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Redox oscillation and benthic nitrogen mineralization within burrowed sediments: An experimental simulation at low frequency

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A B S T R A C T

Possible effects of sediment ventilation by benthic organisms on the nitrogen cycle were investigated using an experimental setup that mimicked stable or relatively low frequency oscillating redox conditions potentially found in bioturbated deposits. Three different conditions inside burrowed sediments were simulated using 2 mm thick sediment layers: 1) continuously oxic sediment exposed to oxygenated overlying bottom water (e.g., burrow walls, surface sediment), 2) continuously anoxic sediment out of reach from either O₂ or NO₃⁻ diffusion and 3) the lining/boundary of burrow structures or sediment pockets (e.g., excavated during feeding) subject to intermittent irrigation and redox fluctuations over several day timescales. Results demonstrated that intermittent redox fluctuations allowed sustained denitrification and episodic nitrification, whereas significant denitrification and both nitrification and denitrification were absent after ~5–10 days from continuously oxidized and anoxic zones respectively. Intermittent redox oscillations enhance metabolic diversity, magnify loss of dissolved inorganic N to solution, and permit sustained coupling between ammonification, nitrification, and denitrification despite lack of a stable stratified oxic-anoxic redox structure. Even relatively low frequency redox oscillations induce greater N loss compared to sediment that is continuously exposed to oxic and anoxic conditions.

Keywords:

Bioturbation
Redox oscillation
Nitrogen cycle
Burrow ventilation
Experimental design
Simulation

1. Introduction

Bioturbation (sensu Kristensen et al., 2012) plays a major role in the early diagenesis of sedimentary organic matter (OM) (Gilbert et al., 1996; Mayer et al., 1996; Aller and Aller, 1998; Sun et al., 1999; Reise, 2002). Particle reworking by benthos directly affects the distribution and fate of particulate organic substrates and adsorbed OM (Boudreau et al., 1998; Gérino et al., 1998; Widdows et al., 1998; Smallwood et al., 1999; Gilbert et al., 2001). Bioirrigation of sediments due to burrow or feeding-pocket ventilation promotes solute exchange across the sediment-water interface, enhances the removal of metabolites from pore water and supplies respiratory reactants such as O₂ and SO₄²⁻ (Jørgensen and Revsbech, 1985; Forster et al., 1999; Timmermann et al., 2006; Behrens et al., 2007). As an example, bioirrigation has been shown to increase both O₂ and NO₃⁻ penetration into deposits (Aller, 1982; Kristensen et al., 1991; Kristensen, 2000). With a few exceptions (e.g. Altmann et al., 2004; Jordan et al., 2009), bioirrigation generally stimulates sedimentary denitrification (Sayama and Kurihara, 1983; Kristensen and Blackburn, 1987; Gilbert et al., 1995, 1998; Rysgaard et al., 1995; Bartoli et al., 2000; Webb and Eyre,

2004). Stimulation results from coupled nitrification-denitrification enhanced by biogenic structure and/or to denitrification fuelled by an increased supply of nitrite and nitrate from the overlying water or from sedimentary nitrification (Aller et al., 1983; Pelegri et al., 1994; Pelegri and Blackburn, 1995; Tuominen et al., 1999; Karlson et al., 2005). Bioirrigation of pore water also promotes the transport of ammonium and other possible inhibiting metabolites from the sediment-pore water system (Nedwell and Walker, 1995; Kristensen and Hansen, 1999; Stief et al., 2013). In addition, ventilation and particle reworking have been found to strongly affect bacterial distributions and activity (Reichardt et al., 1991; Goñi-Urriza et al., 1999).

Thus, sediments subjected to the activities of benthic animals have high spatial and temporal heterogeneity of biogeochemical properties, extending across micro- to macro-scales (Gutiérrez and Jones, 2006; Polerecky et al., 2006; Pischedda et al., 2008). Indeed, ventilation can result in varying solute transport and redox conditions within the different sectors of single large burrows depending on the location and activity patterns of the inhabitants (e.g., *Callinassa*; irrigation frequency range 10× to ~0× d⁻¹; Forster, 1991). A radial geometry can be defined around individual cylindrical burrows or burrow sections to differentiate distinct biogeochemical zones as a function of oxygen penetration (Aller, 1988, 2001). The diagenetic reaction balances and rates related to this radial geometry have been demonstrated to be

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dependent on the spacing between burrows or sections of burrows (Aller and Aller, 1998; Gilbert et al., 2003; Kristensen and Kostka, 2005). Ventilation is also variable with time, and is an intermittent rather than a continuous process. Burrow ventilating species are known to periodically inject overlying water into their burrows (e.g., Wetzel et al., 1995; Matisoff and Wang, 1998; Stief and de Beer, 2006). However, the relative frequency between the ventilation period and other activities (including resting period) is highly variable from one species to another (e.g., Forster and Graf, 1995; Kristensen and Kostka, 2005; Volkenborn et al., 2012). Moreover, for the same species, the periodicity and patterns of ventilation (continuous or periodic) can depend on environmental parameters such as water temperature and food availability (Gérino, 1989; Frenzel, 1990).

Continuous measurements of redox potential in sediment deposits have demonstrated dynamic redox conditions with oscillation frequency timescales of <1 h to >1 day immediately adjacent to natural burrows due to the diffusion of oxygen from the lumina of burrows, or from cavities such as feeding pockets, into the adjacent sediment (Forster and Graf, 1992; Volkenborn et al., 2012). Episodic exposure of anoxic sediment to oxygenated conditions during feeding, burrow construction, or locomotion can also vary over a wide range of timescales from <0.1 days to >1500 days, with typical resting periods (~anoxic) of ~1–100 days for multiple infaunal species (e.g., Myers, 1977; Wheatcroft et al., 1990; Marinelli, 1992). Such redox fluctuations may contribute to making burrow walls and feeding cavities highly reactive microbial sites compared to the surrounding sediments (Henriksen et al., 1983; Aller and Aller, 1986; Jumars et al., 1990; Kristensen and Kostka, 2005).

Previous work on the effects of redox oscillation on rates and dominant pathways during OM remineralization have demonstrated, for example, that Chlorophyll-*a* does not completely degrade under continuously anoxic conditions (Sun et al., 1993), and that periodic re-exposure of sediment to oxygen results in an intermediate or more complete (and sometimes more rapid) decomposition compared with stable oxic or anoxic conditions (Aller, 1994; Hulthe et al., 1998; Sun et al., 1999; Grossi et al., 2003; Caradec et al., 2004). This suggests that redox oscillation and mixing of particles by fauna across redox zonation likely result in an overall stimulated metabolic activity.

In the present study, sediment was incubated together with overlying sea water under diffusively open conditions (sediment plugs; e.g., Aller and Mackin, 1989; Gilbert et al., 2003; Caradec et al., 2004) to further investigate how redox conditions affect rates and pathways of sedimentary nitrogen cycling. In addition to monitoring the evolution of solutes in the pore water and overlying water, aerobic (nitrification: oxidation of NH_4^+ to NO_3^-) and anaerobic (denitrification: reduction of $\text{NO}_3^-/\text{NO}_2^-$ to N_2 and dissimilatory nitrate reduction to ammonium: reduction of $\text{NO}_3^-/\text{NO}_2^-$ to NH_4^+) nitrogen transforming activities were directly measured. The experimental setup, and especially 2-mm thick sediment layers, was designed to mimic stable or relatively low frequency (multi-day) oscillating redox conditions corresponding to three different conditions inside or at the surface of burrowed muddy sediments: 1) oxic sediment continuously exposed to oxygenated overlying bottom water, 2) anoxic sediment out of reach from O_2 or NO_3^- diffusion from the overlying water or from ventilated biogenic structures, and 3) regions of biogenic structures subject to intermittent ventilation and redox fluctuations (Fig. 1). In this latter case, the fluctuation timescales chosen were several days and thus roughly comparable to relatively remote zones of either stable burrow structures (e.g., *Callianassa*; Forster, 1991) or to many feeding-pockets or temporary structures periodically formed and ventilated by mobile infauna (e.g., deposit feeding bivalves, hemichordates, polychaetes; Wheatcroft et al., 1990). The experimental focus was on the effects of unsteady redox conditions over specific, representative time-scales of several days.

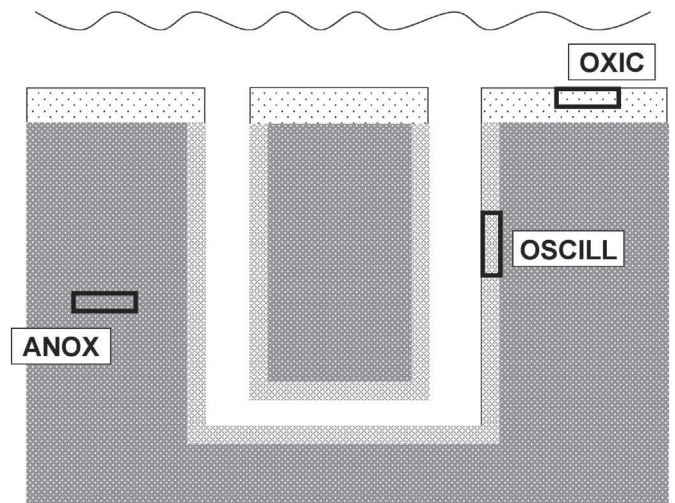


Fig. 1. Schematic representation of the three different parts of a burrowed sediment (with associated environmental conditions) that were mimicked during the experimentation. OXIC: continuously oxygenated surface layer or zones of closely spaced burrow structures, ANOX: continuously anoxic deep layer out of diffusive reach from O_2 or NO_3^- ; OSCILL: wall layer of an inhabited burrow subjected to intermittent irrigation over multiple day timescales, or equivalently, the wall of a recently excavated cavity.

2. Materials and methods

2.1. Experimental setup

Surface (0–2 cm) muddy sediment and seawater were collected at the SOFI Station in the Gulf of Lion (Mediterranean Sea; 43°04N, 5°08E; 170 m depth) in February 2000. This sediment was composed of 34% clay, 46% silt and 20% sand (0–10 cm), had a mean porosity of 0.54 in the two first centimetres (Denis et al., 2001) and was inhabited by the polychaetes *Hyalinoecia bilineata* and *Lumbrineris latreilli*, the molluscs *Abra longicallus* and *Mendicula ferruginosa* and the crustaceans *Eriopisa elongata* and *Natatolana borealis* as major species (Georges Stora, pers. comm.). Sediment was sieved through a 0.25-mm mesh, homogenized and augmented with phytoplankton cells (*Nannochloropsis salina*) cultured in *f/2* medium. The source sediment organic carbon and total nitrogen contents were originally 0.35% and 0% respectively. Organic C was increased to 1.58% following the addition of phytoplankton cells. Unfortunately, N analyses of amended sediment were compromised by a combination of instrument malfunction and sample size limitation but initial values are estimated as ~0.3% based on reported N/C ratios in N-replete *Nannochloropsis* cultures (Flynn et al., 1993).

After collection and manipulation, the surface sediment was incubated under diffusively open conditions in 2 mm thick plugs made of PVC plastic rings fixed on individual circular PVC sheets (Aller and Mackin, 1989; Hulth et al., 1999; Gilbert et al., 2003). Three diameters of plugs were utilized: 3.3 cm (small plugs) for metabolic rates and micro-distributions of oxygen in the pore water, 4.8 cm (medium plugs) for solute concentrations in the pore water, and 6.8 cm (large plugs) for solid phase lipid analyses (Caradec et al., 2004).

All plugs were filled with the mixture of sediment and phytoplankton, and then equally distributed in 3 sets of 3 polycarbonate containers containing 14 L of 0.2- μm filtered seawater (salinity 38.1). Because of destructive sampling, plug areas in containers averaged 299 cm^2 at the start of the experiment and 49 cm^2 at the end. Each container was placed in an individual glove bag and kept in the dark. In one set of containers (termed OXIC), the overlying water was continuously purged with water-saturated air. In another set (termed ANOX), anoxic conditions were maintained by continuous N_2/CO_2 bubbling in the overlying water. The 2.3% CO_2 in N_2 allowed the maintenance of overlying water pH at 8.09 ± 0.04 (mean \pm SD; $n = 6$) during the experiment, as measured every 5 days with a WTW 320 portable pH/mV meter (NIST scale).

In the third set of containers (termed OSCILL), oscillation between oxidizing and reducing conditions was induced by shifting periodically from oxic to anoxic conditions. The overlying water in these containers was first purged with air (OSCILL-OX) which was replaced by N_2/CO_2 (OSCILL-AN) after 5 days. Periodic switching between oxic and anoxic conditions was maintained every 5 days until the end of the experiment (day 35) (Caradec et al., 2004).

Water was continuously stirred by a central Teflon-coated magnetic stirring bar. Incubations were performed in the dark in a temperature controlled room at 15 °C. The overlying water (10 mL) from two containers of each set (OXIC, ANOX and OSCILL) was periodically (1–2 days) sampled with a polypropylene syringe and stored frozen until analysis of nutrients (NH_4^+ , NO_2^- and NO_3^-). Every 5 days, and before the switch between the purging conditions in the OSCILL containers, 4 small plugs (except for days 25 and 30) and one medium plug (except for day 30) were removed from two containers of each treatment. Sediment from the small plugs was then used for the measurement of bacterial nitrogen transformations. Pore water was separated from the sediment of the medium plugs by centrifugation (10 min at $10,000 \times g$), and stored frozen until analysis of nutrients (NH_4^+ , NO_2^- and NO_3^-). Due to the extension of the end of experiment from 25 to 35 days in order to study the stability of the patterns, a decision made after the start of experiment, some sediment samplings were skipped at day 25 and day 30.

2.2. Sedimentary bacterial nitrogen transformations

A combination of acetylene inhibition and isotopic tracer techniques was used to directly measure rates of nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Nitrification and denitrification rates were assessed by the acetylene-blockage method using various partial pressures of acetylene to selectively inhibit nitrification or denitrification (Klemetsson et al., 1988a,b; Kester et al., 1996; Bonin et al., 2001; Marty et al., 2001). DNRA rate measurement was based on the quantification of $^{15}NH_4^+$ produced after introduction of $^{15}NO_3^-$ in the system (Tiedje, 1988).

Sediment subsamples (0.7 mL) were transferred from the plugs into twenty 10 mL Venoject®gas-tight tubes with 2 mL of seawater from each corresponding reservoir. The seawater was then supplemented with chloramphenicol (final concentration $1 g \cdot L^{-1}$) to prevent new bacterial growth during incubation. The tubes were sealed with rubber stoppers. In half of the tubes, acetylene was added at a final concentration of 10 Pa to specifically inhibit the first step of the nitrification process (ammonium oxidation; Berg et al., 1982; Klemetsson et al., 1990). Then, all the tubes were vortexed. Samples were incubated in the dark at the experimental temperature (15 °C) for 0, 1, 3, 5 and 8 h. After incubation, each tube was treated with 0.1 mL of a 1 M $HgCl_2$ solution. Nitrification was calculated as the difference of nitrate production in the absence and presence of acetylene.

Sediment subsamples (0.7 mL) were transferred from the plugs into ten 5 mL Venoject®gas-tight tubes with 2 mL of seawater from each corresponding reservoir. Seawaters were then supplemented with chloramphenicol (final concentration $1 g \cdot L^{-1}$) as mentioned above. Subsamples were inoculated with ^{15}N -nitrate (97.4 atom%, Isotec France) at a concentration lower than ~10% of the estimated nitrate concentration in a given tube. For rate calculations, the actual partitioning was determined after measurement of the nitrate level. No addition of ammonium was necessary as nitrate assimilation was inhibited by the high ammonium content of the sediment ($>100 \mu M$). The tubes were sealed with rubber stoppers and anaerobic conditions obtained by flushing dinitrogen through the tube for 2 min. Acetylene, which inhibits the reduction of nitrous oxide to dinitrogen (Balderston et al., 1976), was injected in the gas phase (final concentration: 15 kPa) and the tubes were vortexed. Samples were incubated in the dark at the experimental temperature (15 °C) for 0, 1, 3, 5 and 8 h, and subsequently treated with 0.1 mL of 1 M $HgCl_2$ solution. Denitrifying activity was considered as the linear initial rate of

nitrous oxide accumulation. According to Tiedje (1988), rates of DNRA activity were determined by monitoring the progressive increase in isotopic enrichment of ^{15}N -ammonium with time as the substrate (nitrate) was used (Gilbert et al., 1997).

2.3. Chemical analyses

Nitrous oxide was quantified by gas chromatography (HP5890, Series II) using an electron capture detector and an automatic injector (Gilbert et al., 2003). Nitrate in the overlying water and pore water was reduced to nitrite on a Cu-Cd column adapted to Technicon II (Treguer and Le Corre, 1975). Nitrite concentrations were determined colorimetrically (Bendschneider and Robinson, 1972). Ammonium was measured using the “OPA” fluorimetric method (Holmes et al., 1999). Water samples were mixed with the working reagent (ratio 1:3, v/v) and incubated for 3 h before being introduced in borosilicate test tubes in which the ammonium content was determined using a spectrofluorometer (Kontron Analytical, SFM 25; λ_{exc} : 350 nm; λ_{em} : 420 nm). Nitrogen stable isotope analysis involved interfacing an automatic N/C analyser (ANCA) to a triple collector isotope-ratio mass spectrometer (ANCA-MS Tracer mass, European Scientific).

2.4. Oxygen profiles

The oxygen distribution in plugs was monitored using micro-electrodes made according to the procedure of Revsbech and Jørgensen (1986). The micro-electrodes were attached to a micromanipulator allowing vertical steps of 200 μm . Ten hours after the first switch between purging conditions in the OSCILL containers, sediment plugs were removed from the different containers (OXIC, ANOX and OSCILL), placed in vials filled with respective container overlying water, and the oxygen profiles measured.

3. Results

3.1. Oxygen profiles

The oxic layer of the intact initial sediment used for incubations was 2.5 mm deep (data not shown). O_2 profiles in the OXIC and OSCILL-OX (10 h after initiation of air purging phase) sediments presented a typical diffusive pattern with an O_2 penetration down to the bottom of the plugs (2 mm) (Fig. 2). Oxygen was not detected in the overlying water or in sediments under the ANOX and OSCILL-AN (10 h after initiation of N_2/CO_2 purging phase) conditions.

3.2. Bacterial activities

The initial rates of nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) were 117 ± 9 , 237 ± 37 , and $0.8 \pm 0.1 \mu mol \cdot L^{-1} d^{-1}$ (mean \pm SD; $n = 2$), respectively (Fig. 3).

Under OXIC conditions, the measured nitrification rate was $51 \mu mol \cdot L^{-1} d^{-1}$ five days after the introduction of the sediment plugs in the water containers (Fig. 3A). This rate remained quite stable during the following 15 days and then decreased slightly to $31 \mu mol \cdot L^{-1} d^{-1}$ by the end of the experiment (35 days). Sediment nitrification under ANOX conditions could not be detected after 5 days. The oscillating conditions (OSCILL) started with air purging (OSCILL-OX). After 5 days, nitrification rate was $43 \mu mol \cdot L^{-1} d^{-1}$, essentially identical to the oxic treatment. During the 5 next days under anoxic conditions (OSCILL-AN), nitrification decreased to below detection. When oxic conditions were resumed, nitrification rates increased to $119 \mu mol \cdot L^{-1} d^{-1}$, and following the next switch to anoxic conditions, nitrification rates were again below detection. During the terminating oxic phase of the OSCILL experiment, nitrification was $61 \mu mol \cdot L^{-1} d^{-1}$. Thus, nitrification rate dynamics in the oscillating treatment closely tracked the changing O_2 conditions in

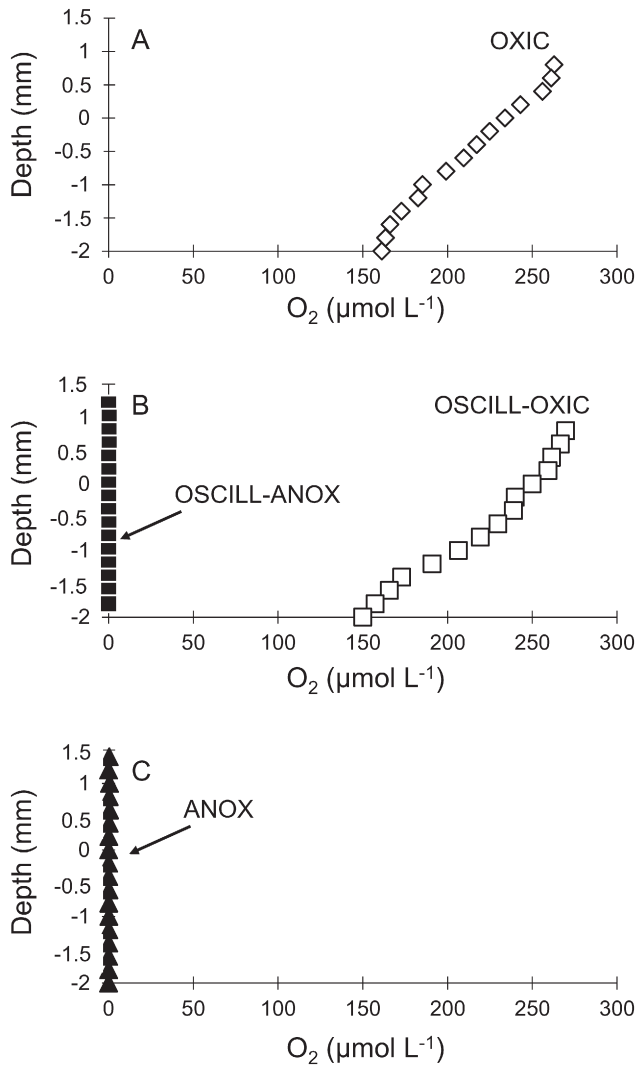


Fig. 2. Sediment oxygen profiles measured for the different experimental conditions. In the OSCILL containers, all the measurements were made 10 h after the switch between purging conditions. The filled symbol indicates anoxic conditions; the open symbol indicates oxenic conditions. A: overlying water continuously purged with water-saturated air (OXIC); B: periodic shifts from oxenic (OSCILL-OXIC, 10 h after shift from anoxic to oxenic) to anoxic conditions (OSCILL-ANOX, 10 h after shift from oxenic to anoxic); C: continual N_2/CO_2 bubbling in the overlying water (ANOX).

overlying water, and when oxenic conditions followed an anoxic period, nitrification rates exceeded those in sediment maintained under continuously oxenic conditions (Fig. 3A). The average measured nitrification rates from 0 to 35 days, calculated as the mean between any two measurements weighted by the corresponding time period, were 53, 45, and 8 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ for the oxenic, oscillating, and anoxic treatments. Ignoring the initial 5 day period, the time weighted averages of nitrification over 5–35 days were 48, 39, and 0 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ respectively.

Under oxenic boundary conditions, denitrification rate was $164 \pm 36 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ after 5 days and progressively decreased to $139 \pm 10 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ after 10 days, $15 \pm 2 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ after 15 days, and thereafter remained at 8–9 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$.

Under anoxic conditions, measured denitrification was $214 \pm 21 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ after 5 days but drastically dropped by day 10 to 4–6 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ thereafter (Fig. 3B). After the first 5 days, the highest rates of denitrification were consistently found in the oscillation treatment at all times but particularly following anoxic periods, with a maximum measured rate of $460 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ at 20 days (Fig. 3B). The average measured denitrification rates from 0 to 35 days, calculated as

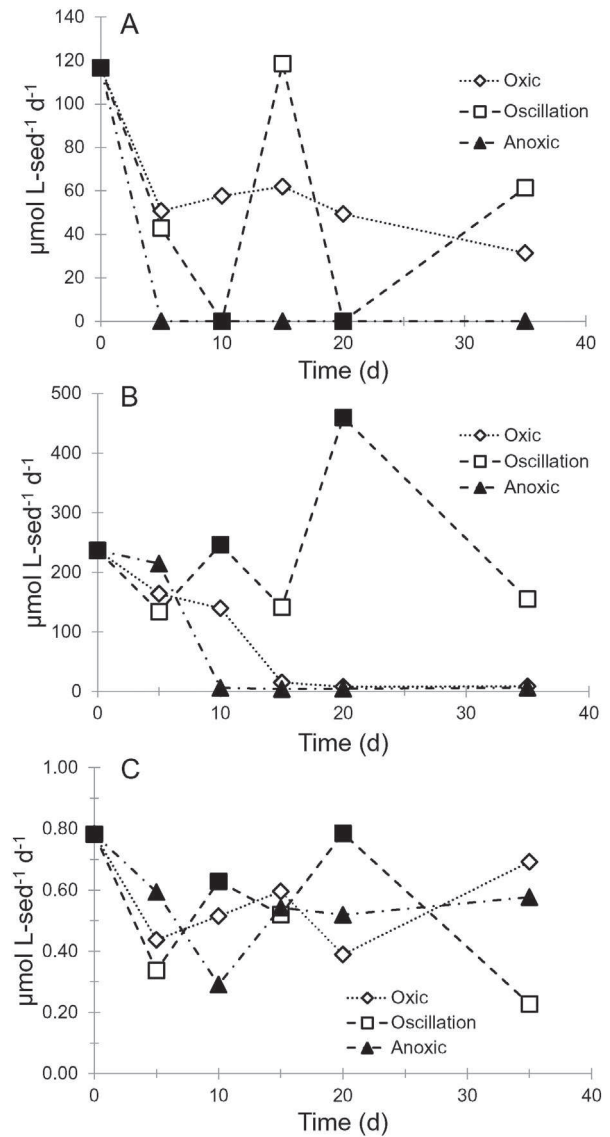


Fig. 3. Time-dependent nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates directly measured in the sediment, for the different experimental conditions. All treatments began with the same initial rates (closed circle). In the case of oscillating overlying water redox conditions, the filled symbol indicates anoxic conditions during the previous 5 days; the open symbol indicates oxenic conditions over the previous 5 days. A: Nitrification rates; B: Denitrification rates; C: DNRA rates. Values are mean \pm SE ($n = 2$) for denitrification and DNRA rates.

the mean between any two measurements weighted by the corresponding time period, were 67, 256, and 57 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ for the oxenic, oscillating, and anoxic treatments. Ignoring the initial 5 day period, the time weighted averages over 5–35 days of denitrification were 44, 268, and 23 $\mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ respectively.

Rates of DNRA were close to or below detection throughout the incubation period ($<0.7 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$) for all treatments. The highest DNRA rate was measured under oscillating conditions at 20 days following an anoxic period ($\sim 0.8 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$). Concomitantly, DNRA was below $0.7 \mu\text{mol}\cdot\text{L}^{-1}\text{d}^{-1}$ throughout the experiment.

3.3. Pore water distributions of DIN

Nitrate concentrations in the pore water continuously increased throughout the OXIC experiment, reaching 36 μM after 35 days (Fig. 4). This increase was slightly slower during the first 15 days than during the last 20 days. Nitrite concentrations ranged from 3 to 7 μM

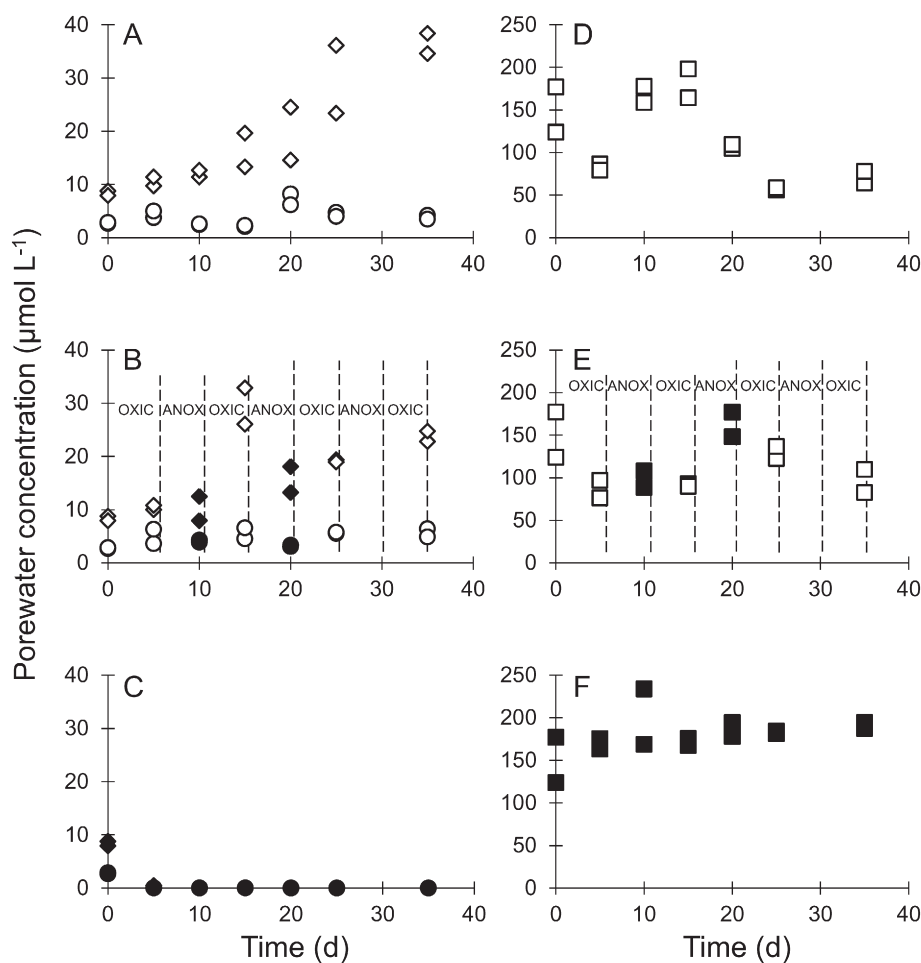


Fig. 4. Time-dependent N-compounds concentrations in the sediment porewater, for the different experimental conditions. NO_3^- , NO_2^- , and NH_4^+ are represented by diamond, circle and square symbols, respectively. The filled symbol indicates anoxic conditions; the open symbol indicates oxic conditions. In the case of oscillating overlying water redox conditions, the filled symbol indicates anoxic conditions during the previous 5 days; the open symbol indicates oxic conditions over the previous 5 days. A and D: overlying water continuously purged with water-saturated air (OXIC); B and E: periodic shifts between oxic and anoxic conditions (OSCILL); C and F: continual N_2/CO_2 bubbling in the overlying water (ANOX).

during the whole experiment. Ammonium concentrations increased from 79 up to 198 μM until day 15, and then decreased to 60 μM until the end of the experiment (Fig. 4).

Nitrate and nitrite concentrations were below detection ($<0.02 \mu\text{M}$) under ANOX conditions during the first 5 days (Fig. 4), whereas ammonium concentration remained steady at 169 μM between day 5 and day 35 (Fig. 4).

Nitrate and nitrite concentrations shared similar patterns under OSCILL conditions with increases during the oxic period (OSCILL-OX) and decreases during the anoxic period (OSCILL-AN) (Fig. 4). Although similar in nature, patterns were more pronounced for NO_3^- which ranged from 8 to 33 μM . Ammonium concentrations followed the inverse patterns with higher concentrations after anoxic periods (Fig. 4). Because overlying water DIN concentrations changed continuously (see below), a portion of the pore water concentration patterns reflect changing boundary conditions in the incubation containers. However, average pore water NO_3^- concentrations exceeded overlying water at all times in the OXIC and OSCILL treatments, and average pore water NH_4^+ exceeded overlying water concentrations at all times in all treatments.

3.4. DIN in the overlying water

Nitrate concentrations in the overlying water differed substantially between treatments. The highest concentrations were attained under OXIC conditions where NO_3^- increased slightly during the first 15 days

(from 2 to 3 μM) and then more rapidly after 20 days, up to a high of 14 μM (Fig. 5A). In contrast, under ANOX conditions, NO_3^- and NO_2^- were below detection (0.03 μM and 0.01 μM respectively) in overlying water after the initial starting period. A highly dynamic and variable NO_3^- concentration pattern characterized the OSCILL treatment (Fig. 5A). NO_3^- concentrations increased by ~5–10 μM immediately (first sample) during OSCILL-OX periods or decreased during OSCILL-ANOX periods.

NH_4^+ in overlying water increased in all treatments with time but, like NO_3^- concentrations, showed very different behaviour as a function of redox conditions (Fig. 5B). In the OXIC treatment, NH_4^+ concentrations were relatively low but increased to ~5–10 μM by 35 days. In the ANOX treatment, NH_4^+ increased at a progressively increasing rate, reaching ~40 μM . NH_4^+ concentrations varied substantially and sharply as a function of oxygenation in the OSCILL treatment but were as high or higher than in the ANOX treatment at all times. The lowest concentrations occurred during oxic periods and the highest during anoxic periods, with a maximum concentration of ~70 μM attained at 30 days (Fig. 5B). Similarities and differences between treatments are evident when the variations in total DIN concentrations ($=\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) are considered. DIN concentrations increase with time in all treatments and at a similar rate in both OXIC and ANOX, although ANOX build ups are slightly higher than OXIC. In contrast, the OSCILL treatment sustains the highest DIN concentrations at all times and shows distinct oscillatory behaviour as a function of redox conditions. The highest DIN build ups occur during anoxic periods. The time

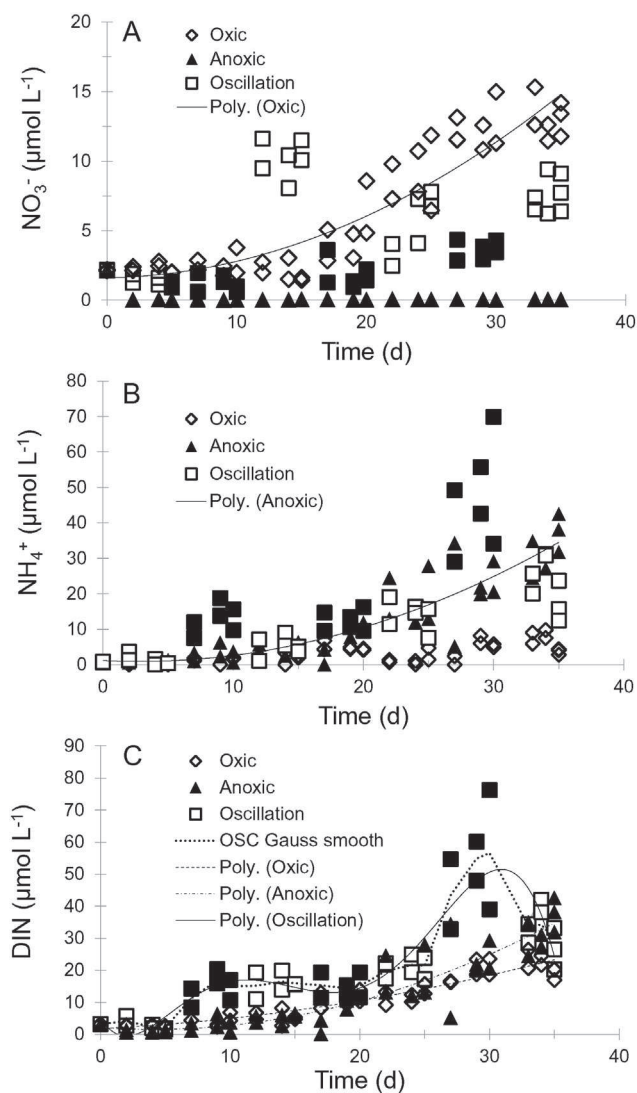


Fig. 5. Time-dependent N-compound (NO_3^- , NO_2^- and NH_4^+) concentrations in the overlying water, for the different experimental conditions. A. NO_3^- ; B. NH_4^+ ; C. DIN ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$). The filled symbol indicates anoxic conditions; the open symbol indicates oxic conditions. Curves represent polynomial and Gaussian smoothed fits to data series to illustrate overall patterns.

averaged DIN concentrations over the 35 day period are: 10.8, 12.6 and 21.2 μM in the OXIC, ANOX, and OSCILL treatments. These concentrations correspond to a net release into overlying water of 151, 177, and 296 $\mu\text{mol N}$.

3.5. Transport – reaction model calculations

The average net nitrification rates and ammonification rates in the sediment plugs at steady state can be estimated using the concentration differences of NO_3^- and NH_4^+ between pore water and overlying water from the relation (Aller and Mackin, 1989):

$$R = 3 D_s (\Delta C) / L^2 \quad (1)$$

where:

- R reaction rate
- D_s whole sediment diffusion coefficient
- ΔC concentration difference between pore water and overlying water
- L sediment plug thickness

Because of the small thickness of plugs (0.2 cm), steady state concentration balances should be attained in <0.5 day at typical values of D_s and assuming an approximately constant overlying water boundary value over the same time period. The rates estimated from these relationships will be minima if additional consumption reactions and biomass synthesis are occurring, for example, denitrification in the case of nitrification. Similarly, reactions such as net ammonification are most accurately estimated under anoxic conditions when nitrification is minimized.

D_s for NO_3^- and NH_4^+ were estimated as 0.554 and 0.568 $\text{cm}^2 \text{d}^{-1}$ ($T = 15 \text{ C}$; $S = 38$; porosity = 0.54) (Boudreau, 1997). The model estimated net ammonification rates, $R\text{-NH}_4^+$, (ANOX only) ranged from 3.9 to 3.6 $\text{mmol L}^{-1} \text{sed}^{-1} \text{d}^{-1}$, decreasing steadily during the experiment (Fig. 6A). Model calculated net nitrification rates, $R\text{-NO}_3^-$, were highest in the OXIC treatment and increased with time to a maximum of $\sim 500 \mu\text{mol L}^{-1} \text{sed}^{-1} \text{d}^{-1}$ (Fig. 6B). Model nitrification rates, while minima because of concomitant denitrification, are $\sim 10\times$ higher than the rates measured using the acetylene block technique. They are in the range of but exceed the measured denitrification rates.

4. Discussion

The concentrations and distribution patterns of oxygen in the pore water under the different experimental conditions confirmed the relative redox states of sediments and the response to changing redox conditions in the overlying water. The rapid (≤ 10 h) and complete penetration of O_2 in the sediments during stationary and periodic air purging, and the absence of O_2 (≤ 10 h) during N_2/CO_2 purging (continuous or alternating) indicated that the use of 2-mm plugs (Gilbert et al., 2003) and the experimental design successfully mimicked at least one mode (a characteristic frequency) of the redox changes and diffusively open transport regimes that surface sediment is naturally exposed to during macrofaunal bioturbation.

The directly measured reaction rates and overlying water compositions show that the dominant pathways of N cycling, the relative rates

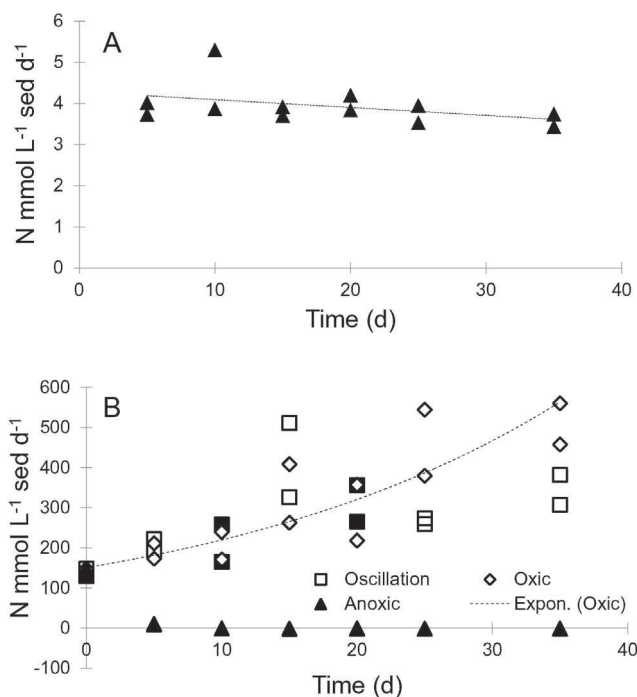


Fig. 6. Transport–reaction model calculated ammonification (A) and nitrification (B) rates at steady state. The filled symbol indicates anoxic conditions; the open symbol indicates oxic conditions. In the case of oscillating overlying water redox conditions, the filled symbol indicates anoxic conditions during the previous 5 days; the open symbol indicates oxic conditions over the previous 5 days.

of reactions, and the eventual fate of N were generally very different among treatments (continuously oxic or anoxic, compared to periodic oscillating conditions) (Figs. 3 and 5). The exception was DNRA, a metabolic pathway linked to very high organic matter loading (Binnerup et al., 1992; Kaspar et al., 1988; Gilbert et al., 1997; Christensen et al., 2000), but which was insignificant under all boundary conditions in the present experiments despite addition of planktonic OM.

The initial measured nitrification and denitrification rates in the experimentally amended sediment were in the range of the values reported for coastal sediments (see Table 3 in Gilbert et al., 1997). Modelled ammonification rates in the ANOX treatment indicate that net remineralization of N during the incubations was quite high, $\sim 3.8 \text{ mmol L}^{-1} \text{ sed}^{-1} \text{ d}^{-1}$, presumably as a result of the addition of fresh planktonic material. A more complete diagenetic model for coupled C, N, and O_2 distributions with nitrification–denitrification in the plug sediment (following Berg et al., 2003; models not presented), indicates that in order to have O_2 extend to the base of the plugs while sustaining such high rates of N remineralization, the C/N ratio of the remineralized component must have been < 5.7 (Redfield average), and likely ~ 4 , consistent with preferential initial decomposition of highly labile N rich material. Because labile planktonic material dominates decomposition in the plugs, the magnitude of N remineralization is presumably similar in all treatments (Lee, 1992). These calculated ammonification rates could potentially support NH_4^+ fluxes of $\sim 7.6 \text{ mmol m}^{-2} \text{ d}^{-1}$, or given the plug areas ($\sim 300 \text{ cm}^2$) and overlying water volumes (14 L), an expected rate of increase of DIN in overlying water of $\sim 16 \mu\text{M d}^{-1}$. Such rates of DIN increase were not observed until late in the experimental period in the OXIC and ANOX treatments and only immediately following redox oscillations in the OSCILL treatment (Fig. 5).

Thus, overlying water DIN patterns indicate that over much of the experiment most remineralized DIN released in sediment was taken up either at the very surface of the sediment or in the overlying water + container walls. This pulsed release of DIN during sudden redox changes may reflect a “nutrient flushing” phenomenon associated with metabolic switching and/or death of microbial populations in surface sediment or overlying water (e.g., Marumoto et al., 1982; Aller, 1994; Valdemarsen and Kristensen, 2005). In any case, the result of oscillation is clearly greater net and episodic loss of DIN ($\sim 300 \mu\text{mol}$ versus $\sim 150\text{--}170 \mu\text{mol}$ or $\sim 1.7\text{--}2\times$) from remineralized substrate or accumulated biomass (immobilized N) relative to stable oxic or anoxic redox conditions in the sediment–water complex (Fig. 5C).

In both the OXIC and OSCILL treatments, pore water NO_3^- exceeded overlying water NO_3^- at all times (Figs. 4 and 5), and the NO_3^- concentrations also increased in magnitude during the experiment, particularly under continuously oxic conditions (Fig. 4). These facts demonstrate that any denitrification activity in sediment under both treatment modes was supported by sedimentary nitrification, that there was a net flux of NO_3^- into overlying water, and that sediment became steadily more oxidized with time, that is, labile substrate decreased with time. Directly measured nitrification rates in sediment were more or less constant through time under continuously oxic conditions or decreased slightly (Fig. 3A). In contrast, nitrification rates calculated from transport models for the same sediment were systematically higher ($\sim 10\times$) and tended to increase with time (Fig. 6B). This relative time dependent pattern of directly measured rates is likely real but the difference in absolute rates between nitrification rate estimates may reflect a systematic underestimation of nitrification by the acetylene block method. Because sedimentary nitrification clearly supports any denitrification in these experiments, the nitrification rates must be equal to or greater than denitrification. Of the two estimates of nitrification, the magnitude of the modelled nitrification rate is more consistent with the measured denitrification rates. The modelled nitrification rate is a net rate, however, so its apparent time dependence could be affected by variations in denitrification. If denitrification is relatively higher in basal sediment in the OXIC treatment during the first part of the

experiment, the estimated net nitrification rate would be calculated as relatively lower compared to later times as reactive substrate is depleted. The overall interpretations are that the overall rate of N remineralization decreases slightly during the experiment in all treatments (Fig. 6A), that nitrification rates are nearly constant or decrease slightly with time in the OXIC treatment (Fig. 3A), and that net nitrification increases and pore water NO_3^- concentrations increase as denitrification contributions decrease due to overall lower remineralization rate and greater inhibition of denitrification from higher basal O_2 concentration at later times (Fig. 6B).

Directly measured nitrification rates in the OSCILL treatments underwent extreme excursions, varying from undetectable after 5 days of anoxia to $\sim 2\times$ the maximum magnitude observed in the OXIC treatment after switching to oxic conditions (Fig. 3A). These excursions to very high nitrification rates likely represent a combination of utilization of high concentrations of both reduced (NH_4^+) and oxidized (O_2) species that are suddenly brought together shortly after oxygenation, as well as to growth of nitrifiers over a several day period of enhanced respiration. The rapid increase of NO_3^- in overlying water associated with oxygenation events presumably reflects the increased production and associated flux of NO_3^- from sediment into overlying water and, perhaps, a pulsed release of stored microbial intracellular NO_3^- following renewed access to O_2 (Stief et al., 2013; Piña-Ochoa et al., 2010).

Under continuously anoxic conditions, the lack of oxygen or other energetically favourable oxidants such as MnO_2 rapidly prohibits nitrification (Caffrey et al., 2003). Nitrate disappears from the sediment–overlying water complex, and there is an eventual loss of denitrification due to lack of oxidant (Hulth et al., 2005). In such anoxic systems, nitrification inhibition by sulphide may also occur (Joye and Hollibaugh, 1995). In the present experiments, detectable NO_3^- was absent in both the pore water and overlying water of the ANOX treatment after the initial 5 days. Correspondingly, there was no measureable nitrification or denitrification using the acetylene block technique for the remainder of the ANOX incubation ($t > 5$ days) (Fig. 3B). Thus, ammonification represents the primary mode of N remineralization in the ANOX treatment, assuming production of dissolved organic N is of relatively minor importance. As noted earlier, this N remineralization rate is likely similar in all treatments.

A primary observation of the present study is that denitrification rates are highest in the oscillating system and sustained as such throughout the experimental period (Fig. 3B). For the present sedimentary organic matter reactivity, the 5-day oscillation frequency thus allowed maintenance of both nitrification and denitrification capacities in the system. It seems very likely that a similar conservation of both capacities occurs not only at the high frequencies found in actively irrigated burrows (Kristensen et al., 1991; Mayer et al., 1995), but also at the far slower multiple-day frequencies mimicked here, such as observed in large complex burrow systems or episodic excavation of biogenic cavities during burrowing and feeding. Metabolic diversity is clearly enhanced even by such low frequency oscillations. Loss of N as N_2 is also clearly enhanced. In the present experiment, measured denitrification rates in the oscillating system averaged 5–10 \times those in continuously oxic or anoxic boundary conditions (~ 268 vs 44 and 23 $\mu\text{mol L}^{-1} \text{ sed}^{-1} \text{ d}^{-1}$). As a percentage of remineralized N, losses of N through denitrification apparently represent only ~ 7 , 1, and 0.6% for the oscillating, oxic, and anoxic treatments assuming an ammonification rate of 3.9 $\text{mmol L}^{-1} \text{ sed}^{-1} \text{ d}^{-1}$. If much of the initially remineralized N is immobilized into biomass, as indicated by the minimal build up of DIN in overlying water despite remineralization (Fig. 5C), the impact of denitrification on net exported dissolved N would be far greater (i.e., ratioed to a lower total N remineralization rate).

From a quantitative point of view, the absolute and relative reaction rates documented here cannot be compared exactly with reaction balances in natural burrow walls and in surrounding sediments because oxygen availability, organic matter reactivity, and redox oscillation

frequencies have been shown to be significantly variable within irrigated structures and different regions of sedimentary deposits (Forster, 1991; Kristensen et al., 1991; Mayer et al., 1995; Wenzhöfer and Glud, 2004; Pischedda et al., 2012). Burrow radial geometry is also a variable factor determining oxygen penetration and N-transformation reaction balances into burrow walls (Aller, 1988; Kristensen et al., 1991). However, it is clear that even at relatively low frequencies of redox oscillation (present study: 5 day period), the N cycle in organic-rich sediment is affected by enhanced net DIN release and denitrification. As noted previously, a wide spectrum of redox oscillation frequency is found in bioturbated sediments and exactly how multiple frequencies that are characteristic of different faunal activities impact the coupling of biogeochemical reactions, elemental cycling, and microbial communities remains a rich area of investigation. For example, recent work demonstrates that cable bacteria can become a significant microbial component when oxic–anoxic boundary conditions are stabilized for periods of ~10 days or more, further complicating possible decomposition patterns as a function of redox structure and time (e.g., Marzocchi et al., 2014; Schauer et al., 2014; Risgaard-Petersen et al., 2015).

5. Conclusions

Redox oscillations enhance the net release of DIN and denitrification even at relatively low frequencies of fluctuation. The coupling between different possible reaction pathways that accompany oscillations must enhance metabolic diversity and induce a more efficient loss of immobilized N. A next step to further understand the mechanisms that control the N-cycle in bioturbated deposits would be to integrate microbial community composition and functional data (Yazdani Foshtomi et al., 2015). It is essential to determine whether the N-cycle microbial communities in anoxic and oxic sediments are different under static or fluctuating redox conditions as demonstrated in tropical humid soils (Pett-Ridge et al., 2006). This will help us to differentiate between the hypotheses of (i) the establishment of specialized communities (strictly aerobes, strictly anaerobes and fluctuating) incompatible with multiple redox conditions and (ii) the functional adaptation of the community to fluctuating redox properties through facultative tolerance to brief periods of potentially unfavourable oxic or anoxic conditions in burrows and feeding structures.

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