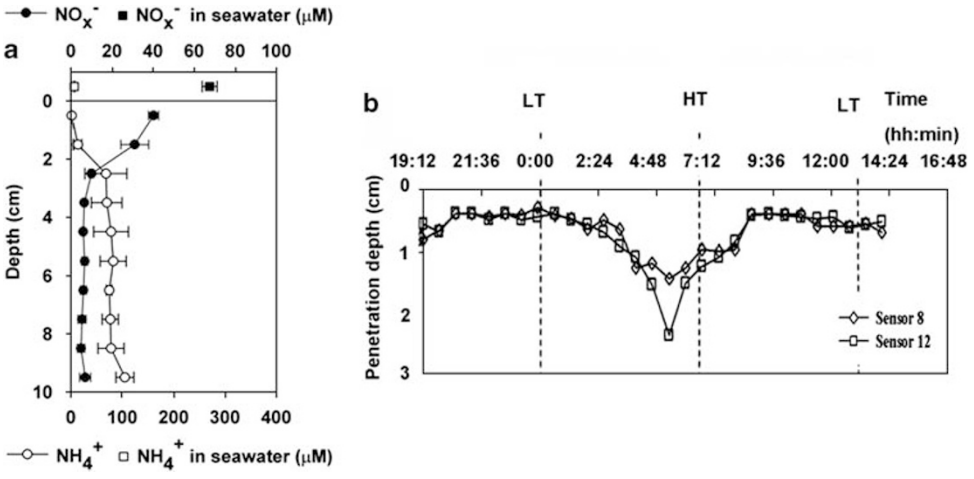

Figure 1 Distribution of dissolved inorganic nitrogen species (NH_4^+ and NO_x^- ($NO_3^- + NO_2^-$)) and O_2 penetration in sediments from the upper flat at Janssand. (**a**) NO_x^- (closed circles) and NH_4^+ (open circles) concentration profiles in permeable sediments in March 2007 during exposure. The daily mean values of NO_x^- and NH_4^+ concentrations in overlying seawater are depicted as closed and open squares, respectively. (**b**) O_2 penetration depth during a tidal cycle measured by two oxygen sensors in March 2006 (modified from Jansen *et al.*, 2009).

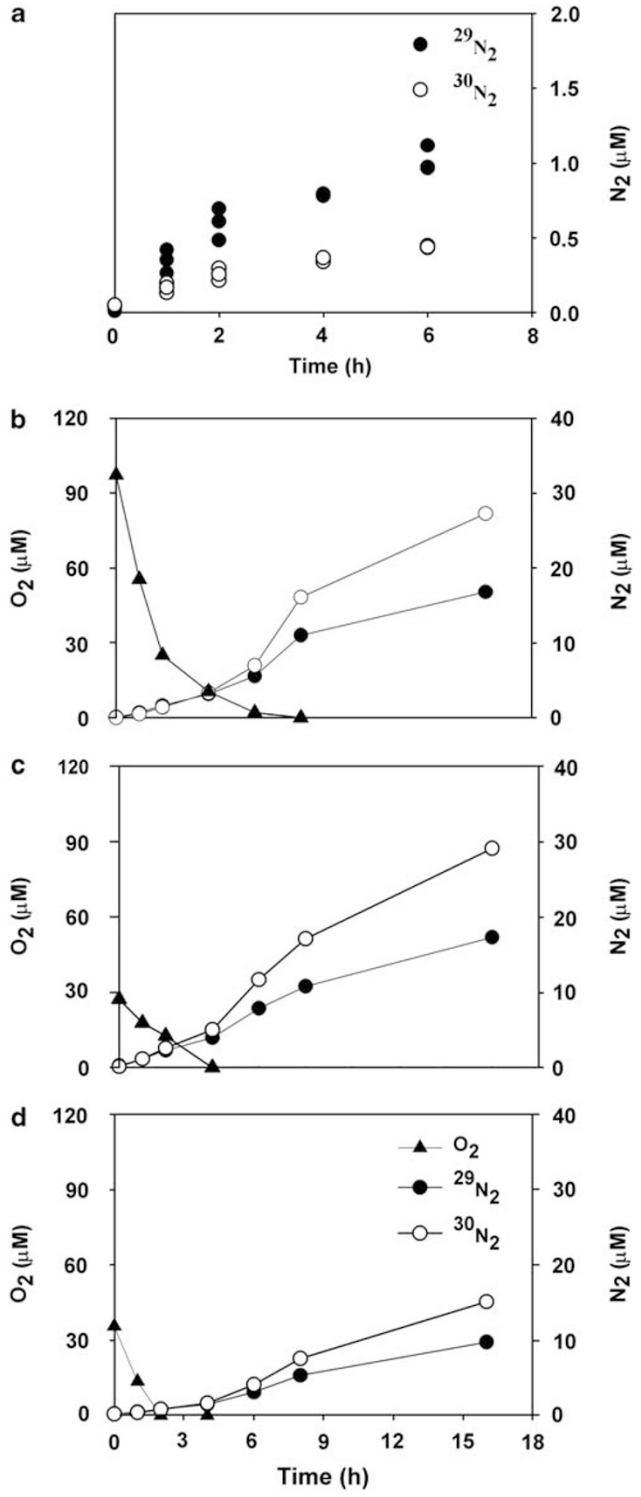

Figure 2 ^{15}N-labeled N_2 production and oxygen consumption in whole-core and bag incubations. (**a**) $^{29}N_2$ (black circles) and $^{30}N_2$ (open circles) production from $^{15}NO_3$ amendments in percolated whole-core incubations. O_2 consumption and ^{15}N-labeled N_2 production versus time in $^{15}NO_3^-$-amended, oxic, gastight bag incubations with sediment from (**b**) 0 to 2 cm, (**c**) 2 to 4 cm and (**d**) 4 to 6 cm depth intervals.

initial O_2 concentrations of ~95, 30 and 35 μM were observed, respectively. Higher $^{29}N_2$ and $^{30}N_2$ production rates were observed in incubations from the 0 to 4 cm depth intervals where higher NO_x^- concentrations are observed in sediment pore waters (Figures 1a and 2b–d). No significant change in the denitrification rates was observed in the incubations under oxic conditions (during the first 4 h) in comparison to anoxic conditions (during the last 12 h) (Table 1). In the incubation from the deepest depth interval (4–6 cm) where NO_x^- was depleted, the lowest denitrification rates were observed whereas O_2 depleted earliest (~2 h) (Table 1). Moreover at 4–6 cm depth, the rate under anoxic conditions was 2.3 times higher as that under oxic conditions (Figure 2d and Table 1). When extrapolated over the 0–5 cm depth interval, potential denitrification rates measured in percolated intact cores and bag incubations were in the same range (Table 1).

Microelectrode and biosensor measurements

Similar to the observations in the bag incubations, time series measurements by microsensors upon percolation of air-saturated and NO_3^--rich seawater showed that NO_x^- and O_2 were consumed simultaneously at 0.5–1 cm depth in intact sediment cores (Figure 3; Table 1). O_2- and NO_x^--rich seawater was transported downwards into the sediment by percolation, which increased concentrations at 0.5 cm to ~240 μM O_2 and 50 μM NO_x^-, and at 1 cm to ~230 μM O_2 and 40 μM NO_x^-. Under these high initial O_2 concentrations, a slight accumulation of 3–6 μM NO_x^- was detected after 2–3 min, followed by a substantial linear decrease in NO_x^- over the next 0.5–1.0 h of incubation in the presence of O_2. NO_x^- was consumed at a higher rate at 1 cm than at 0.5 cm under oxic conditions (Table 1). After O_2 was completely consumed, the NO_x^- reduction rate increased slightly at 0.5 cm depth; however, NO_x^- consumption rates showed no significant difference under oxic and anoxic conditions at 1 cm depth (Table 1), which corresponded to the results observed in the bag incubations with sediments from 0 to 4 cm depth.

Aerobic denitrification in an FTSRR

To provide further evidence for the simultaneous consumption of NO_x^- and O_2 in permeable sediments, we conducted an incubation in a stirred retention reactor, in which the slurry was vigorously and continuously mixed. Under constant mixing, substantial $^{30}N_2$ production was again observed by real-time MIMS measurements in the presence of 32 μM O_2 (Figure 4). $^{15}NO_3^-$ was amended to the continuously stirred chamber 20 min after the start of the incubation in the presence of 128 μM O_2. Online MIMS analyses indicated that after an initial lag period of 1.1 h, significant $^{30}N_2$ production occurred in the presence of 40 μM O_2. Concomitantly, O_2 consumption slowed below that concentration. Simultaneously, there was a slight accumulation of NO_x^- (data not shown) during $^{30}N_2$

Table 1 Summary of denitrification rates measured in all incubations

Measurement	*Investigated depth (cm)*	*Denitrification/NOx consumption ($mmol\,N\,m^{-3}_{sediment}\,h^{-1}$)*	
		Oxic	*Anoxic*
Intact core incubation	5	4.60 ± 0.46	
	Integrated to 5	0.23 ± 0.02[a]	
Intact core by multi-microsensors	0.5	15.5 ± 0.04	21.9 ± 0.05
	1	22.0 ± 0.04	21.5 ± 0.04
Slurry incubation	0–2	6.40 ± 0.37	10.57 ± 3.20
	2–4	8.27 ± 0.32	9.63 ± 1.15
	4–6	2.72 ± 0.16	6.28 ± 0.84
	Extrapolated to 5	0.32 ± 0.01[a]	0.47 ± 0.07[a]
Constant mixing, flow-through retention reactor incubation	0–3	6.23 ± 0.07	
	Extrapolated to 3	0.187 ± 0.002[a]	

The mean porosity of sediments in upper flat is 35% (Billerbeck *et al.*, 2006b).
[a]The unit is $mmol\,N\,m^{-2}_{sediment}\,h^{-1}$.

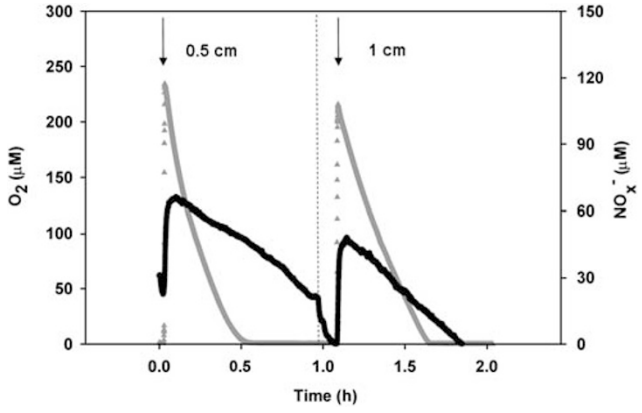

Figure 3 Time series of O_2 (gray) and NO_x^- (black) concentrations in an intact sediment core after percolation (indicated by arrows) with air-saturated overlying seawater containing ~60 µM NO_x^-. O_2 and NO_x^- microsensor tips were adjusted on a horizontal axis and measurements were carried out simultaneously. The first percolation treatment started at 0 h when sensors were positioned at 0.5 cm depth. The sensors were moved from 0.5 to 1 cm after 0.9 h and the second percolation began at 1.1 h when sensors were positioned at 1 cm depth.

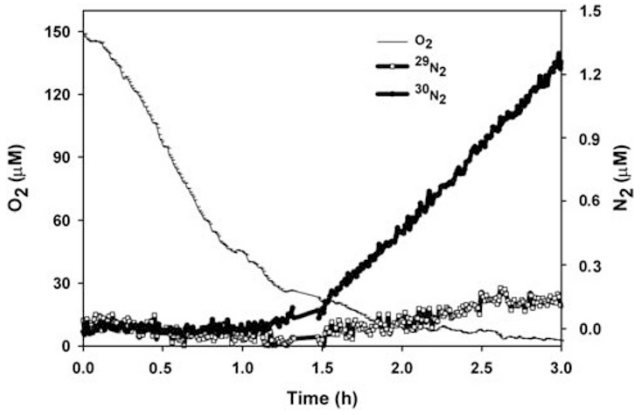

Figure 4 ^{15}N-labeled N_2 production and oxygen concentration versus time during the incubation of permeable sediments in the flow-through stirred retention reactor (FTSRR). Sediments were sampled from the 0 to 3 cm depth interval of the upper flat during April 2008. $^{29}N_2$, $^{30}N_2$ and O_2 concentrations are depicted as open circles, black squares and open triangles, respectively.

production. However, during that period, $^{29}N_2$ production was not concurrent with $^{30}N_2$ production and the increase of NO_x^-. In contrast, $^{29}N_2$ began to accumulate only when NO_x^- decreased at 1.5 h of incubation in parallel with a sevenfold higher rate of $^{30}N_2$ production (Figure 4).

Discussion

In permeable marine sediments of the Wadden Sea, zones of NO_x^- and O_2 penetration often overlap to several centimeters depth due to pore water advection (Figure 1) (Werner *et al.*, 2006; Billerbeck *et al.*, 2006a, b; Jansen *et al.*, 2009). Further, previous O_2 percolation experiments that incorporated pore water advection, showed that during the spring season when NO_x^- is at high concentration in the overlying seawater, O_2 persisted in the bulk pore water over the first 1–2 h of incubation in intact cores of Wadden Sea sediments (Polerecky *et al.*, 2005; Billerbeck *et al.*, 2006b). From these observations, it could be inferred that where NO_x^- and O_2 co-occur, O_2 may not act as the primary or exclusive control of N_2 production in permeable sediment environments. To test this assumption, we investigated N loss by denitrification in relation to O_2 dynamics. Several lines of independent evidence collected with multiple experimental approaches under near *in situ* conditions showed that denitrification occurs in the presence of oxygen. We observed the immediate and rapid consumption of NO_x^- under air-saturated pore water in the intact core, and the directly determined production of ^{15}N-labeled N_2 in the presence of up to 90 µM O_2 in slurry incubations. Further, the rapid production of labeled N_2 was not diminished in a vigorously stirred system—FTSRR. Thus, our results strongly

suggest that aerobic denitrification makes a substantial contribution to N loss in permeable marine sediments.

The rates and mechanisms of N removal in permeable marine sediments remain in question. Few studies have quantified N_2 production in coastal permeable sediments, and the rate measurements in this small but growing database vary widely, ranging from 0.1 to 3 mmol m^{-2} per day (Laursen and Seitzinger, 2002; Vance-Harris and Ingall, 2005; Cook *et al.*, 2006; Rao *et al.*, 2007, 2008). However, researchers have now become aware of the fact that in experiments where pore water advection is absent or impeded, a realistic determination of diagenetic processes is not achieved (Jahnke *et al.*, 2000; Cook *et al.*, 2006). At present, at least two mechanisms have been proposed to explain denitrification in the presence of oxygen: (1) co-respiration of NO_x^- and O_2 (Bateman and Baggs, 2005), and (2) closely coupled nitrification–denitrification in microenvironments isolated from bulk sediment pore water (Rao *et al.*, 2007). Bateman and Baggs (2005) provided one of the few observations of the contribution of aerobic denitrifying bacteria to denitrification potential in the environment. Using a combined stable isotope and acetylene inhibition approach, they were able to distinguish the relative contribution of nitrification and denitrification to N_2O production in arable soil. The results suggested that aerobic denitrification occurred at 20% water-filled pore space.

Although biogeochemical evidence exists for denitrification in the presence of oxygen in the marine environment (Hulth *et al.*, 2005; Hunter *et al.*, 2006; Brandes *et al.*, 2007; Rao *et al.*, 2007), significant rates of aerobic denitrification have not been verified until now. New techniques such as NO_x biosensors and stable N isotope tracers applied in conjunction with MIMS allowed for the further confirmation of aerobic denitrification. Rao *et al.* (2007, 2008) incorporated the effects of pore water advection, and in corroboration with our results, observed high rates of N_2 production in flow-through columns of oxic permeable sediments. In continental shelf sediments of the South Atlantic Bight, Rao *et al.* (2007) observed that pore water nitrate was only above detection in the oxic zone. N released as N_2 accounted from 80% to 100% of remineralized N, and the C:N ratio of regeneration supported the interpretation of N_2 produced by denitrification. In the study by Rao *et al.* (2007), the addition of ^{15}N-nitrate caused only a small and gradual rise in $^{29}N_2$ and $^{30}N_2$ production in sediment columns over up to 12 days of incubation. Only columns with anoxic outflow showed substantial $^{29}N_2$ or $^{30}N_2$ production. Thus, Rao *et al.* (2007) concluded that their evidence for aerobic denitrification was equivocal, and N_2 production more likely occurred from coupled nitrification–denitrification in microenvironments.

In this study, we observed the rapid and immediate production of ^{15}N-labeled N_2 in the presence of O_2 under a variety of experimental conditions. Oxygen and NO_x^- dynamics were directly determined in real time under well-mixed conditions in sediment slurries and intact core incubations. Microsensor measurements showed that NO_x^- and O_2 consumption occurred simultaneously in intact cores (Figure 3). Further direct evidence for the co-respiration of O_2 and NO_x^- was provided using ^{15}N tracer experiments in slurries that were constructed with sediments from different depths. Successive incubation experiments showed the reliability and uniformity of aerobic denitrification rates, despite the fact that the experimental setup differed (including the amount of sediments, volume of associated water and starting concentration of labeled nitrate; Supplementary Table 1). Although the concentrations of $^{29}N_2$ and $^{30}N_2$ in the associated water varied, the denitrification rates normalized to sediment volume were in the same range, with the exception of the higher rate measured by the microsensor, which incorporated NO_3^- assimilation as well as denitrification (Table 1). Under the experimental conditions used, $^{29}N_2$ could be attributed to coupled nitrification–denitrification or anammox in the slurry (Thamdrup and Dalsgaard, 2002). In contrast, $^{30}N_2$ would only be produced by complete denitrification. Anammox was shown to comprise only a small percentage of N_2 production in parallel slurry experiments conducted in gastight bags (Gao *et al.*, in preparation). Therefore, we conclude that the ^{15}N-labeled N_2 production is mainly contributed by denitrification, and the occurrence in the presence of O_2 provided evidence for aerobic denitrification.

At each depth examined in slurry incubation, the potential denitrification rate under aerobic conditions was similar to that measured under anaerobic conditions. Moreover, the maximum denitrification rate was not observed in the deepest depth interval with the lowest initial O_2 concentration, but rather in the surface 0–4 cm depth (Figures 2b-d). This suggests that the overlapping NO_x^- concentration may act together with O_2 to control the denitrification rate. On the Janssand tidal flat during winter/spring, rapid denitrification is likely to be supported by the constant supply of NO_x^- advected from the overlying seawater (Gao *et al.*, in preparation). In short, O_2 dynamics did not strongly affect N loss by denitrification in the presence of abundant NO_x^-, but rather denitrification coexisted with O_2 respiration in permeable Wadden Sea sediments affected by advection.

To further exclude the possibility of anoxic microniches forming in our sediment incubations, we conducted an experiment in a vigorously mixed FTSRR. The initial production of $^{30}N_2$ in the presence of 40 μM O_2 (Figure 4) provided evidence for the process of aerobic denitrification. The concomitantly suppressed O_2 consumption may indicate that nitrate acts as a competitive electron acceptor to facultatively aerobic denitrifying bacteria.

Whereas at lower O_2 concentrations later in the incubation (where an increased ratio of unlabeled NO_3^- was observed), the production of $^{29}N_2$ indicated denitrification coupled to nitrification. Due to the variability in the mass abundance signals, we cannot exclude the possibility that some $^{29}N_2$ was also produced in the early stages of the FTSRR incubation. Thus, we observed the mechanism for rapid denitrification under oxic conditions depended on two pathways—aerobic denitrification and denitrification coupled to nitrification.

During the FTSRR incubation, the bulk pore water was vigorously flushed by aerated seawater and the labeled isotope ratio was kept constant. Thus, the possibility that denitrification occurred in anoxic microzones can be completely excluded. In corroboration with our results, previous studies on the formation of anoxic microzones in particles and aggregates showed that the respiration capacity is simply not sufficient to create anoxia under high ambient O_2 concentration, and anoxic microzones more likely form at around 10% of air saturation (under $\sim$25 μM O_2 in the bulk phase; Schramm *et al.*, 1999; Ploug, 2001). In our study, at O_2 concentrations of $\sim$20% air saturation and above, the establishment of anoxic microzones would be unlikely. Given the larger grain sizes present in marine sands, O_2 transport is enhanced by advection/interstitial fluid flow, which produces less steep O_2 gradients at the sediment–water interface and within particles/aggregates compared with those that develop under pure diffusion conditions (Ploug, 2001). The above-mentioned experiments were conducted in a vertical flow system under nonturbulent uniform flow conditions. Thus, for the coarse-grained sediments in our well-mixed retention reactor experiments where the sediment slurry is exposed to turbulent aerated flow, anoxic microzones would not form. Therefore, we conclude that substantial N loss occurs by aerobic denitrification in the permeable Wadden Sea sediments.

Denitrification has long been considered as an anoxic biogeochemical process in marine and aquatic environments, and oxygen has been shown to inhibit denitrifying enzyme activity (Tiedje *et al.*, 1982; Hulth *et al.*, 2005; Brandes *et al.*, 2007). However, a phylogenetically and physiologically diverse group of microorganisms has been shown to denitrify in the presence of oxygen in laboratory studies (Zehr and Ward, 2002; Hayatsu *et al.*, 2008). Bacteria capable of aerobic nitrate respiration were cultured in abundance from freshwater soils and sediments (Carter *et al.*, 1995). Aerobic denitrifiers were further isolated from a variety of managed and natural ecosystems (Patureau *et al.*, 2000). Thus, the influence of oxygen on nitrate respiration activity appears to vary between microorganisms, with some strains able to respire nitrate at or above air saturation (Lloyd *et al.*, 1987; Hayatsu *et al.*, 2008). Microbiological studies have gone so far as to suggest that the capacity for denitrification under aerobic conditions is the rule rather than the exception amongst ecologically important denitrifying microbial communities (Lloyd *et al.*, 1987).

Previous studies indicate that the diversity, as well as the metabolic activity, of bacterial communities is high in permeable sediment environments, likely due to increased transport of growth substrates and the removal of metabolites by advective exchange with the overlying water column (Hunter *et al.*, 2006; Mills *et al.*, 2008; Boer *et al.*, 2009). Denitrification in the marine environment is believed to be mediated by a group of facultative anaerobes that display a wide range in phylogenetic affiliation and metabolic capabilities (Zehr and Ward, 2002). In pristine ecosystems, nitrate concentrations are typically too low to select for large populations of denitrifying organisms, and denitrifiers are thought to rely on aerobic heterotrophy in conjunction with their denitrification capacity (Tiedje, 1988). In permeable marine sediments, up- and downwelling of pore water associated with sandy sediment ripples generates redox oscillations that may promote the microbially mediated oxidation and reduction of N species.

Although the consensus is that low or no O_2 is required for the initiation of denitrification, most information on the O_2 level at which denitrification starts comes from pure cultures. Denitrification has been observed in the laboratory at O_2 concentrations approaching air saturation (Zehr and Ward, 2002), but previous environmental studies are equivocal with regard to the impact of O_2 dynamics on denitrification. Large differences are observed in the expression and regulation of denitrification genes between species studied in pure culture (Shapleigh, 2006). The expression of denitrification genes was shown to require O_2 in some cases, and the presence of denitrification intermediates may impact the denitrification rate in the presence of O_2. A possible explanation is that the accumulation of intermediates slows O_2 respiration, particularly at low O_2 levels, thereby slowing down the aerobic–anaerobic transition and allowing the expression of O_2-requiring denitrification genes (Bergaust *et al.*, 2008).

We hypothesize that the co-respiration of nitrate and O_2 represents an adaptation of denitrifiers to recurrent tidally induced redox oscillations in permeable sediments of the Wadden Sea. Some evidence from pure cultures of denitrifying bacteria supports this hypothesis. For example, when the selective pressure of environmental redox changes was removed, the aerobic denitrification ability of *Paracoccus denitrificans* decayed (Dalsgaard *et al.*, 1995; Robertson *et al.*, 1995). Further, Bergaust *et al.* (2008) proposed that denitrifiers adapt to recurrent oscillations in oxygen concentrations through a protection mechanism, which consists of the coordinated expression and activity of the denitrification enzymes for survival during the rapid transition from oxic to anoxic conditions. A 'bottleneck effect' was also proposed, whereby nitrifying

and denitrifying bacteria react to oxygen and nitrate in the environment by coordinating their respective activities. Schmidt *et al*. (2003) observed that the onset of the aerobic denitrification did not depend on oxygen sensitivity of the corresponding enzymes, but rather on regulation of redox-sensing factors at the transcriptional level. Our biogeochemical evidence corroborates microbiological studies to indicate a clear need to elucidate the significance and the controls of aerobic denitrification in permeable marine sediments.

In contrast to the paradigm that denitrification is an exclusively anaerobic process, our experiments point to aerobic denitrification and indicate that O_2 may not act as a primary or exclusive control of N_2 production in permeable marine sediments. We propose that the availability of NO_x^- as well as O_2 limit the denitrification rate at depths of marine sands that are impacted by pore water advection. We can only speculate on the mechanism of aerobic denitrification at this time. Co-metabolism would imply that both NO_x^- and O_2 are used simultaneously as electron acceptor in a single organism. Alternatively, separated denitrifying and oxygen respiring populations may be active within the community. In the first case, one would expect a competition for electrons within the electron transport chain, thus an enhanced denitrification upon oxygen depletion. In the second case, denitrification would be uncoupled entirely from the presence of oxygen, as denitrification is not kinetically inhibited by oxygen, nor can oxygen compete for electrons. In the FTSRR, we observed a pronounced effect of oxygen on denitrification rate whereas in other incubations less of an effect was found, indicating that both mechanisms may be present. Further research is needed to elucidate the true mechanisms of aerobic denitrification in permeable marine sediments.

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