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Short exposure to oxygen and sulfide alter nitrification, denitrification, and DNRA activity in seasonally hypoxic estuarine sediments

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One sentence summary: In a chronically hypoxic basin, nitrifiers rapidly recovered from anoxic and sulfidic conditions, denitrifiers had a more muted response while DNRA bacteria were only active when sulfide was high.

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ABSTRACT

Increased organic loading to sediments from eutrophication often results in hypoxia, reduced nitrification and increased production of hydrogen sulfide, altering the balance between nitrogen removal and retention. We examined the effect of short-term exposure to various oxygen and sulfide concentrations on sediment nitrification, denitrification and DNRA from a chronically hypoxic basin in Roskilde Fjord, Denmark. Surprisingly, nitrification rates were highest in the hypoxic and anoxic treatments (about $5 \mu\text{mol cm}^{-3} \text{d}^{-1}$) and the high sulfide treatment was not significantly different than the oxic treatment. Denitrification in the hypoxic treatment was highest at $1.4 \mu\text{mol cm}^{-3} \text{d}^{-1}$ and significantly higher than the high sulfide treatment. For DNRA, the rate in high sulfide treatment was $2 \mu\text{mol cm}^{-3} \text{d}^{-1}$. This was significantly higher than all oxygen treatments that were near zero. In this system, nitrifiers rapidly recovered from conditions typically considered inhibiting, while denitrifiers had a more muted response. DNRA bacteria appear to depend on sulfide for nitrate reduction. Anammox was insignificant. Thus, in estuaries and coastal systems that experience short-term variations in oxygen and sulfide, capabilities of microbial communities are more diverse and tolerant of suboptimal conditions than some paradigms suggest.

Keywords: nitrification; denitrification; dissimilatory nitrate reduction; estuary; oxygen; sulfide

INTRODUCTION

Eutrophication is a significant problem in estuaries and coastal zones around the world (Nixon 1995; Howarth et al. 2011). In sediments, the response to eutrophication can create positive

feedbacks as hypoxia or anoxia reduces aerobic decomposition and increases anaerobic decomposition, particularly sulfate reduction and the concomitant production of hydrogen sulfide (Marvin-DiPasquale, Boynton and Capone 2003; Howarth et al. 2011 Rabalais et al. 2014). This in turn may alter the

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balance between nitrogen removal processes, such as coupled nitrification and denitrification and nitrogen recycling within the system (Kemp *et al.* 1990), potentially enhancing dissimilatory nitrate reduction to ammonium (DNRA), a pathway that short-circuits N removal through denitrification, as its end-product is ammonium and not N_2 (Gardner *et al.* 2006; Bonaglia *et al.* 2014).

Our understanding of the linkages between oxygen, sulfur and nitrogen biogeochemistry has primarily emphasized interpreting field results using correlative approaches, making it a challenge to identify causal factors. We focus on an experimental approach manipulating oxygen and sulfide concentrations to examine three key nitrogen transformations: aerobic nitrification, denitrification and DNRA. Aerobic nitrification requires molecular oxygen for the oxidation of ammonia to nitrite and nitrite to nitrate; thus, loss of oxygen in sediments when bottom waters become hypoxic or anoxic results in reduced nitrification rates (Kemp *et al.* 1990; Caffrey *et al.* 2003; Abell *et al.* 2011). A generally accepted paradigm that oxygen stimulates while sulfide inhibits nitrification (Joye and Anderson 2008) is supported by experimental evidence showing that sulfide and other sulfide compounds can be potent inhibitors of nitrification and ammonium oxidizing bacteria (Oremland and Capone 1988; Joye and Hollibaugh 1995; McCarty 1999). However, significant rates of nitrification have been measured in some sulfide-rich environments such as eelgrass beds (Iizumi, Hattori and MeRoy 1980), salt marsh sediments (Dollhopf *et al.* 2005) and the hypoxic waters of the Baltic Sea (Berg *et al.* 2015). In contrast, responses of nitrate reducers to sulfide and oxygen are often quite different.

Nitrite and nitrate reduction pathways are diverse and include denitrification, anaerobic ammonium oxidation (anammox), DNRA and even nitrification when oxygen is low. Denitrifiers are primarily facultative anaerobes, whereas anammox and DNRA are considered strict anaerobic processes. Nitrate reduction activity occurs in anoxic or microoxic conditions (Dalsgaard *et al.* 2014; Bonaglia *et al.* 2016) and is inhibited by oxygen (Mohan and Cole 2007), at concentrations as low as 1–3 μM (Canfield, Kristensen and Thamdrup 2005). While the effects of oxygen on nitrate reduction processes have been examined in a variety of studies (Becker *et al.* 1996; Zumft 1997; Dalsgaard *et al.* 2014), whether sulfide inhibits or stimulates these processes is still under debate. Generally, sulfide has an inhibitory effect on heterotrophic denitrification (Sørensen, Tiedje and Firestone 1980) and on anammox activity at micromolar concentrations (Jensen *et al.* 2008), but serves as an electron donor for chemolithotrophic denitrifying and DNRA bacteria (Brunet and Garcia-Gil 1996; Zumft 1997).

We examine nitrification, denitrification and DNRA simultaneously using an experimental approach examining their response to short-term (hours) exposure to variable oxygen and sulfide concentrations, in contrast to most studies that focus on a single process and correlate rates with environmental conditions. The presence of anammox was determined in a separate experiment. Experiments were conducted with sediments from Roskilde Fjord, Denmark, in a location periodically exposed to hypoxia. We expected the following results: (i) that nitrification would be somewhat inhibited at low/no oxygen levels and completely inhibited by sulfide, (ii) denitrification and DNRA would be inhibited by oxygen and (iii) denitrification would be inhibited by sulfide while DNRA would be enhanced. We examine how pre-existing environmental conditions influence the results of the oxygen and sulfide manipulation.

MATERIALS AND METHODS

Study area

Roskilde Fjord is a 30 km long, shallow estuary in Denmark with average water depth of 3 m and basins of 15 m or greater. Circulation is controlled by freshwater flow and wind mixing, since tides in the estuary are minimal. Throughout the 1970–80s, excessive nutrient loading reduced water clarity, shifting the ecosystem to a phytoplankton-dominated system (Borum and Sand-Jensen 1996; Conley *et al.* 2000). Since 1990, upgrades of sewage treatment facilities have reduced nutrient loading, reducing annual chlorophyll levels, and improving Secchi depth (Riemann *et al.* 2016; Staehr, Testa and Carstensen 2017). Despite the shallow water depths and improvements in water quality, seasonal hypoxia still occurs in the deeper basins (>15 m) annually (J. Carstensen, pers. comm.).

Water column and sediment sampling and processing

Water column and sediment samples were collected from ROS 52 (55° 40.632'N, 12° 1.123'E) on April 5, 2016 and on April 26, 2016. Water depth was about 17 m at ROS 52. Long term monitoring (Conley *et al.* 2000) and other studies (Clarke, Juggins and Conley 2003) have been conducted at this location. A Kajak corer was used to collect sediment cores (7 cm inner diameter). Salinity, temperature, dissolved oxygen and pH profiles were measured in the water column using an YSI Professional Plus multi-meter at 2 m intervals on April 5 and 1 m intervals on April 26.

Porewater profiles

Water content was measured in April 5 sediments. Sediment cores were sliced under N_2 atmosphere, centrifuged and analyzed for porewater sulfide⁻, ammonium (NH_4^+), nitrite (NO_2^-) and nitrate + nitrite ($NO_3^- + NO_2^-$) (Fig. S1, Supporting Information). Samples for sulfide analysis were preserved with zinc acetate (Fonselius, Dyrssen and Yhlen 2007). Nutrient and sulfide analyses for overlying water, porewater and experiments were conducted using some modifications to standard methods (Parsons, Maita and Lalli 1984; Fonselius, Dyrssen and Yhlen 2007; Schenetger and Lehnert 2014), which are described in detail in the supplemental information.

Three microprofiles of dissolved oxygen (O_2), pH and hydrogen sulfide were measured in an intact core using a motorized micromanipulator (MM33–2; Unisense, DK), and microsensors with a tip diameter of 50 μm (OX-50, pH-50, H_2S -50; Unisense, DK). Overlying water column (~4 cm) was stirred by means of a gentle flow of air across the water surface. Before determinations, OX-50 microsensor was calibrated using a two-point (oxic-anoxic) calibration, pH-50 was calibrated with pH standards of 4.01, 7.00 and 10.01 and H_2S -50 was calibrated in fresh Na_2S solutions, according to the manufacturer's recommendation (Unisense, DK). Total dissolved sulfide ($\Sigma H_2S = H_2S + HS^- + S^{2-}$) was calculated from the measurement pairs of H_2S and pH for each depth interval, knowing the 1st and 2nd dissociation constant of H_2S/HS^- and HS^-/S^{2-} , pK_1 and pK_2 , respectively. However, since $pH < 9$ and no S^{2-} was expected, we only used pK_1 for the calculations (Jeroschewski, Steuckart and Kühl 1996), which for our temperature and salinity was 6.84 (Millero, Plese and Fernandez 1988).

Nitrification

Potential nitrification was measured as $\text{NO}_3^- + \text{NO}_2^-$ and NO_2^- production in NH_4^+ amended sediment slurries at room temperature with shaking. For the April 5 experiment, sediment cores were sliced into 0–1 and 1–2 cm sediment layers. Two grams of sediment and 50 mL GF/F filtered bottom water were amended with 1 mL of a 25 mM solution of NH_4Cl (NH_4^+ spike concentration: 500 μM) in triplicate. Samples were collected at 0 and after 24 h.

For the April 26 experiment, the 0–2 cm layer was homogenized and 5 cm^3 of sediment was dispensed into 250 mL bottles. Six replicates per treatment had 250 mL of GF/F filtered bottom water with a spike addition of 500 μM NH_4^+ (4 mL of 25 mM solution of NH_4Cl). Sediment slurries were exposed to 1 of the 5 treatments: oxic, hypoxic (<70 μM O_2), anoxic (0 μM O_2), anoxic + low sulfide (100 μM), anoxic + high sulfide (1 mM) for a 4-h period.

Samples were collected at 0 and 4 h (beginning and end of exposure period) and then at 7, 16 and 23 h. Hypoxic or anoxic conditions within bottles were maintained during the 4-h exposure using a gassing manifold to add N_2 to the headspace when samples were removed at 0 and 4 h for all treatments except the oxic treatment. Sulfide concentrations were measured in all treatments at 0, 4 and 23 h. Oxygen concentrations were measured at 0 and 4 h in the oxic and hypoxic treatments, but not in anoxic or sulfide treatments to minimize oxygen contamination, then all treatments were measured at 23 h. Following the 4-h exposure, ~50 mL samples of the slurry were removed for denitrification and DNRA experiments as described below (Fig. S1, Supporting Information). Then, all 250 mL bottles were opened to the atmosphere. We used time points between 8 and 23 h to calculate rates of ammonium oxidation (production of $\text{NO}_3^- + \text{NO}_2^-$) and nitrite oxidation (production of NO_3^-).

Denitrification and DNRA

Denitrification and DNRA experiments were conducted on sediment slurries exposed to different oxygen and sulfide treatments. Approximately, 50 mL of slurry from each of the replicate bottles ($n = 30$) was dispensed into a series of 4 Exetainers® vials (12 mL gas-tight glass vials; Labco, UK; from now on referred as Exetainers), which contained a 4 mm glass bead. Vigorous shaking of the bottle while filling the Exetainers maintained slurry homogeneity. Exetainers ($n = 120$) were filled to the top and capped immediately to avoid bubbles or any headspace. Slurries were kept homogeneous on a rotating stirrer and preincubated for 15 h to consume residual O_2 or NO_3^- . Prior to addition of $^{15}\text{NO}_3^-$, O_2 concentrations were measured in 20 haphazardly chosen Exetainers using a precalibrated microelectrode and all O_2 concentrations were below detection limits (<1 μM). Each Exetainer received 100 μL of an anoxic 18 mM $\text{Na}^{15}\text{NO}_3^-$ solution ($^{15}\text{NO}_3^-$ spike concentration: 150 μM) for measuring activity of denitrification and DNRA. A ZnCl_2 solution (200 μL ; 7 M) was injected with a needle through the septum into 30 Exetainers, i.e. one vial from each replicate, for T_0 . The remaining Exetainer samples ($n = 90$) were incubated on the rotating stirrer for up to 4.5 h. Thirty Exetainer vials were sacrificed by injection with ZnCl_2 at regular intervals (~1.5 h) until the experiment was terminated.

In parallel to the April 26 experiments, the presence/absence of anammox activity was tested using incubations of anoxic slurries amended with $^{15}\text{NH}_4^+$. The topmost 2 cm sediment of an additional sediment core was extruded and homogenized and 5 mL of this sediment was transferred into a 250 mL bottle, which was filled with anoxic bottom water (Fig. S1, Supporting Infor-

mation). This slurry was dispensed into 20 Exetainers using the procedure described above. Eight Exetainers received 100 μL of an anoxic solution of 18 mM $^{15}\text{NH}_4\text{Cl}$ solution ($^{15}\text{NH}_4^+$ spike concentration: 150 μM) and the other 8 Exetainers received 100 μL of an anoxic 18 mM $^{14}\text{NO}_3^- + ^{15}\text{NH}_4^+$ solution ($^{14}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ spike concentrations: 150 μM each). Four Exetainers did not receive any ^{15}N and served as controls. Slurries were incubated for up to 4.5 h on a rotating stirrer and sampled in duplicate (the control was sampled once each time) using the procedure described above. All Exetainers were stored upside down in a refrigerator until analysis of isotopic compositions of N_2 .

The isotopic composition of the N_2 in the headspace of denitrification and anammox experiments were determined using gas chromatography-isotope ratio mass spectrometry (GC-IRMS, DeltaV plus, Thermo). Slopes of the linear regression of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ concentration against time were used to calculate rates of $p^{29}\text{N}_2$ and $p^{30}\text{N}_2$ production in the $^{15}\text{NO}_3^-$ amended treatments, from which D_{14} ($^{14}\text{NO}_3^-$ denitrification) and D_{15} ($^{15}\text{NO}_3^-$ denitrification) and total potential denitrification ($D_{14} + D_{15}$) could be calculated as in Nielsen (1992).

Concentrations of labelled ammonium ($^{15}\text{NH}_4^+$) were quantified in 3 mL water samples taken from each Exetainer after N_2 analyses by oxidation of NH_4^+ to N_2 with alkaline hypobromite (Warembourg, 1993). Samples were analyzed by GC-IRMS as for labeled N_2 analysis described above. Slopes of the linear regression of $^{15}\text{NH}_4^+$ concentration against time were used to calculate production rates of labeled ammonium ($p^{15}\text{NH}_4^+$), which corresponds to the potential DNRA rate.

Statistical analyses

Non-parametric tests were used, since rates of nitrogen processes were not normally distributed (Helsel and Hirsh 2002). Differences in rates of ammonium oxidation by depth on April 5 were evaluated using a Kruskal–Wallis test ($\alpha = 0.05$). For each nitrogen process (ammonium oxidation, nitrite oxidation, denitrification, DNRA), we conducted a Kruskal–Wallis test to see if there were differences among the treatments (oxic, hypoxic, anoxic, anoxic + low sulfide, or anoxic + high sulfide). If treatment was significant, a Wilcoxon rank sum test with a Bonferroni correction was done to examine pairwise differences between treatments. All analyses were done using the stats package (version 3.3.2) in R (R Core team 2018).

RESULTS

ROS 52 was strongly stratified during April 5 with a surface salinity of 11 and temperature of 8.4°C, while bottom water salinity was 14 and temperature was 3.8°C (Table 1, Fig. S2, Supporting Information). The depth of the pycnocline was ~9 m. The water column was generally well mixed on April 26 and bottom water

Table 1. Bottom water column characteristics at station ROS 52 in Roskilde Fjord on April 5 and 26, 2016.

	April 5	April 26
Water Depth (m)	18	16
Bottom Salinity	14	11
Bottom Temperature°C	3.8	9.1
Bottom DO mg L^{-1} (% saturation)	4.8 (40.4)	1.0 (9.4)
pH	7.7	8.1
$\text{NO}_3^- + \text{NO}_2^-$ μM	27.9	5.1
NO_2^- μM	0.97	0.2
NH_4^+ μM	6.4	5.3

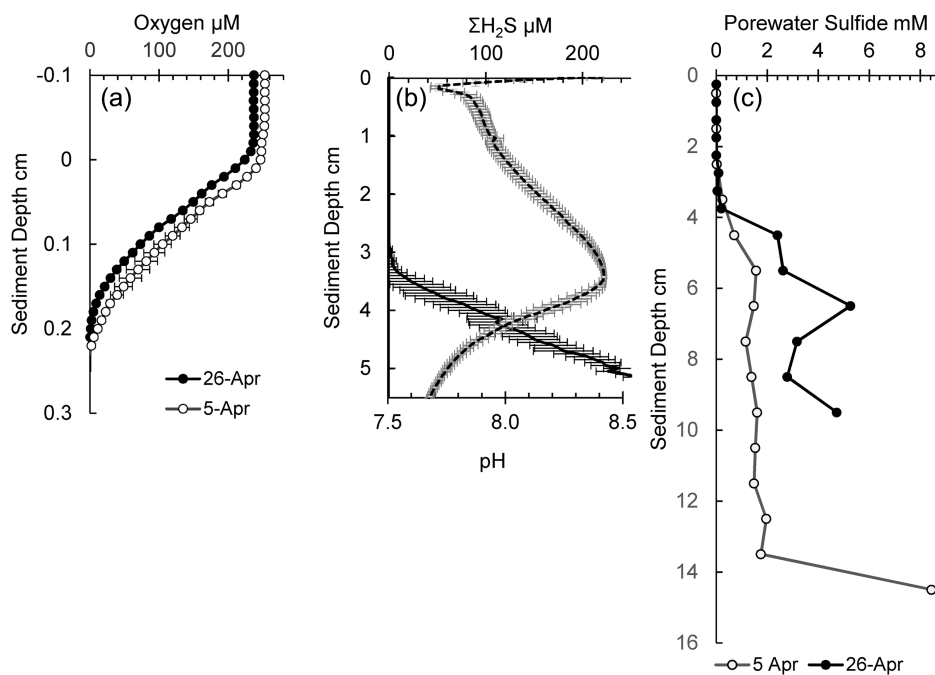


Figure 1. Sediment microelectrode profiles of oxygen (μM) (a), $\Sigma\text{H}_2\text{S}$ (μM) concentrations (solid) and pH (dashed) (b). Deeper porewater sulfide (mM) profiles determined by sediment slicing (c). All profiles from sediment samples at ROS 52, Roskilde Fjord from April 5 and 26. Note the change in sediment depth scale for each profile.

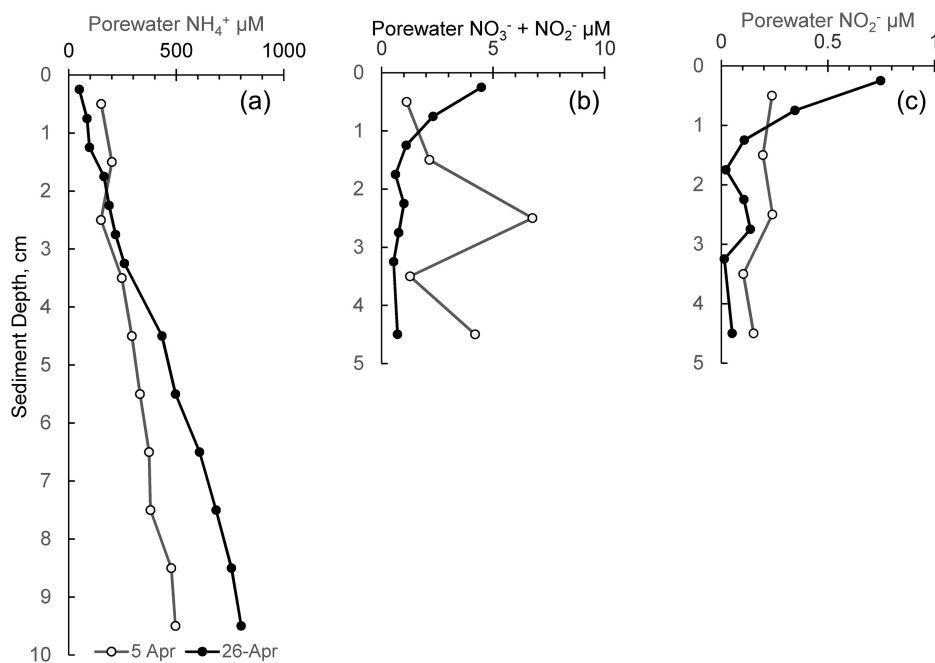


Figure 2. Porewater ammonium (a), nitrate + nitrite (b) and nitrite (c) concentrations in μM with depth from sediment samples at ROS 52, Roskilde Fjord from April 5 and 26.

temperature was 9.1°C (Fig. S2, Supporting Information). Bottom water dissolved oxygen concentrations were at 40% saturation during April 5, but only 10% in bottom waters during April 26 (Table 1). pH declined from 8.8 in surface waters to 7.7 below the pycnocline on April 5 and was 8.1 in the bottom waters on April 26 (Fig. S2, Supporting Information).

Sediments were fine grained with water content of 88% or greater to a depth of 5 cm (data not shown). Oxygen microelectrode profiles were similar between two dates, with a penetra-

tion depth of 1.8 mm (Fig. 1a). The profile of pH declined from a high of 8.4 at the surface to 7.7 at 0.25 cm, increasing up to 8.4 by 3.5 cm before gradually declining (Fig. 1b). Porewater sulfide was less than 10 μM in top 2.5 cm increasing to over 1000 μM by 5 cm sediment depth (Fig. 1c). NH_4^+ concentrations increased linearly with depth on both sampling dates (Fig. 2a). There was a subsurface peak of 6.8 μM $\text{NO}_3^- + \text{NO}_2^-$ between 2 and 3 cm on April 5. Concentrations were highest in 0–0.5 cm layer (4.5 μM) on April 26 and declined with depth (Fig. 2b). Porewater NO_2^-

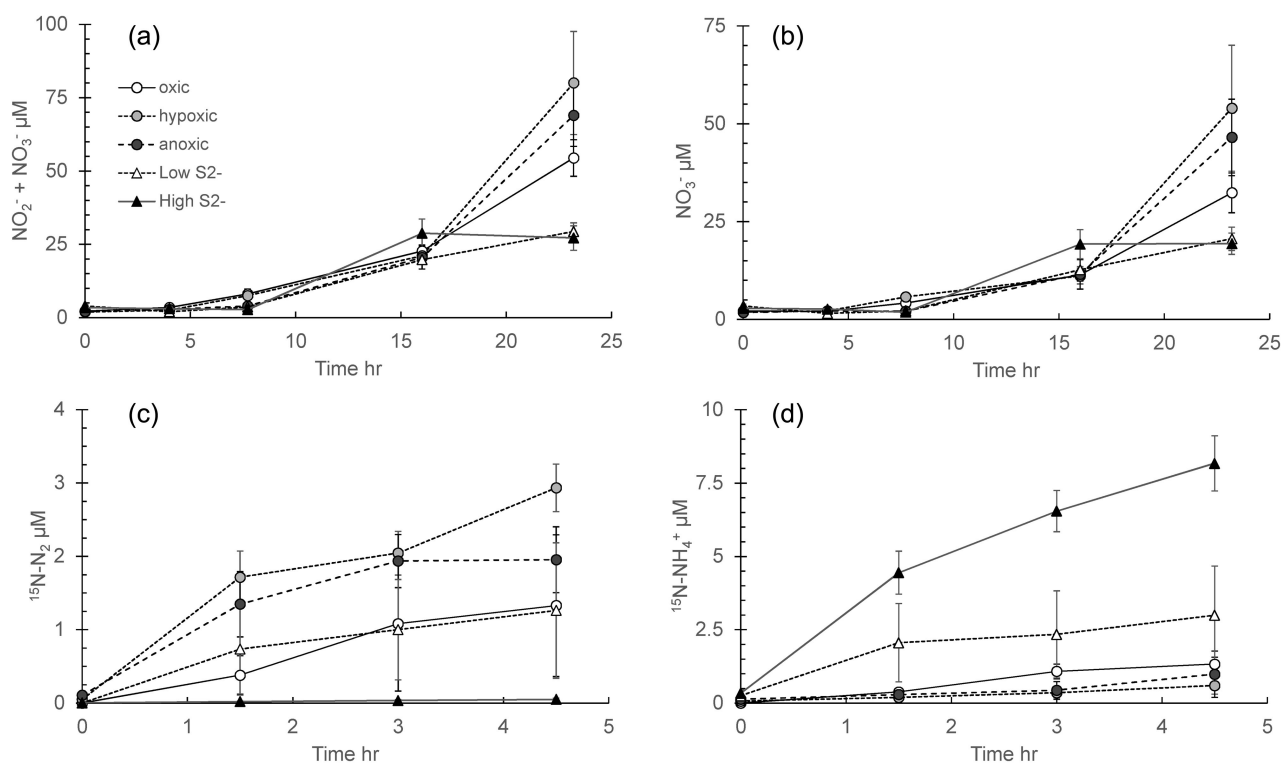


Figure 3. Average $\text{NO}_3^- + \text{NO}_2^-$ (a), NO_3^- (b), $^{15}\text{N-N}_2$ (c) and $^{15}\text{N-NH}_4^+$ concentrations (d) from sediment slurry experiments over time from each treatment. Mean \pm S.E. ($n = 6$). Changes in $\text{NO}_3^- + \text{NO}_2^-$ and NO_3^- concentrations between 8 and 23 h were used to calculate rates of ammonium oxidation and nitrite oxidation, respectively. Changes in $^{15}\text{N-N}_2$ and $^{15}\text{N-NH}_4^+$ over 4.5 h period used to calculate rates of denitrification and DNRA.

concentrations were less than $0.3 \mu\text{M}$ on April 5 and about $0.8 \mu\text{M}$ in the surface 0–0.5 cm layer on April 26 and declined with depth (Fig. 2c).

Potential nitrification from ROS 52 during April 5 in the 1–2 cm layer was significantly higher than either the 0–1 cm layer ($P = 0.001$). Potential nitrification in 1–2 cm layer was twice the 0–1 cm layer at ROS 52 (Table S1, Supporting Information). During the April 26 experiment at ROS 52, $\text{NO}_3^- + \text{NO}_2^-$ and NO_3^- concentrations increased in all treatments following the exposure period (Fig. 3a and b). Sulfide concentrations in the oxygen treatments were below 2 during the entire 23 h time course (Table S2, Supporting Information). Sulfide concentrations at the end of the 4 h treatment period were 52 and $570 \mu\text{M}$ in the low and high sulfide treatments and less than $4 \mu\text{M}$ at 23 h (Table S2, Supporting Information). Treatment was a significant factor explaining differences in ammonium oxidation rates (Kruskal-Wallis $P < 0.001$, Fig. 4a), with the Wilcoxon *post-hoc* test showing significantly lower rates ($P < 0.05$) in the low sulfide compared to the other treatments (Fig. 4a). Nitrite oxidation rates were not significantly different between treatments ($P = 0.09$, Fig. 4b).

Labeled $^{15}\text{N}_2$ in potential denitrification experiments increased over the incubation period (Fig. 3c). Differences between treatments were statistically significant ($P = 0.004$) (Fig. 4c) with higher rates in the hypoxic and oxic treatments than high sulfide treatment ($P = 0.05$). In contrast, potential anammox activity was negligible because labeled N_2 did not increase in either series of incubations with $^{15}\text{NH}_4^+$ (with or without NO_3^-) (data not shown). The increase in $^{15}\text{NH}_4^+$ over the course of the incubation was higher in the sulfide treatments than other treatments (Fig. 3d). Rates of potential DNRA were highest in the high sulfide treatment which was significantly higher than oxic, hypoxic, anoxic treatments ($P < 0.003$; Fig. 4d).

DISCUSSION

Effects of oxygen and sulfide on nitrogen transformations

Our results were consistent with the paradigms that oxygen is essential for nitrification, but inhibitory to heterotrophic denitrification, while sulfide is inhibitory to nitrification and denitrification but stimulatory to DNRA (Mohan and Cole 2007; Joye and Anderson 2008). The rapid recovery of nitrification activity following anoxia has been observed in previous studies (Bodelier et al. 1996; Nikolausz et al. 2008) and attributed to the ability of nitrifiers to use other substrates (Geets, Boon and Verstraete 2006). Our observations of sulfide inhibition of denitrification at high sulfide concentrations are consistent with Porubsky, Weston and Joye (2009). Potential DNRA rates increased exponentially with increasing sulfide concentrations (Figs 4d and 5), similar to other studies showing higher DNRA under hypoxic environments when porewater sulfide concentrations can be high (An and Gardner 2002; Gardner et al. 2006; Bonaglia et al. 2014). High DNRA rates from the high sulfide treatment are consistent with mechanism that sulfide is an electron donor for chemolithotrophic bacteria carrying out the DNRA (Brunet and Garcia-Gil 1996). However, some responses of nitrifiers and denitrifiers were unexpected.

One surprising result was that nitrification was not significantly inhibited by sulfide, even at concentrations of $570 \mu\text{M}$. Although nitrification rates in low and high sulfide treatments were about 73% of the rate in the oxic treatment, only the low sulfide treatment was significantly different than the oxic treatment. It suggests that the nitrifying community in Roskilde Fjord was relatively tolerant of sulfide in porewater, compared to Tomales Bay where previous experiments showed complete

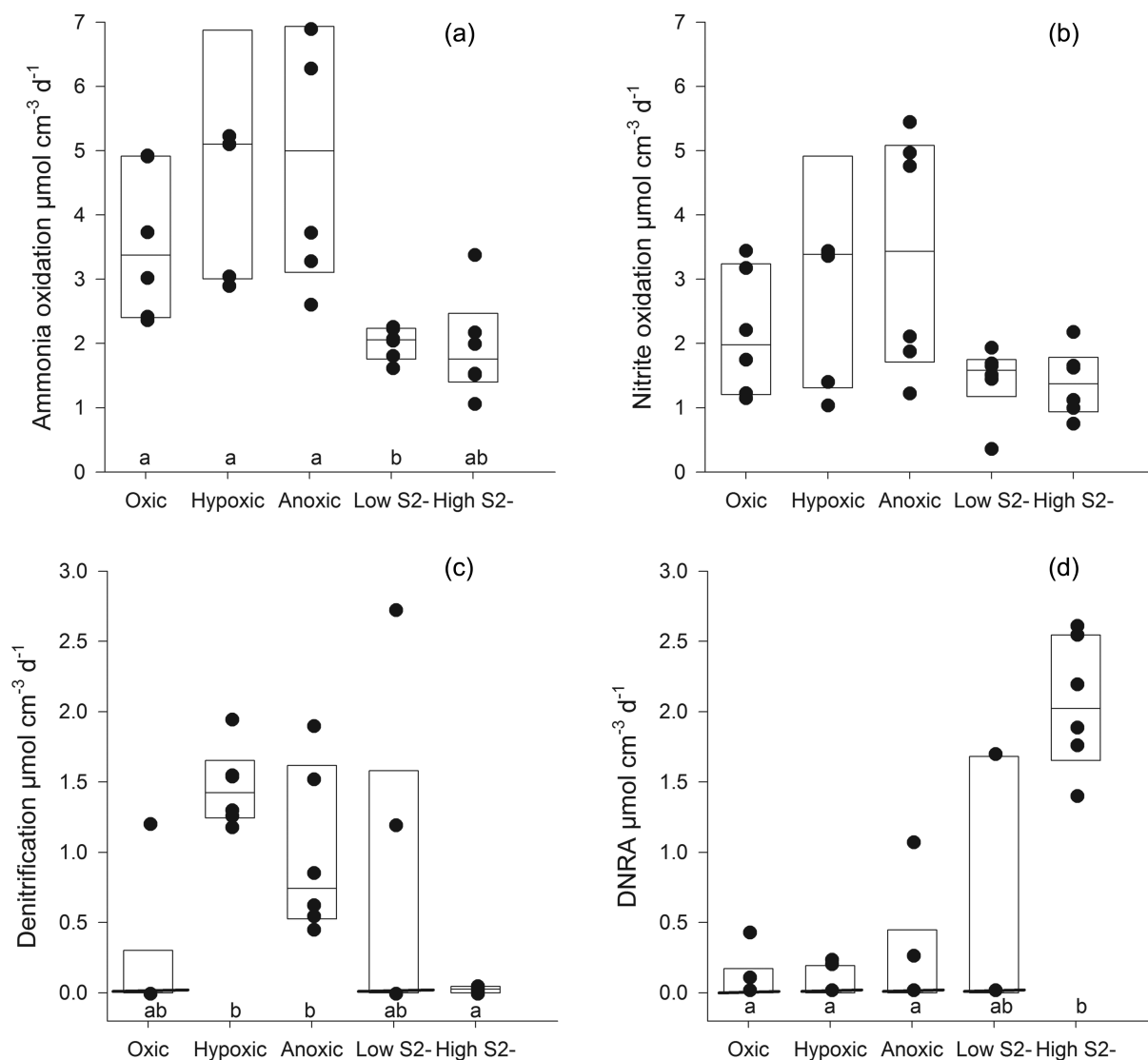


Figure 4. Potential ammonium oxidation (a), nitrite oxidation (b), denitrification (c) and dissimilatory nitrate reduction (DNRA) (d) in $\mu\text{mol cm}^{-3} \text{d}^{-1}$ from sediment slurry experiments. Individual measurements represented by dots with line in box indicating median, bottom and top of box representing 25th and 75th percentiles, respectively. For each rate measurement, treatments sharing the same letter were not significantly different from one another. Note change in scale between top and bottom panels.

inhibition of nitrification at $100 \mu\text{M}$ sulfide (Joye and Hollibaugh 1995). Periodic exposure to sulfide diffusing up from deeper layers may have selected for a more sulfide tolerant microbial community in Roskilde Fjord as has been observed in other sulfidic sediments (Caffrey et al. 2010). In contrast, sulfide is absent from porewater in Tomales Bay, despite significant sulfate reduction rates in the Bay (Chambers et al. 2000). This suggests that nitrifiers from estuaries with variable oxygen and sulfide concentrations can begin nitrifying shortly after oxygen concentrations increase and sulfide disappears.

Higher rates of potential nitrification in the hypoxic or anoxic treatment compared to the oxic treatment were also unexpected. A brief (several hours) exposure to hypoxia or anoxia seemed to prime nitrifiers, such that when oxygen was present, rates were higher, about double the control rates. The experiments from April 5 are consistent with this hypothesis in that the sediments in the 1–2 cm layer, well below the depth of oxygen penetration, had higher potential nitrification rates than surface sediments. This suggests that nitrifiers may be able to

recover rapidly from low oxygen events. Higher nitrification under fluctuating oxygen levels has been observed in a variety of environments (Diab, Kochba and Avnimelech 1993; Park et al. 2006; Pett-Ridge et al. 2013) including periodic intrusion of oxygen from marsh plants, seagrasses or macrofaunal bioirrigation, which enhances nitrification deep in sediments (Caffrey and Kemp 1990; Dollhopf et al. 2005; Beman et al. 2012). Differences in community composition, whether ammonia oxidizing archaea or ammonia oxidizing bacteria, may also play a role. Various studies have shown that ammonia oxidizing archaea tolerate and may thrive at low oxygen concentrations (Abell et al. 2011; Qin et al. 2017) and may form a consortium with sulfide oxidizing bacteria (Park et al. 2010). In these sediments, ammonia oxidizing archaea represented 54% of the ammonia oxidizers (J. T. Hollibaugh, pers. comm.). In addition, physiological adaptations to hypoxic or anoxic conditions may also occur, with some studies showing that ammonia oxidizing bacteria can oxidize ammonium using nitrite instead of oxygen (Geets, Boon and Verstraete 2006).

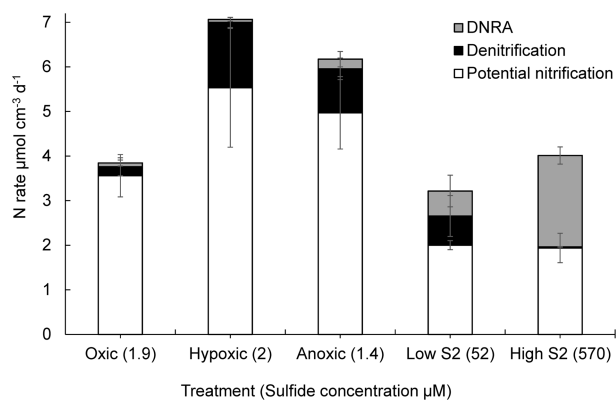


Figure 5. Rates of potential nitrogen transformation from sediment slurry experiments by treatment with sulfide concentrations during 4-h treatment period given in parentheses

Denitrification in the oxic treatment, while about 14% of them hypoxic or anoxic treatments, was not significantly different from those treatments. Based on the rates of oxygen consumption and the 15-h pre-incubation period (prior to $^{15}\text{NO}_3^-$ addition), preservation of oxic spots from previous exposure to oxic conditions seems unlikely, although they may have been generated next to the Exetainers rubber septa (De Brandere et al. 2012). Oxygen inhibits the synthesis of nitrate and nitrite reductase and denitrifiers switch from aerobic to anaerobic metabolism when oxygen falls within the range of 1–7 μM (Becker et al. 1996), although Dalsgaard et al. (2014) suggested that 50% of denitrification activity in the Baltic Sea water column is inhibited at 0.3 μM O_2 . Lack of inhibition at low sulfide treatment (63 μM H_2S) contrasts with Porubsky, Weston and Joye (2009), who observed inhibition at sulfide concentration around 15 μM . Higher sulfide tolerance by denitrifiers in our study may be explained by prior hypoxic conditions, contrary to Porubsky, Weston and Joye (2009) study, which likely had oxic overlying water.

Prior oxygen exposure did not have a significant effect on DNRA in this study (Fig. 3). This contrasts with the general

paradigm that oxygen represses DNRA (Mohan and Cole 2007; Joye and Anderson 2008). It is consistent with significant rates of DNRA in sediments with oxic water columns (Roberts et al. 2014).

Relationship to environmental conditions

These experiments represent a snapshot in time; thus, understanding the environmental context is critical. Samples were collected during the spring transition during rapidly warming water temperatures (4°C–10°C) and set up of annual hypoxia, as bottom water O_2 declined from 4.8 to 1.0 mg/L. Despite hypoxic bottom waters, rates of potential nitrification, both ammonia oxidation and nitrite oxidation, were about twice as high as rates for nitrate reduction pathways, denitrification and DNRA (Fig. 5). It is possible that these nitrification rates are underestimates if some of the nitrate or nitrite were reduced within anoxic microsites within the sediment slurries. Nitrification rates were likely higher than nitrate reduction pathways because surface sediments from 0 to 2 cm were used for the experiments and that abundances of anaerobes were likely lower in this zone. A less likely alternative is that anaerobes require more recovery time from the 4-h treatment than 15-h pre-incubation period prior to the start of denitrification and DNRA experiments.

While these are potential rates, they do provide insight into the ability of the microbial community to respond to changing environmental conditions. Comparisons of potential nitrification and denitrification rates made on the same sample are inconsistent, with some studies showing 5- to 10-fold higher rates of potential nitrification to denitrification (Iizumi, Hattori and MeRoy 1980; Caffrey and Kemp 1990; Beman 2014) and some with comparable rates (Dollhopf et al. 2005). Potential nitrification has sometimes been used as a proxy for nitrifier abundance (Caffrey et al. 2007; Beman, Popp and Francis 2008; Bernhard et al. 2010; Damashek et al. 2015). These high nitrification rates in Roskilde Fjord suggest an active and abundant community. However, we have no explanation why these rates are much higher than other estuaries (Table 2) that have comparable oxygen, nutrient and sediment characteristics.

Table 2. Comparison of potential nitrification rates in different estuaries. Median (range) reported.

Location	Potential nitrification $\mu\text{mol cm}^{-3} \text{d}^{-1}$	Reference
Roskilde Fjord	7.2 (2.6–27.7)	This study
Kysing Fjord	1.3 (0.6–3.3)	Hansen, Henriksen and Blackburn 1981
Danish waters	0.9 (0.1–1.6)	Henriksen, Hansen and Blackburn 1981
Aarhus Bay	0.8 (0–2.3)	Hansen, Henriksen and Blackburn 1981; Henriksen, Hansen and Blackburn 1981; Caffrey (unpublished data)
South San Francisco Bay	0.8 (0.3–3.4)	Caffrey (unpublished data)
North San Francisco Bay	0.6 (0–4.4)	Damashek et al. 2015; Caffrey (unpublished data)
Chesapeake Bay	0.5 (0–2.1)	Kemp et al. 1990
Apalachicola Bay	0.6 (0–1.8)	Caffrey et al. 2007
Pensacola Bay	0.5 (0–1.9)	Caffrey et al. 2007
Rookery Bay	0.5 (0–2.1)	Caffrey et al. 2007
Bering-Chukchi shelf	0.4 (0.3–0.8)	Henriksen et al. 1993
Sapelo Island Tidal creek	0.4 (0–1.4)	Caffrey et al. 2007
Skidaway Island marsh	0.32 (0.17–0.65)	Dollhopf et al. 2005
Weeks Bay	0.2 (0–8.2)	Caffrey et al. 2007
Elkhorn Slough	0.1 (0–11.8)	Caffrey 2002; Caffrey et al. 2003; Caffrey et al. 2010
Plum Island Sound	0.01 (0–0.32)	Bernhard et al. 2007
Barn Island, CT	0.01 (0–0.12)	Moin et al. 2009

Potential rates of denitrification and DNRA are generally difficult to compare because of the different methods used for incubation (e.g. concentration of NO_3^- added) or used to calculate N_2 and NH_4^+ production. Potential denitrification rates measured in the Yarra River estuary in Australia were similar to our rates (Fig. 4), while potential DNRA rates from this location were up to one order of magnitude lower than ours (Robertson et al. 2016). In the East China Sea, rates increased with depth, with surface sediments similar to our anoxic sediments and 6–8 cm layer sediments similar to our low sulfide treatment (Song et al. 2013). The positive relationship between DNRA rates and sulfide concentrations (Fig. 5) is in agreement with a study in anoxic Baltic Sea waters (Bonaglia et al. 2016), but contrasts with results from tidal creek sediments (Porubsky, Weston and Joye 2009).

CONCLUSIONS

Sediments from this mesohaline and hypoxic basin in Roskilde Fjord appear to be particularly well suited to nitrifiers such that they can rapidly respond to short-term variations in sulfide and oxygen. In contrast, denitrifiers from Roskilde Fjord, while tolerant of low oxygen and sulfide, were unable to recover from short-term exposure to oxidic or highly sulfidic conditions. DNRA bacteria appear to depend on sulfide for nitrate reduction. Thus, microbial communities exposed to diurnal fluctuations in oxygen and sulfide may be more tolerant of suboptimal conditions than earlier studies suggest such that they can rapidly respond when conditions become optimal.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSLE](#) online.

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Conflict of interest. None declared.

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