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The denitrification paradox: The role of O₂ in sediment N₂O production

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ABSTRACT

We designed a novel laboratory sediment flux chamber in which we maintained the headspace O₂ partial pressure at preselected values, allowing us to experimentally regulate “in-situ” O₂ to evaluate its role in net N₂O production by an intertidal estuarine sediment (Tyne, UK). In short-term (30 hour) incubations with 10 litres of overlying estuarine water (1-3cm depth) and headspace O₂ regulation (headspace: sediment/water ratio ~9:1), net N₂O production was highest at 1.2 % O₂ (sub-oxic; 32.3 nmol N₂O m⁻² d⁻¹), an order of magnitude higher than at either 0.0% (anoxic; 2.5 N₂O nmol m⁻² d⁻¹) or 20.85% (ambient; 2.3 nmol N₂O m⁻² d⁻¹) O₂. In a longer-term sealed incubation (~490 hour) without O₂ control N₂O behaviour was highly non-linear with distinct phases: (i) an initial period of no or little change in O₂ or N₂O up to ~ 100 hours; (ii) a quasi-linear, inverse correlation between O₂ and N₂O to ~360 hours, in which O₂ declined to ~2.1% and N₂O rose to ~7800 natm; (iii) over the following 50 hours a slower O₂ decline, to ~1.1%, and a more rapid N₂O increase, to ~12000 natm; (iv) over the next 24 hours a slowed O₂ decline towards undetectable levels and a sharp fall in N₂O to ~4600 natm; (v) a continued N₂O decrease at zero O₂, to ~3000 natm by ~ 490 hours. These results show clearly that rapid N₂O consumption (~115 nmol m⁻² d⁻¹), presumably via heterotrophic denitrification (HD), occurs under fully anoxic conditions and therefore that N₂O production, which was optimal for sub-oxic O₂, results from other nitrogen transformation processes. In experiments in which we amended sediment overlying water to either 1mM NH₄⁺ or 1mM NO₃⁻, N₂O production rates were 2-134 nmol N₂O m⁻² d⁻¹ (NH₄⁺ addition) and 0.4-2.2 nmol N₂O m⁻² d⁻¹ (NO₃⁻ addition). Processes involving NH₄⁺ oxidation (nitrifier nitrification; nitrifier denitrification; nitrification-coupled denitrification) are principally responsible for N₂O production in Tyne sediments. Our data afford an insight into the O₂ effect on N₂O fluxes from Intertidal sediments. Highest N₂O production occurred under sub-oxic headspace (~1.2 O₂%) incubations. Anoxic sediments where HD was isolated acted as periodic N₂O sinks or relatively small sources of N₂O.

1. Introduction

Nitrous oxide (N₂O) accounts for ~6% of tropospheric radiative forcing by well-mixed greenhouse gases (Myhre et al. 2013) and is implicated in O₃ consumption in the stratosphere that results in NO and NO₂ production (Ravishankara et al. 2009). Tropospheric N₂O growth of 0.73 ± 0.03 ppbv yr⁻¹ is in part linked to increasing emissions from soils, freshwaters and estuaries. Estuarine dissolved concentrations and atmospheric emissions of N₂O correlate strongly with inputs of dissolved inorganic nitrogen (DIN: NH₄⁺ + NO₃⁻ + NO₂⁻) via rivers (Barnes and Upstill-Goddard, 2011), consistent with the increasing mobilisation of anthropogenic reactive nitrogen (Davidson, 2009; Syakila and Kroeze 2011). Consequently, river and estuarine N₂O emissions are now classified as anthropogenic by the IPCC (Ciais et al. 2013).

N₂O is thought to arise via several microbial pathways. In classical nitrification, a two-stage process carried out by autotrophic bacteria and archaea, NH₄⁺ is first oxidised aerobically to NO₂⁻ via hydroxylamine (NH₂OH) using ammonia monooxygenase, followed by NO₂⁻ oxidation to NO₃⁻ (NH₄⁺ → NH₂OH → NO₂⁻ → NO₃⁻). After the first stage, N₂O can be produced directly by two NH₄⁺ oxidation pathways: nitrifier nitrification (NN: NH₂OH → N₂O) and nitrifier denitrification (ND: NO₂⁻ → NO → N₂O). Nitrification also provides NO₃⁻ for heterotrophic denitrification, a process referred to as nitrification-coupled denitrification (NCD: NO₃⁻ → NO₂⁻ → NO → N₂O) (Kool et al., 2011). Classical denitrification (or heterotrophic denitrification, HD), in which NO₃⁻ is the terminal electron acceptor (NO₃⁻ → NO₂⁻ → NO + N₂O → N₂), is carried out by facultative heterotrophic anaerobes in anoxic soil, sediment and water; indeed, the inhibition of denitrifier activity even at very low levels of dissolved O₂ is well known (Allison et al. 1960; Knowles 1982). Only 2% of the denitrifying capacity of intact soil cores was found to be expressed at dissolved O₂ concentrations > 0.03 atm (Parkin and Tiedje, 1984). Even so, aerobic denitrification (co-respiration) is carried out by some microbes that are able to simultaneously

use O_2 and NO_3^- as electron acceptors and chemo-denitrification, which utilizes electron donors other than organic carbon has also been documented (Van Cleemput and Samater, 1995) although this is not a significant source of N_2O in coastal sediment.

Importantly, the supply of dissolved N_2 and/or O_2 strongly regulates N_2O during denitrification and may give rise to substantial N_2O consumption. For example, in pristine mangrove sediments with low ambient nitrogen Maher et al. (2016) found N_2O uptake rates $> 1 \mu\text{mol m}^{-2} \text{d}^{-1}$. In the complete absence of O_2 , N_2O can be enzymatically reduced to gaseous N_2 by nitrous oxide reductase (Wrage et al. 2001). We found a large N_2O sink in the intensely anoxic middle reaches of a highly polluted sub-tropical estuary during summer low freshwater flow, when dissolved N_2O declined to below the atmospheric equilibrium value ($\sim 8 \text{ nM}$). Supersaturation was observed only further upstream and downstream where dissolved O_2 was detectable (Rajkumar et al. 2008).

Despite its clearly important role in N_2O cycling, dissolved O_2 was not widely measured in previous experimental studies, which may have partly compromised their conclusions. Work in agricultural soils for example, adopted the fraction of total pore space occupied by water (WFPS) as an O_2 proxy; denitrification was assumed to dominate N_2O production for $\text{WFPS} > 0.7$ and nitrification was assumed to dominate at $\text{WFPS} < 0.7$ (Dobbie et al. 1999). Early experiments designed specifically to isolate the heterotrophic denitrification contribution to N_2O production frequently failed to ensure complete anoxia at the start and routinely used sealed chambers in which the sediment redox status was susceptible to rapid changes (e.g. Seitzinger et al. 1984). Resulting estimates of sediment O_2 demand or total O_2 uptake (TOU) (e.g. Glud, 2008) may thus be erroneous and this may be amplified in estuarine sediments where higher TOU rates than are found in coastal or open ocean sediments reflect large organic fluxes and periodic sediment resuspension. TOU

estimates for estuarine sediments span more than an order of magnitude; from $\sim 1 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Nedwell and Trimmer, 1996) to up to $14 \text{ g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ (Hopkinson et al. 1999). How well this range approximates TOU variability *in situ* can be difficult to assess given the sealed nature of the experiments. Relating declining O_2 concentrations to N_2O production and/or consumption can therefore be problematic when redox conditions change due to sediment O_2 consumption. Clearly, an experimental design ensuring continuous replenishment of O_2 in a closed headspace to offset O_2 losses by sediment consumption and thereby mimicking the O_2 status of the sediment exposed to atmospheric air *in situ*, would be an important advance.

Studies that have avoided using sealed chambers tend to contradict the conclusions of sealed chamber incubations that ascribed N_2O production either solely or principally to HD. In freshwater lake sediments for example, no evidence was found for HD-mediated N_2O release under complete sediment anoxia and it was instead concluded that dissolved O_2 in the overlying water regulated sediment N_2O emission (Liikanen and Martikainen 2003). Supporting this, Jia et al. (2013) attributed N_2O production at very low O_2 to ND and Zhu et al. (2013) found that with decreasing O_2 availability N_2O production via NH_4^+ oxidation increased, consistent with results found earlier in pure culture (Goreau et al. 1980).

Given the recognition that denitrification can be a net N_2O sink in lake and marine sediments, in oceanic O_2 minima and in estuaries (LaMontagne et al. 2003; Liikanen and Martikainen 2003; Rajkumar et al., 2008; Vieillard and Fulweiler 2014) we examined experimentally, the N_2O response of an estuarine sediment to varying dissolved O_2 under controlled conditions. We did this using a novel custom-designed, closed laboratory sediment flux chamber in which we maintained headspace O_2 at pre-selected partial pressures and which we describe in some detail. By manipulating “in-situ” O_2 in this manner we evaluated the role played by dissolved O_2 during N_2O production by an estuarine sediment.

2. Materials and Methods

2.1. Study site

Sampling took place in the Tyne estuary, UK (Figure 1), which receives an annual freshwater discharge $\sim 7.8 \times 10^8 \text{ m}^3$ from a catchment area $\sim 2.5 \times 10^3 \text{ km}^2$. The Tyne is a mesotidal, partially mixed estuary that experiences periodic strong stratification and has mean springs and neaps tidal ranges of 4.3 m and 2.1 m respectively. A weak turbidity maximum zone (TMZ) occurs in the middle to upper estuary during summer (Upstill-Goddard et al. 2000); this has typical suspended particulate matter (SPM) concentrations $\sim 0.3\text{--}0.6 \text{ g l}^{-1}$ and low dissolved O_2 (Barnes and Upstill-Goddard 2011). In the River Tyne dissolved inorganic nitrogen (DIN: $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) is dominated by NO_3^- (50–80 μM). River Tyne NH_4^+ is generally low (1–2 μM) but in the TMZ $\sim 30\text{--}60 \mu\text{M}$ is typical (Barnes and Upstill-Goddard 2011) and 30–270 μM has been observed in the middle to lower estuary (salinity 20–30) due to the discharge of untreated and partially-treated sewage (Spencer et al. 2007). Summer nitrification converts the NH_4^+ to NO_3^- and leads to N_2O super-saturation and a small NO_3^- maximum in the TMZ, although overall NO_3^- remains quasi-conservative throughout the estuary (Barnes and Upstill-Goddard 2011).

2.2. Sample collection

Sediments for the incubation experiments were collected from an intertidal site near the Tyne estuary TMZ (Newburn: $54^\circ 58.8' \text{ N}$, $1^\circ 44.7' \text{ W}$, Figure 1). In these freshwater-dominated upper reaches of the Tyne estuary sediments have relatively homogeneous compositions and grain size distributions (Barnes, 2003). Sampling dates are listed in Table 1. “Blocks” of sediment $\sim 40 \times 20 \text{ cm} \times 5\text{--}10 \text{ cm}$ deep were removed using a spade and immediately transferred on-site to a gas-tight, $0.48 \text{ m} \times 0.70 \text{ m} \times 0.50 \text{ m}$ (h x l x w) incubation chamber, via a removable hatch (Figure 2). 8–10 “Blocks” of sediment were

collected from an area of about 50 metres squared. The chamber, constructed from robust 12 mm acrylic (Bay Plastics Ltd, UK) and equipped with integral headspace and water sampling ports, was commissioned specifically for this study. Sediment was added to a depth ~ 5-10cm avoiding disturbance and taking care to avoid any sediment fouling of the water sampling ports. The hatch was then sealed. Overlying estuarine water (~15 litres) was collected in a clean carboy and a portable probe (WTW Multi 350i, Germany) was used to record its salinity and temperature. Typical precisions were ± 0.2 salinity and $\pm 0.1^\circ\text{C}$. Subsamples (0.1 L) were filtered on site (Whatman 0.4 μm) and stored on ice in clean glass bottles for subsequent DIN analysis.

2.3 Experimental system

In the laboratory, the chamber hatch was removed and 10 litres of the estuarine water were carefully decanted onto the sediment surface to fully cover it to a depth ~1- 3 cm, taking care to ensure minimal disturbance of the sediment-water interface. Sediments were typically 100% inundated with estuarine water representing a high tide state. The chamber was then re-sealed to give an internal headspace volume $\sim 0.14 \text{ m}^3$, which was calculated via a graduated scale on the tank wall. This resulted in a headspace to sediment/water ratio ~9:1. The tank headspace was connected in a continuous loop to a gas chromatograph (GC: Shimadzu GC-14B) configured for the analysis of N_2O (Upstill-Goddard et al., 1996), and a single set-point O_2 monitor-controller equipped with a Clarke-type (Pt) O_2 sensor (Pro-Ox 110 and E703 sensor; BioSpherix Ltd, USA). This facilitated the pre-selection and control of headspace O_2 partial pressure via connections to cylinders of high purity compressed O_2 and N_2 (Figure 2). The E703 continuously monitors O_2 in the range 0-0.21 atm. to $\pm 0.1\%$ accuracy. A 2m long stainless-steel capillary line vented the chamber to air to maintain its internal pressure at ambient whilst precluding any measureable headspace loss during

subsequent experiments. In initial tests of system integrity the loss of a N₂O enriched headspace (30 ppmv) was <1 % over 14 days.

The chamber, GC and O₂ monitor-controller thus formed a sealed circuit around which the headspace was continuously circulated via a small diaphragm pump, through a 1 ml GC sample loop that was automatically switched onto the GC detector line as required, via a motor-driven, stainless steel, chromatography valve (Valco: Vici AG, Switzerland). All system tubing (1.75 mm i.d.) and chamber connections were of stainless steel. The filled tank and ancillaries thus constituted a mesocosm experiment that could be used to both reproduce and modify, in situ estuarine conditions.

2.1. Incubation experiments

We carried out seven sediment incubation experiments on individual sediments, all at a laboratory temperature of $22 \pm 0.5^\circ\text{C}$ (Table 1). Three were at regulated headspace O₂ partial pressures of 0.00 % (anoxic), 1.20 % (sub-oxic) and 20.85 % (ambient). For the anoxic experiment, the chamber headspace was pre-purged with O₂-free N₂ at 5 l min⁻¹ for ~ 3 hours. We additionally incubated sediment without O₂ regulation to reproduce conditions in traditional sealed incubations (O₂-unregulated experiment). In two further experiments we examined the potential regulation of sediment N₂O by NH₄⁺ or NO₃⁻ under a 20.85 % O₂ (ambient) headspace, adjusting the overlying water to 1mM N by adding solutions of dissolved NH₄⁺ (as NH₄Cl) or NO₃⁻ (as NaNO₃). We sampled from these experiments regularly over the first ~50 hours but more infrequently thereafter. We also ran an accompanying control experiment at 20.85 % O₂ without N addition.

2.2. Sampling and analysis

At intervals during the experiments ~50 ml of the overlying estuarine water was removed via a chamber side port for the analysis of DIN components (NO_3^- , NO_2^- , NH_4^+), into 100 ml gas tight glass syringes (SAMCO; Camlab.co.uk) fitted with three-way polycarbonate stopcocks. Sub-samples were analysed for DIN using pre-calibrated ion selective electrodes (NICO200 Ltd: ELIT 8021 for NO_3^- ; ELIT 8071 for NO_2^- ; ELIT 8051 for NH_4^+). Electrode pre-calibration used a series of known concentration standards prepared by serial dilution from 0.055M standard solutions. Limits of detection were all 1 μM and analytical precisions were $\pm 0.5 \mu\text{M}$.

GC analysis of N_2O in tank headspace involved isothermal separation (60°C) on a 1.75 mm id. 80-100 mesh Porapak Q column (5m), with additional in-line $\text{Mg}(\text{ClO}_4)_2$ and NaOH columns for the removal of H_2O vapour and CO_2 . Carrier gas (flow rate $\sim 25 \text{ cm}^3 \text{ min}^{-1}$) was ultra-high purity (UHP) N_2 . N_2O analysis used an Electron Capture Detector (ECD) with a ^{63}Ni source at 350°C . Calibration used a mixed secondary standard prepared by pressure dilution in UHP N_2 (Upstill-Goddard et al. 1996) and pre-calibrated against a commercially sourced mixed standard certified to an accuracy of $\pm 1\%$ (Air Products). Analytical precision for N_2O was $\pm 0.8\%$ based on the repeat analysis of the standard.

From the temporal trends in tank headspace partial pressures vs time we estimated N_2O emissions in all experiments and O_2 consumption in the O_2 -unregulated experiment, by applying linear fits to selected time intervals in each case (Table 1).

3. Results

3.1 Sediment nutrient fluxes

Nutrient fluxes, determined by monitoring temporal changes in concentrations with time in the overlying water, were always dominated by NH_4^+ (>95% of total observed DIN; data not shown); values were $\sim 20 \mu\text{mol m}^{-2} \text{ d}^{-1}$ at 20.85% O_2 (ambient), $\sim 52 \mu\text{mol m}^{-2} \text{ d}^{-1}$ at

1.2% O₂ (suboxic) and ~71 $\mu\text{mol m}^{-2} \text{d}^{-1}$ at 0.0% O₂ (anoxic). By contrast NO₃⁻ and NO₂⁻ concentrations were comparatively low (NO₂⁻ < 1 μM ; NO₃⁻ < 5 μM) and showed little or no observable change in the overlying water. We therefore conclude that any consumption of overlying water NO₃⁻ and/or NO₂⁻ by sediment HD and /or dissimilatory processes was offset by their production from nitrification, which could also fuel denitrification via NCD. An alternative possibility is that the rate of denitrification was too low to establish a measureable NO₃⁻ concentration gradient that would enable NO₃⁻ to diffuse across the sediment-water interface. In previous work we found that Humber estuary (UK) sediments consumed only ~2% of water column dissolved NO₃⁻ despite its concentration being >100 μM (Barnes and Owens 1999). This estimate, which was based on measured experimental sediment denitrification rates and nutrient fluxes, was subsequently confirmed via a mass balance (Jickells et al. 2000). In the light of these findings it is perhaps not surprising that Tyne sediment with a relatively low concentration of overlying water NO₃⁻ (< 5 μM) are evidently not a major NO₃⁻ sink.

3.2 Time dependent behaviour of N₂O and O₂, and emission and uptake fluxes

Figure 3 shows the time dependencies of headspace N₂O and O₂ partial pressures in the O₂-unregulated experiment in which headspace O₂ was monitored but not regulated. During the initial ~48 hours of the experiment N₂O and O₂ partial pressures changed only modestly. Following this initial “lag-phase”, O₂ partial pressure progressively decreased quasi-linearly with time to 2.1% after ~360 hours, mirrored by an increase in N₂O to ~7800 natm. N₂O and O₂ were highly linearly correlated during this period ($r^2 = 0.94$; $n=12$). N₂O then increased more dramatically, to ~12000 natm after 410 hours, accompanied by a slightly lowered rate of O₂ decline, to 1.1% after 410 hours. Over the following 24 hours N₂O decreased sharply to ~4600 natm, by which point O₂ was no longer detectable, and it continued to decline linearly

but more slowly, to ~3000 natm at the end of the experiment (491 hours). These time dependent changes in N₂O and O₂ partial pressures and their trends are directly reflected in the rates of emission/consumption estimated for these time intervals as shown in Table 1. Optimal headspace O₂ partial pressures for maximum intertidal sediment N₂O production were from 2.1% to 1.1% (~ 32.3 nmol N₂O m⁻² d⁻¹). This was always more than an order of magnitude higher than sediment N₂O production at all higher headspace O₂ partial pressures. However, for a headspace O₂ below 1% and at full anoxia, tank headspace N₂O fluxes were lower and the sediment/water consumed headspace N₂O. Evidently, Tyne estuarine sediments function as a strong N₂O source at all sub-ambient O₂ partial pressures down to ~1% but as a potential headspace N₂O sink under conditions of increasing anoxia, the N₂O consumption presumably being a consequence of HD.

In the O₂ regulated experiments (Figure 4) the largest changes in headspace N₂O partial pressure were under sub-oxic headspace (1.2% O₂) conditions. Based on Henry's law at laboratory temperature this corresponds to an overlying water O₂ concentration of 0.1 mg l⁻¹ (4.6 μM). Corresponding sediment N₂O emission was an order of magnitude higher than under either anoxic or ambient O₂ (20.845%) conditions (Table 1) and is almost identical to the rate derived for the O₂-unregulated experiment for the period 362-412 hours. During this period we observed the highest rate of increase in headspace N₂O, when headspace O₂ was within the sub-oxic range, decreasing from 2.1% to 1.1% (Figure 3, Table 1). The relatively small difference in N₂O emission between ambient and anoxic conditions (Table 1) clearly indicates that rates of sediment N₂O production under ambient conditions increase only modestly under full anoxia as compared to the suboxic situation. Even so, we contend that the small difference we observed between ambient and anoxic conditions may at least in part reflect our experimental procedure. Our reasoning is that even though for the anoxic experiment we pre-flushed the flux chamber headspace with N₂O-free N₂, the possibility of

some residual N₂O in the sediment pore water cannot be excluded and this could have resulted in at least part of the measured N₂O emission. Notwithstanding this potential artefact, our data inescapably emphasise the much greater importance of sub-oxic headspace O₂ partial pressures (~1%) than conditions of full anoxia for maximal N₂O production by an estuarine sediment.

Figure 5 shows changes in tank headspace N₂O for overlying estuarine bottom water both un-amended and adjusted to a concentration of either 1 mM NH₄⁺ or 1 mM NO₃⁻. Following NO₃⁻ amendment headspace N₂O increased linearly but only modestly over the first ~30 hours and thereafter showed little change to 50 hours (Figure 5a), as reflected in a relatively low N₂O emission rate (Table 1). Later in the experiment (140-190 hours) N₂O again increased linearly but the corresponding emission was low. Overall the NO₃⁻ amended sediment/water to headspace N₂O fluxes were not measurably higher than those in the un-amended (control) experiment (Table 1). In the NH₄⁺ amended experiment N₂O initially increased in a broadly similar fashion with corresponding emission rates somewhat higher than for NO₃⁻ addition over the first 30-50 hours (Table 1). Headspace N₂O then declined from 50-70 hours in the un-amended experiment. In contrast, in the case of NH₄⁺ amendment N₂O increased with time and this was particularly evident ($r^2=0.998$) during the period 257-306 hours, for which the emission rate was an order of magnitude higher than for the sub-oxic experiment (Figure 5b, Table 1). Presumably, prior to this linear relationship the curvature indicates the adaption of sediment nitrifiers (population structure) to the increased NH₄⁺ concentration in the incubation which is typically 2 orders of magnitude higher than in the estuarine water column (Barnes 2003). The implication of these results is that N₂O emissions from generic upper Tyne estuary sediments are stimulated by bottom water DIN enrichment. While it appears that NH₄⁺ enrichment has a substantially greater effect than NO₃⁻ enrichment, on observed sediment/water to headspace N₂O fluxes we should add a note

of caution. Given the delayed onset of maximum N₂O emissions, which presumably reflects the response time of sediment pore waters via diffusion, and the mismatch in experimental timescales (Table 1), large increases in headspace N₂O between 190 and ~300 hours in the NO₃⁻ amended experiment, had we monitored them, cannot be discounted. Nevertheless, we note that the overall effect of NO₃⁻ enrichment on N₂O emissions was not measurably different from the un-amended case over the duration of the measurements (Table 1).

4 Discussion

Our experimental results (Figures 3 and 4) demonstrate that sediment N₂O emissions are closely regulated by headspace O₂ partial pressures. However, the time-dependent relationships we observed were non-linear and non-intuitive. Sub-oxic headspaces (~1-2% O₂) led to about an order of magnitude increase in sediment/water to headspace N₂O fluxes compared with anoxic headspaces or those maintained at higher oxygen partial pressures, including those comparable to atmospheric air. Anoxic sediments in an anoxic atmosphere consumed enriched headspace N₂O by HD. Observed increases in headspace N₂O after addition of 1mM NO₃⁻ to the overlying water were smaller than those for 1mM NH₄⁺ amendment. This implied that processes other than HD, occurring under suboxic conditions, for which we found the highest N₂O emissions (Figure 3), are mainly responsible for sediment N₂O production (e.g. Liikanen and Martikainen, 2003). Dundee and Hopkins (2001) showed that the chemoautotrophic nitrifying bacterium *Nitrosomonas europaea*, which has been identified as a major component of the ammonium oxidising bacteria (AOB) in European estuaries (de Bie et al. 2002), was capable of N₂O production across a wide range of O₂ concentrations and that rates were comparatively high under conditions of low O₂. These findings are consistent with *N. europaea* being capable of both ND, as previously

indicted by Poth and Focht (1985), and N₂O production during NH₄⁺ oxidation. Enhanced N₂O production has also been observed under sub-oxic conditions in lakes, coincident with N₂O under-saturation in anoxic waters (Mengis et al. 1997), implying N₂O consumption by HD. A similar sensitivity of N₂O production to O₂ was also identified for terrestrial soils, in which the cessation of HD under 100% O₂ stimulated N₂O production (Robertson, 1987).

The results of our nitrogen addition experiments (Figure 5) clearly demonstrate a substantial capacity for the bacterial populations in Tyne sediments to rapidly turn over nitrogen in the form of NH₄⁺ but a much lower such capacity in the case of NO₃⁻. Consequently, the speciation of nitrogen inputs, which in the Tyne are dominated by NH₄⁺ (Barnes and Upstill-Goddard, 2011), must play a substantial role in sediment nitrogen cycling and in the sediment N₂O budget of the estuary. This conclusion is broadly supported by the results of previous work that was carried out in mangrove sediments (Muñoz-Hincapié et al., 2002). These authors found greatly enhanced N₂O emissions from mangrove sediment slurries following both NH₄⁺ and NO₃⁻ addition. Relative to un-amended controls, enhancements were up to ~2800-fold following NH₄⁺ addition and up to ~1800-fold following NO₃⁻ addition, with highest emissions in both cases observed during conditions of low tide with air exposure that favour nitrification, although the possibility of some additional N₂O production with additional NO₃⁻ addition was also recognised (Muñoz-Hincapié et al., 2002). These results prompted the conclusion that although N₂O emissions following NH₄⁺ addition may have resulted from both nitrification and denitrification, the former accounted for most of the observed N₂O emission (Muñoz-Hincapié et al., 2002).

Taking account of our experimental findings and the several other lines of evidence discussed above, we contend that sediment N₂O production dictated by NN, ND and NCD fuelled by NH₄⁺ is likely in intertidal sediments. Under anoxic conditions these sediments

could function as sinks for N₂O although this phenomenon has seldom been previously reported. However, there is recent strong evidence for ecosystem-scale sediment N₂O sinks both in coastal and open ocean settings (Vieillard and Fulweiler, 2014; Townsend-Small et al. 2014; Foster et al. 2016). Although some studies have explained the existence of a net sediment N₂O sink via HD as a result of low mineral nitrogen availability (Chapuis-Lardy et al. 2007; Conen and Neftel 2007), we previously found a strong a N₂O sink via HD in the anoxic sediments of a highly perturbed tropical estuary subject to large anthropogenic fluxes of DIN, mostly as NH₄⁺ (Rajkumar et al. 2008). The Tyne estuary also has high ambient DIN (Barnes and Upstill-Goddard, 2011) but although our results strongly imply N₂O removal under anoxic conditions (Figure 4), presumably also due to HD, Tyne sediments are an overall N₂O source. We propose that this is due to a close co-existence of NN, ND and NCD that generate N₂O fuelled by the high NH₄⁺ availability, alongside HD, and that this coexistence likely reflects strong gradients in dissolved O₂, not only vertically but also laterally due to small-scale sediment heterogeneity.

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Figure captions

Figure 1. Location of the Tyne Estuary, UK, and the sediment sampling site at Newburn.

Figure 2: Schematic of the sediment incubation tank used to estimate sediment N₂O emissions at pre-selected headspace partial pressures of O₂.

Figure 3. Partial pressures of N₂O (black circles) and O₂ (grey circles) in sediment flux chamber headspace during a 490 hour “control” experiment in which headspace O₂ was not regulated (Pro-Ox 110/ E703 O₂ regulator-sensor disconnected). The headspace to sediment volume ratio was approximately 9:1.

Figure 4. Time-dependent behaviour of headspace N₂O during short-term (~24 hour) incubations of Tyne sediment under pre-selected headspace O₂ partial pressures: anoxic (0% O₂); sub-oxic (1.2% O₂); ambient (20.85 % O₂). The headspace to sediment volume ratio was approximately 9:1. Note the differing scales on the N₂O axes.

Figure 5. Partial pressures of N₂O in a sediment flux chamber headspace of 20.8 % O₂ partial pressure during incubations. a) data for the first ~50 hours: □, control experiment; ●, estuarine bottom water augmented with NH₄Cl to 1 mmol N l⁻¹; ●, estuarine bottom water augmented with NaNO₃ to 1 mmol N l⁻¹. (b) Results for NH₄Cl amendment (1 mmol N l⁻¹) for the period ~260-310 hours. Note the differing scales and that technical issues precluded any analyses during the time period ~50-260 hours.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1. Details of incubation tank experiments carried out, and corresponding rates of N₂O emission and O₂ consumption. Negative values of N₂O emissions indicate its consumption. For all experiments the laboratory temperature was 22 ± 0.5 °C.

Experiment	Salinity	Temperature in situ (°C)	Duration (hours)	Sediment collection date	Time interval (hours)	N ₂ O emission (nmol m ⁻² d ⁻¹)	O ₂ consumption mmol m ⁻² d ⁻¹
O ₂ unregulated	< 0.2	7.3	491	01.05.2013	0-46	0.4	5.3
					138-362	11.9	195
					362-412	32.9	44
					412-415		163
					412-438	-115	
					460-491	-9.9	
Anoxic: 0.00 % O ₂	< 0.2	7.3	23	22.11.2014	0-23	2.5	
Sub-oxic: 1.20 % O ₂	< 0.2	7.0	25	14.11.2013	0-25	32.3	
Ambient: 20.85 % O ₂	< 0.2	7.0	27	05.12.2013	0-27	2.1	
NH ₄ ⁺ addition to 1 mM N ₂	< 0.2	10.3	306	04.10.2013	0-32	2.0	
					40-48	6.5	
					257-306	134	
NO ₃ ⁻ addition to 1 mM N ₂	< 0.2	7.2	190	25.11.2014	0-50	0.4	
					140-190	2.2	
DIN un-amended (control)	< 0.2	7.3	177	22.11.2013	0-46	2.1	
					50-70	-3.0	
					70-177	0.3	