Isotopomer labeling and oxygen dependence of hybrid nitrous oxide production

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Abstract. Nitrous oxide (N2O) is a potent greenhouse gas and ozone depletion agent, with a significant natural source from marine oxygen-deficient zones (ODZs). Open questions remain, however, about the microbial processes responsible for this N2O production, especially hybrid N2O production when ammonia-oxidizing archaea are present. Using 15N-labeled tracer incubations, we measured the rates of N2O production from ammonium (NH4+), nitrite (NO−2), and nitrate (NO−3) in the eastern tropical North Pacific ODZ and the isotopic labeling of the central (α) and terminal (β) nitrogen (N) atoms of the N2O molecule. We observed production of both doubly and singly labeled N2O from each tracer, with the highest rates of labeled N2O production at the same depths as the near-surface N2O concentration maximum. At most stations and depths, the production of 45N2Oα and 45N2Oβ were statistically indistinguishable, but at a few depths there were significant differences in the labeling of the two nitrogen atoms in the N2O molecule. Implementing the rates of labeled N2O production in a time-dependent numerical model, we found that N2O production from NO−3 dominated at most stations and depths, with rates as high as 1600 ± 200 pM N2O d−1. Hybrid N2O production, one of the mechanisms by which ammonia-oxidizing archaea produce N2O, had rates as high as 230 ± 80 pM N2O d−1 that peaked in both the near-surface and deep N2O concentration maxima. Based on the equal production of 45N2Oα and 45N2Oβ in the majority of our experiments, we infer that hybrid N2O production likely has a consistent site preference, despite drawing from two distinct substrate pools. We also found that the rates and yields of hybrid N2O production were enhanced at low dissolved oxygen concentrations ([O2]), with hybrid N2O yields as high as 20% at depths where [O2] was below detection (880 nM) but nitrification was still active. Finally, we identified a few incubations with [O2] up to 20 µM where N2O production from NO−3 was still active. A relatively high O2 tolerance for N2O production via denitrification has implications for the feedbacks between marine deoxygenation and greenhouse gas cycling.

1 Introduction

Nitrous oxide (N2O) is one of the lesser-known greenhouse gases, yet its potential to warm the environment on a per-molecule basis is immense. N2O has a global warming potential 273 times that of carbon dioxide (Smith et al., 2021), and its atmospheric mixing ratio is increasing at a rate of 0.85 ± 0.03 ppb yr−1 (Tian et al., 2020). In the ocean, hotspots of N2O production and flux to the atmosphere occur in marine oxygen-deficient zones (ODZs), where steep redox gradients allow multiple N2O production processes to overlap (Codispoti and Christensen, 1985). ODZs have expanded over the last 60 years (Breitburg et al., 2018; Stramma et al., 2008) and will likely continue to do so as the oceans warm.
Much of the N₂O cycling in ODZs is linked to denitrification. In low-oxygen waters, denitrifying organisms produce N₂O as an intermediate during organic matter remineralization (Dalsgaard et al., 2014; Naqvi et al., 2000; Zunft, 1997). Both direct rate measurements (Frey et al., 2020; Ji et al., 2015, 2018) and natural abundance isotope measurements (Casciotti et al., 2018; Kelly et al., 2021; Monreal et al., 2022; Toyoda et al., 2023) indicate that N₂O production directly from nitrate (NO₃⁻), i.e., without exchange with extracellular nitrite (NO₂⁻) or nitric oxide (NO) pools, is the primary source of N₂O in ODZs. N₂O production from extracellular NO₂⁻, meanwhile, tends to occur at lower rates (Frey et al., 2020; Ji et al., 2015, 2018). Historically, N₂O production from denitrification was thought to cease at dissolved oxygen concentrations above 2–3 µM (Dalsgaard et al., 2014), but more recent data suggest that N₂O production from NO₃⁻ can occur at ambient oxygen levels as high as 30 µM (Frey et al., 2020; Ji et al., 2018). N₂O consumption via denitrification is more sensitive to oxygen than N₂O production via denitrification, leading to an oxygen window in which denitrification is a source but not a sink of N₂O (Babin et al., 2015; Dalsgaard et al., 2014; Farías et al., 2009; Frey et al., 2020), although the oxygen inhibition constant for N₂O consumption remains difficult to define (Sun et al., 2021a). N₂O may also be consumed through N₂O fixation, although the importance of N₂O fixation in the ocean has yet to be determined (Farías et al., 2013; Si et al., 2023).

Nonetheless, a significant fraction of the N₂O in the oxygen clines above and below ODZs may be derived from archaeal nitrification. When NO₃⁻ is present, isotopic evidence continues to suggest that ammonia-oxidizing archaea can produce N₂O via a hybrid mechanism that combines nitrogen (N) derived from NO₂⁻ and ammonium (NH₄⁺) to form the N₂O molecule (Frame et al., 2017; Frey et al., 2020, 2023; Stieglmeier et al., 2014; Trimmer et al., 2016). New evidence indicates that ammonia-oxidizing archaea can produce N₂O both as a by-product of hydroxylamine oxidation and via hybrid N₂O production and that the ratio of these processes depends on the ratio of NH₄⁺ to NO₂⁻ available to the archaea (Wan et al., 2023b). The exact mechanism and enzymology of archaeal N₂O production remains unknown (Carini et al., 2018; Stein, 2019) but may involve a reaction between hydroxylamine and NO, which occur as intermediates during archaeal ammonia oxidation (Kozlowski et al., 2016; Lancaster et al., 2018; Martens-Habbema et al., 2015; Vajrala et al., 2013). In anaerobic conditions, ammonia-oxidizing archaea are also capable of NO dismutation to O₂ and N₂, which may involve N₂O as an intermediate (Kraft et al., 2022). Ammonia-oxidizing bacteria, more common in regions that are nutrient-replete, produce N₂O as a byproduct of hydroxylamine oxidation (Cohen and Gordon, 1979) and via nitrifier denitrification as oxygen concentrations decline (Goreau et al., 1980; Stein and Yung, 2003; Wrage et al., 2001) and nitrite concentrations rise (Frame and Casciotti, 2010).

The stable natural abundance nitrogen and oxygen isotopes of N₂O can provide quantification of – and distinction among – potential N₂O cycling mechanisms (Kim and Craig, 1990; Rahn and Wahlen, 2000; Toyoda and Yoshida, 1999). For example, natural abundance N₂O isotopocule studies have indicated that the high near-surface N₂O accumulations in the eastern tropical North Pacific (ETNP) ODZ are 80% derived from denitrification and 20% derived from nitrification (Kelly et al., 2021). The isotopic content of the individual N and oxygen (O) atoms in the N₂O molecule are expressed in delta notation, defined as δ(15N) or δ(18O) = (Rsample / Rstandard − 1), where Rstandard for δ(15N) and Rstandard for δ(18O) are the ratios 15N / 14N of air and 18O / 16O of Vienna Standard Mean Ocean Water (VSMOW), respectively (Kim and Craig, 1990; Rahn and Wahlen, 2000; Toyoda and Yoshida, 1999). In addition to the bulk N and O isotope ratios in N₂O, we can measure the isotopic content of the inner (α) N atom and the outer (β) N atom in N₂O (Brenninkmeijer and Röckmann, 1999; Toyoda and Yoshida, 1999). The difference in the 15N content of these two atoms is often referred to as the “site preference” and is defined as δ(15N)sp = δ(15Nα) − δ(15Nβ). In natural abundance studies, δ(15N)sp is particularly useful because it exhibits distinct values for different N₂O production processes, which are independent of the isotopic value of the substrate (Frame and Casciotti, 2010; Sutka et al., 2003, 2004, 2006; Toyoda et al., 2002, 2005). This allows partitioning between different N₂O sources and has been used extensively to quantify N₂O cycling in the ocean (Bourbonnais et al., 2017, 2023; Casciotti et al., 2018; Farías et al., 2009; Kelly et al., 2021; Monreal et al., 2022; Popp et al., 2002; Toyoda et al., 2002, 2005, 2019, 2021, 2023; Westley et al., 2006; Yamagishi et al., 2007). As we elaborate upon in the discussion, however, the premise that δ(15N)sp exhibits a unique and consistent value depends on the assumption that both N atoms in N₂O are derived from a singular substrate pool. Thus, hybrid N₂O production may complicate traditional interpretations of natural abundance N₂O isotopocules.

Previous studies have used 15N tracer experiments to measure N₂O production rates in ODZs (Frey et al., 2020, 2023; Ji et al., 2015, 2018). These studies used the accumulation of 45N₂O and 46N₂O resulting from the addition of 15N-labeled substrates such as 15N–NH₄⁺ and 15N–NO₂⁻ to measure N₂O production rates. To our knowledge, the isotopomer measurement has never been applied to 15N tracer experiments to track 15N from different substrates into the α and β positions of the N₂O molecule. Here, we present data showing the production of N₂O isotopomers with 15N in the α position (45N₂Oα) and 15N in the β position (45N₂Oβ) from
were overflowed in a secondary container filled with sub-


to minimize bubble-free, with no headspace, using gray butyl rubber septa

bles (WHEATON) using Tygon tubing. Incubation bottles
directly from Niskin bottles into 160 mL glass serum bot-

ble ODZ, secondary chlorophyll deep oxyclines, oxic–anoxic interfaces above and below the

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2.2 Sample collection

Laboratory as part of a prior study (Kelly et al., 2021).

5.00 µM and with

amended with Na

amended with

three tracers. For each station and depth, nine samples were

were produced at each experimental depth, comprised

of triplicate samples for each of the three time points and

were thus produced at each experimental depth, comprised

were overflowed in a secondary container filled with sub-

oxic water from the same depth, and Niskin bottles were

vented with carbon dioxide gas to displace the withdrawn

water. The butyl rubber stoppers were deoxygenated in a He-

flushed anaerobic chamber for ~1 week prior to sampling.

After sample collection, a 2 mL He headspace was created in each bottle by displacing the 2 mL sample from the bottle with He. At most (all but two) anoxic depths at stations PS2 and PS3, samples were sparged with He gas for 90 min at a flow rate of at least 100 mL min−1, equivalent to 56 volume exchanges, to remove potential oxygen contamination introduced during sampling. Depths with low but non-zero ambient dissolved oxygen were not sparged with He gas. After sparging, 100 µL of 1030 ppm N2O in He (4 nmol N2O) in gaseous form was introduced back into each bottle for a final concentration of 26 nM to provide a constant background of N2O for later isotopic analysis (Fig. S4a). The isotopic content of this N2O carrier, measured independently via IRMS (Kelly et al., 2023; McIlvin and Casciotti, 2010), was δ(15N) = −1.5 ± 0.2 ‰, δ(13N) = 0.2 ± 0.4 ‰, δ(15Nbulk) = −0.65 ± 0.08 ‰, and δ(18O) = 37.4 ± 0.3 ‰.

Time series were constructed by sacrificing triplicate bottles over a time course, rather than by resampling the incubation bottles over time. A total of 27 incubation samples were thus produced at each experimental depth, comprised of triplicate samples for each of the three time points and three tracers. For each station and depth, nine samples were amended with 15NH4Cl (98.8 atm % 15N; Sigma-Aldrich) to a final concentration of 0.501 µM and with Na14NO2 to a final concentration 1.01 µM. Nine samples were amended with Na15NO2 (98.8 atm % 15N; Sigma-Aldrich) to a final concentration of 5.00 µM and with 14NH4Cl to a final concentra-

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tion of 0.510 µM. Finally, nine samples were amended with K\(^{15}\)NO\(_3\) (98.8 atm % \(^{15}\)N; Sigma-Aldrich) to a final concentration of 1.00 µM, plus 1.01 µM Na\(^{14}\)NO\(_2\) and 0.510 µM \(^{14}\)NH\(_4\)Cl. Note that the Na\(^{15}\)NO\(_3\) tracer was added at a higher concentration than the other tracers or the Na\(^{14}\)NO\(_2\) carrier; this discrepancy was due to a miscalculation that was caught midway through the cruise, but the high tracer addition was retained for the sake of consistency. The NO\(_2\) and NH\(_4\)\(^+\) tracer and carrier additions were confirmed via [NO\(_2\)] and [NH\(_4\)\(^+\)] measurements of sample aliquoted from each bottle immediately before samples were measured for N\(_2\)O isotopic content, using colorimetric and fluorometric techniques (Grasshoff et al., 1999; Holmes et al., 1999). The Na\(^{14}\)NO\(_2\) and \(^{14}\)NH\(_4\)Cl amendments served two purposes: (1) to provide enough total NO\(_3\) for isotopic analysis of \(^{15}\)NO\(_2\) produced from \(^{15}\)NH\(_4\)\(^+\) and (2) to minimize isotope dilution of the substrate pool, which can cause underestimation of rates with low substrate additions. The final atm % \(^{15}\)N of the substrate pools was thus 56%–100% for \(^{15}\)N–NH\(_4\)\(^+\), 65%–100% for \(^{15}\)N–NO\(_2\), and 2%–92% for \(^{15}\)N–NO\(_3\) experiments. Three samples for each tracer were terminated immediately after tracer addition with the addition of 100 µL saturated mercuric chloride (HgCl\(_2\)) solution. These also served as abiotic controls. The remaining samples were incubated at 12 °C in the dark; three samples per tracer were terminated at 12 h and at 24 h with 100 µL saturated HgCl\(_2\). All samples were incubated at 12 °C, which was chosen as an intermediate temperature that approximated subsurface conditions. After termination, samples were stored at room temperature (~20 °C) in the dark until isotope analysis.

2.3 Chemiluminescent optode oxygen measurements

Eight 160 mL glass serum bottles were prepared with a chemiluminescent oxygen optode spot (PyroScience) affixed to the inner glass wall with silicone glue. These bottles were incubated alongside experimental bottles to monitor dissolved [O\(_2\)] during incubations. At stations PS2 and PS3, two optode bottles per depth were filled, purged, amended with the N\(_2\)O carrier, and incubated without the addition of tracer or HgCl\(_2\). At each time point, [O\(_2\)] was measured in each sensor bottle for at least 10 min using fiber-optic cables paired to the oxygen optode spot mounted inside the bottle (PyroScience). The fiber-optic cables were calibrated with a two-point measurement of (1) a sodium sulfite solution (30 g L\(^{-1}\) in DI, or 0.24 M) and (2) surface seawater saturated with air at 12 °C (270 µM [O\(_2\)], based on a salinity of 35 psu and a temperature of 12 °C) (Garcia and Gordon, 1992). The two calibration bottles, each containing its own optode spot, were used to calibrate all four of the fiber-optic cables, effectively correcting them to the same scale. Differences in detection limit between sensor spots were accounted for by first performing this two-point calibration procedure to correct for differences between fiber-optic cables and then by measuring the minimum oxygen concentration measured by each sensor spot in purged seawater (purged at 100 mL min\(^{-1}\). for 90 min, equal to 56 volume exchanges). Those detection limits were specific to each optode spot and varied from 146–880 nM [O\(_2\)].

The optode [O\(_2\)] measurements were adjusted for the detection limit specific to each sensor spot; optode [O\(_2\)] for each experiment was calculated as the mean measured [O\(_2\)] at each of the three time points. No optode measurements were made at station PS1, since this station lacked a secondary NO\(_3\) maximum; thus incubations performed at low-oxygen depths were not expected to occur under functional anoxia. Optical oxygen sensors are susceptible to interference from NO, which could result in an overestimate of [O\(_2\)] in experiments with especially high rates of NO production (Kraft et al., 2022). Given maximum ammonia oxidation rates of 4.68 ± 0.07 nM N d\(^{-1}\), the release of equivalent amounts of NO would result in an [O\(_2\)] overestimate of 0.745 nM during a 24 h incubation, based on the interference curve calculated by Kraft et al. (2022) ([O\(_2\)] overestimate = 0.159 × [NO]). Because of this small potential error and the lack of relevant NO measurements, no correction was applied for NO interference.

Optode [O\(_2\)] generally agreed with ambient [O\(_2\)] measured by the Sea-Bird oxygen sensor attached to the rosette (Fig. S1). Two important exceptions were in the experiments at the base of the ODZ and the deep ODZ core at station PS2, which were not purged before tracer addition. As a result, the ambient [O\(_2\)] at these depths was below detection on the Sea-Bird sensor, but the optode [O\(_2\)] measurements in the incubation bottles from these depths were 17.7 ± 0.1 and 19.2 ± 0.8 µM, respectively (Fig. S1, Table S1). Additionally, two depths that were suboxic (and thus not sparged prior to tracer addition) had higher optode [O\(_2\)] than ambient [O\(_2\)]; in the deep oxycline at station PS2, ambient [O\(_2\)] was 6.8 µM and optode [O\(_2\)] was 14.8 ± 0.2 µM; at the oxic–anoxic interface at station PS2, ambient [O\(_2\)] was 6.5 µM and optode [O\(_2\)] was 9.48 ± 0.09 µM (Fig. S1, Table S1). Because of these few exceptions, we always report both optode and ambient [O\(_2\)] in the following figures and text.

2.4 Nitrous oxide isotopocule measurements

Two steps were taken to prepare incubation samples for N\(_2\)O isotopocule analysis immediately prior to measurement. Firstly, a 5 mL aliquot was removed from each sample by syringe and replaced with He gas. These aliquots were refrigerated until analysis for [NO\(_2\)] and [NH\(_4\)\(^+\)] to check tracer and carrier additions, as mentioned above. After this aliquot was removed, 100 µL of \(^{14}\)NH\(_4\)Cl, Na\(^{14}\)NO\(_2\), or K\(^{14}\)NO\(_3\) carrier was added to each sample at final concentrations of 54, 262, or 27 µM, respectively, to bring \(^{15}\)N tracer levels below 5000 ‰. Note that these carrier additions were different from the \(^{14}\)N carrier added to each incubation alongside the \(^{15}\)N tracer; the purpose of the later carrier additions was to pre-
vent exposure of the IRMS system to highly $^{15}$N-enriched substrates.

Samples were measured for N$_2$O concentrations and $^{15}$N isotopocouples on a custom-built purge and trap system coupled to a Thermo Finnigan DELTA V Plus IRMS, which was run in continuous flow mode and configured to measure $m/z$ 30, 31, 44, 45, and 46 (McIlvin and Casciotti, 2010). These measurements were made under normal operating conditions, using an ionization energy of 124 eV, emission current of 1.50 mA, and accelerating voltage of 3 kV. Samples were analyzed alongside reference materials (B6, S2, and atmosphere-equilibrated seawater) to calibrate the IRMS for scrambling in the ion source with the pyisotopomer software package in Python (Kelly et al., 2023). The number ratios of isotopomers $^{14}$N$^{15}$NO and $^{15}$N$^{14}$NO were calculated as in Kelly et al. (2023), with the following modifications to account for the contribution of $^{15}$N$^{15}$NO to the molecular ion number ratios 46/44 ($^{46}$R) and 31/30 ($^{31}$R), which, while negligible at natural abundance, becomes important in tracer experiments.

In natural abundance samples, pyisotopomer solves the following four equations to obtain $^{15}$R$^\alpha$ and $^{15}$R$^\beta$:

$$45^R = 15^R + 15^R\beta + 17^R$$  
(1)

$$46^R = \left(15^R + 15^R\beta\right)17^R + 18^R + 15^R\alpha 15^R\beta$$  
(2)

$$17^R/17^R_{VSMOW} = \left(18^R/18^R_{VSMOW}\right)^\beta \left[D(17^O) + 1\right]$$  
(3)

$$31^R = \frac{1 + 15^R\alpha + 15^R\beta - \left(15^R - 17^R\right)}{1 + 15^R\alpha + 15^R\beta}$$  
(4)

where $45^R$, $46^R$, and $31^R$ are the molecular ion number ratios 45/44, 46/44, and 31/30, $^{15}$R$^\alpha$, $^{15}$R$^\beta$, $^{17}$R, and $^{18}$R denote the number ratios of $^{14}$N$^{15}$N$^{16}$O, $^{15}$N$^{14}$N$^{16}$O, $^{14}$N$^{15}$O, and $^{15}$N$^{14}$O, respectively, to $^{14}$N$^{16}$O. Here, $D(17^O)$ was assumed to be zero. In these equations, the term $(15^R\alpha)/\beta$ represents the statistically expected contribution of $^{15}$N$^{15}$N$^{16}$O to the $^{46}$R and $^{31}$R ion number ratios, based on the probabilities of forming $^{15}$N$^{15}$N$^{16}$O. The probability of getting $^{15}$N in N$^\alpha$ is given by $15^R\alpha$, and the probability of getting $^{15}$N in N$^\beta$ is given by $15^R\beta$; furthermore, the two probabilities are assumed to be independent, so the probability of getting $^{15}$N in both positions would be $(15^R\alpha)(15^R\beta)$ (Kaiser et al., 2004). Predicting the concentration of $^{15}$N$^{15}$N$^{16}$O from the distribution of $^{15}$N in the singly labeled molecules ($^{15}$R$^\alpha$ and $^{15}$R$^\beta$) is a reasonable assumption for natural abundance samples, where the concentration of $^{15}$N$^{15}$N$^{16}$O is extremely low (Kanterová et al., 2022; Magyar et al., 2016).

For $^{15}$N-labeled samples, however, we cannot predict $^{15}$N$^{15}$N$^{16}$O from the singly labeled molecules ($^{15}$R$^\alpha$ and $^{15}$R$^\beta$). This is because the relationship between the formation of $^{15}$N$^{15}$N$^{16}$O, $^{14}$N$^{15}$N$^{16}$O, and $^{15}$N$^{14}$N$^{16}$O depends on production mechanism and the atom fraction of the substrate. For example, in $^{15}$N$^{−}$N$^{−}$O$^{−}$ experiments with denitrification occurring, there may be far more $^{15}$N$^{15}$N$^{16}$O molecules produced than the amount predicted from the production of $^{14}$N$^{15}$N$^{16}$O and $^{15}$N$^{14}$N$^{16}$O. To account for this, we added a term to the equations for $^{46}$R and $^{31}$R to account for the potential of excess $^{15}$N$^{15}$N$^{16}$O production ($^{15}$N$^{15}$N$^{16}$O$^{\text{excess}}$) in tracer experiments:

$$46^R = \left(15^R\alpha + 15^R\beta\right)17^R + 18^R + 15^R\alpha 15^R\beta$$  
(5)

$$31^R = \frac{1 + 15^R\alpha + 15^R\beta - \left(15^R - 17^R\right)}{1 + 15^R\alpha + 15^R\beta}$$  
(6)

To quantify $^{15}$N$^{15}$N$^{16}$O$^{\text{excess}}$ in tracer samples, we assumed that any increase in $^{46}$R over the course of the experiment is due to added $^{15}$N$^{15}$N$^{16}$O, i.e., that $\delta^{15}(O)$ remains constant. This should be a reasonable assumption—while denitrification and N$_2$O consumption could cause natural-abundance-level increases in $\delta^{15}(O)$ and thus $^{46}$R (10s of per mil), N$_2$O production from $^{15}$N-labeled substrates are expected to cause much greater increases in $^{46}$R (100s to 1000s of per mil). We calculated the term $^{15}$N$^{15}$N$^{16}$O$^{\text{excess}}$ by subtracting the mean $^{46}$R at $t_0$ from the measured $^{46}$R at later time points using the pyisotopomer template designed for tracer experiments (Kelly, 2023). Then, we used the “Tracers” function in pyisotopomer, which takes this $^{15}$N$^{15}$N$^{16}$O$^{\text{excess}}$ into account, to calculate $^{15}$R$^\alpha$ and $^{15}$R$^\beta$.

The concentration of $^{44}$N$^{2}$O in each sample was calculated from $m/z$ 44 peak area and from a linear conversion factor divided by the sample volume (McIlvin and Casciotti, 2010). The concentrations of $^{45}$N$^{2}$O$^\alpha$, $^{45}$N$^{2}$O$^\beta$, and $^{46}$N$^{2}$O were finally calculated by multiplying $^{15}$R$^\alpha$, $^{15}$R$^\beta$, and $^{46}$R by the average [$^{15}$N$^{2}$O] across all time points for that tracer experiment. Average values of [$^{15}$N$^{2}$O] were used to avoid aliasing random variability in [$^{15}$N$^{2}$O] over increases in $^{15}$R$^\alpha$, $^{15}$R$^\beta$, and $^{46}$R. The analytical precisions for N$_2$O isotopocouple measurements, based on the pooled standard deviations of reference materials run alongside samples, were $\delta(15N^{2}O) = 4.4 \text{‰}$, $\delta(15N\beta) = 3.4 \text{‰}$, $\delta(15N^{bulk}) = 3.5 \text{‰}$, and $\delta^{18}(O) = 2.1 \text{‰}$. The analytical precision was poorer than that in a similar natural abundance dataset (Kelly et al., 2021) due to minor $^{15}$N carry-over in some of the standards analyzed immediately following highly enriched samples.

### 2.5 Nitrite and nitrate isotope measurements

After N$_2$O analysis, approximately 2 mL of sample remained in each bottle, which was prepared for the analysis of $\delta^{15}$N$^{−}$N$^{−}$O$^{−}$, $\delta(15N^{−}$N$^{−}$O$^{−})$, or $\delta(15N^{−}$N$^{−}$O$^{−}−$), to determine the rates of NH$_3$ oxidation, NO$_3^{−}$ oxidation, and NO$_3^{−}$ reduction, depending on the tracer experiment. Samples incubated with $^{15}$N–NH$_4^{+}$ were prepared for $\delta(15N^{−}$N$_2$O$^{+}$)
analysis using the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001), with updates from McIlvin and Casciotti (2011), to determine rates of NH3 oxidation. These samples were run on a Thermo Finnigan DELTAPLUS XP IRMS alongside a process blank and reference materials USGS32, USGS34, and USGS35 (Böhlke et al., 2003) to obtain δ(15N–NO3−) and δ(15N–NO2−).

Samples incubated with 15N–NO3− were first treated with 5% sulfamic acid (weight by volume, or 10 mM final concentration) to remove 15N–NO3− (Granger and Sigman, 2009) then prepared with the denitrifier method for δ(15N–NO3−) analysis (Casciotti et al., 2002; McIlvin and Casciotti, 2011; Sigman et al., 2001) to determine rates of NO3− oxidation. For these analyses, reference materials USGS32, USGS34, and USGS35 (Böhlke et al., 2003) were also treated with 5% sulfamic acid and prepared with the denitrifier method alongside incubations with low ambient [NO3−] that had high 16Oδ(15N) values (>1000‰; Fig. S2). This is likely because NO3− is produced when sulfamic acid is added to NO3− (Granger and Sigman, 2009), so the sulfamic treatment probably chemically converted some 15N–NO3− tracer to 15N–NO3−; additionally, 15N–NO3− is a possible contaminant of the 15N–NO3− tracer solutions. Regardless, this would have shifted all three time points equally and thus should not introduce a bias into the slope of δ(15N–NO3−) with time and the rates calculated from there.

Finally, samples incubated with 15N–NO3− were prepared for δ(15N–NO3−) isotopic analysis with the azide method (McIlvin and Altabet, 2005) to determine rates of NO3− reduction to NO2−. The 2 mL of remaining sample was transferred into 20 mL vials, where it was prepared alongside reference materials RSIL-N23, -N7373, and -N10219 (Casciotti et al., 2007). Reference materials were diluted from 200 mM working stocks into 3 mL NO3−-free seawater in 5 and 10 nmol quantities of NO3− to correct for the contribution of a consistent blank to a range of sample sizes. The analytical precisions for δ(15N–NO3−), δ(15N–NO2−), and δ(15N) were 0.9‰, 1.2‰, and 0.4‰, respectively. The δ(15N) analytical precision for the denitrifier and azide methods is typically better (McIlvin and Altabet, 2005; Sigman et al., 2001), but tracer measurements tend to have lower analytical precision than natural abundance measurements.

The rates of NH4+ and NO3− oxidation were calculated using a weighted least-squares linear regression through product 15N vs. incubation time (Fig. S3). Each sample was weighted by its uncertainty, which was calculated based on the slope and intercept of the calibration curve, blank peak area, and sample peak area (Appendix A). Although using this uncertainty calculation is complex, it allows the assessment of relative error and the inclusion of low-peak-area samples that had high enough δ(15N) enrichments such that the relative error remained below 10% (and in most cases 1%). A weighted least-squares regression was used in place of an ordinary least-squares regression to prevent samples with high uncertainties from biasing the slope estimate (e.g., two samples in Fig. S3b). Then, the rate was calculated by

$$m \left( \frac{15N}{d^{-1}} \right) = \frac{m \left( \frac{15F_{\text{product}}}{P} \right)}{15F_{\text{substrate}}}$$

where $m(15F_{\text{product}})$ is the slope of the atom fraction of 15N in the product vs. incubation time, $|P|$ is the mean product concentration (e.g., NO3− in an NO2− oxidation experiment), and $15F_{\text{substrate}}$ is the atom fraction of 15N in the substrate (e.g., NO3− in an NO2− oxidation experiment). Our method of estimating individual uncertainties was developed to deal with low NH3 oxidation rates, which generated low peak areas in δ(15N–NO3−) samples. Since the rates of NO3− reduction were generally much higher than the rates of NH3 oxidation (Table S2), a parallel method was not needed to estimate individual uncertainties in samples measured with the azide method, i.e., δ(15N–NO3−) measurements, so rates of NO3− reduction were with an ordinary least-squares regression in Eq. (7) instead of a weighted least-squares regression.

### 2.6 Modeling N2O production mechanisms

A time-dependent model was constructed to infer the rates and mechanisms of N2O production from the measured isotopologue time courses in each incubation experiment. While it is possible to calculate rates of hybrid and bacterial N2O production with linear regressions of 45N2O and 46N2O with time (Trimmer et al., 2016), these calculations cannot take into account 15N transfer between substrates and, more importantly, produce separate rate estimates for separate tracer experiments. They also do not leverage the additional information provided by N2O isotopomers. We sought to solve for a common set of N2O production rate constants across the three parallel tracer experiments at a given station and depth, wherein the only differences between each tracer experiment were the starting concentrations of 14N and 15N in NH4+, NO2−, and NO3− (Fig. 2). The model encoded four different N2O-producing pathways: (1) production solely from NH4+, which includes N2O from hydroxylamine oxidation (referred to as Pathway 1 in Wan et al., 2023b), hybrid production using cellular NO2− (referred to as Pathway 2 in Wan et al., 2023b), and nitrifier denitrification using cellular NO2−; (2) hybrid production using extracellular NO2− (referred to as Pathway 3 in Wan et al., 2023b); (3) production from NO2−, i.e., denitrification or nitrifier denitrification using extracellular NO2−; and (4) production from NO3−, i.e., denitrification using cellular NO3− (Fig. 2). Using this model, the relative importance of each of these pathways was determined at each incubation depth based on the production of 15N-labeled N2O isotopocules in parallel experiments supplied with different 15N substrates.

The concentration of each nitrogen species was modeled as

$$N_{t+1} = N_t + \Delta t \left( \sum_{n=1}^{i} f_{\text{source}} - \sum_{n=1}^{k} f_{\text{sink}} \right),$$

where $f_{\text{source}}$ and $f_{\text{sink}}$ are the source and sink terms for each nitrogen species at time $t$. The source terms are calculated based on the production of N2O isotopocules from the different pathways, and the sink terms are calculated based on the consumption of N2O isotopocules by other processes. The model was solved using a numerical integration method, such as the Runge-Kutta method, to obtain the concentration of each nitrogen species at each time step. The model was validated using the observed N2O isotopologue time courses from the incubation experiments.
process multiplied by the probability of forming each isotopocule at time $t$ regardless of which substrate is labeled. The model is optimized to the overall rates and mechanisms of N\textsubscript{2}O transfer between substrate pools, as described in the main text. Finally, N\textsubscript{2}O transfer (dashed black arrow) is modeled as first-order rates to account for \textsuperscript{15}N transfer between substrate pools, as described in the main text.

The model is optimized against the observed \textsuperscript{46}N\textsubscript{2}O, \textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textalpha}, \textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textbeta}, and \textsuperscript{44}N\textsubscript{2}O at each time point in each tracer experiment (black box).

where $N_i$ is the concentration of a given N species (e.g., NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{2}\textsuperscript{-}, NO\textsubscript{3}\textsuperscript{-}, or N\textsubscript{2}O) at time $t$, $N_{i+1}$ is its concentration at time $t+1$, $\Delta t$ represents the model time step (days), $\sum_{n=1}^{i} J_n^{\text{source}}$ is the sum of $i$ individual source processes of that species (nM d\textsuperscript{-1}), and $\sum_{n=1}^{k} J_n^{\text{sink}}$ is the sum of $k$ individual sink processes of that species (nM d\textsuperscript{-1}).

The pattern of N\textsubscript{2}O isotopocule production for a given process was set by the total rate $J$ of N\textsubscript{2}O production for that process multiplied by the probability of forming each isotopocule from a given pair of substrates. The probabilities of forming each isotopocule were based on the atom fractions of the two substrates from which the nitrogen atoms in N\textsubscript{2}O are derived:

$$P\left(\textsuperscript{46}N\textsubscript{2}O\right) = \left(\textsuperscript{15}F_1\right)\left(\textsuperscript{15}F_2\right)$$

$$P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textalpha}\right) = f \left(\textsuperscript{15}F_1\right) \left(1-\textsuperscript{15}F_2\right)$$

$$+ \left(1-f\right) \left(1-\textsuperscript{15}F_1\right)\left(\textsuperscript{15}F_2\right)$$

$$P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textbeta}\right) = \left(1-f\right) \left(\textsuperscript{15}F_1\right) \left(1-\textsuperscript{15}F_2\right)$$

$$+ f \left(1-\textsuperscript{15}F_1\right)\left(\textsuperscript{15}F_2\right)$$

$$P\left(\textsuperscript{44}N\textsubscript{2}O\right) = \left(1-\textsuperscript{15}F_1\right) \left(1-\textsuperscript{15}F_2\right)$$

where $P\left(\textsuperscript{46}N\textsubscript{2}O\right)$, $P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textalpha}\right)$, $P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textbeta}\right)$, and $P\left(\textsuperscript{44}N\textsubscript{2}O\right)$ are the probabilities of forming each isotopocule; $\textsuperscript{15}F_1$ is the atom fraction of \textsuperscript{15}N in substrate 1; $\textsuperscript{15}F_2$ is the atom fraction of \textsuperscript{15}N in substrate 2; $f$ is the proportion of N\textsubscript{2}O derived from substrate 1, and $1-f$ is the proportion of N\textsubscript{2}O derived from substrate 2. Assuming a 1:1 pairing of substrates 1 and 2, $f$ also represents the proportion of N\textsubscript{2}O derived from substrate 2 and $1-f$ represents the proportion of N\textsubscript{2}O derived from substrate 1. Processes that derive both nitrogen atoms from the same substrate pool are a special case of Eqs. (9)–(12), where $\textsuperscript{15}F_1=\textsuperscript{15}F_2$. Measuring bulk \textsuperscript{45}N\textsubscript{2}O production instead of individual isotopomers (Trimmer et al., 2016) is also a special case of Eqs. (9)–(12), where $P\left(\textsuperscript{45}N\textsubscript{2}O\right) = P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textalpha}\right) + P\left(\textsuperscript{45}N\textsubscript{2}O\textsuperscript{\textbeta}\right)$ and $f$ cancel out.

To represent each N\textsubscript{2}O-producing $J$ term in the model, the rates of N\textsubscript{2}O production were modeled as second order:

$$J_i = k_i [\text{substrate}_1][\text{substrate}_2],$$

where $J_i$ is the rate of N\textsubscript{2}O production process $i$ in nM N d\textsuperscript{-1}, $k_i$ is a second-order rate constant for that process, $[\text{substrate}_1]$ is the concentration of substrate 1 for process $i$, and $[\text{substrate}_2]$ is the concentration of substrate 2 for process $i$. Each rate constant $k_i$ was optimized in the model for each station and depth. Again, N\textsubscript{2}O production processes that draw both nitrogen atoms from the same substrate are a special case, where $[\text{substrate}_1] = [\text{substrate}_2]$. $J$ was multiplied by 1/2 to convert the rate from nM N d\textsuperscript{-1} to nM N\textsubscript{2}O d\textsuperscript{-1}, which was then multiplied by Eqs. (9)–(12) to obtain the rates of production of each isotopocule (note that rates are reported in pM d\textsuperscript{-1}). For example, the rate of hybrid \textsuperscript{46}N\textsubscript{2}O production was represented as

$$J_{\text{hybrid}}^{\text{46}N\textsubscript{2}O} = 1/2 \left( k_{\text{hybrid}} \left[\text{NH}_4^+\right]\left[\text{NO}_2^-\right] \right) \left(\textsuperscript{15}F_{\text{NH}_4^+}\right) \left(\textsuperscript{15}F_{\text{NO}_2^-}\right),$$

where $J_{\text{hybrid}}^{\text{46}N\textsubscript{2}O}$ is the rate of \textsuperscript{46}N\textsubscript{2}O production via hybrid production in nM N\textsubscript{2}O d\textsuperscript{-1}.

To relate the $J$ terms to consumption of the substrate pools (NH\textsubscript{4}\textsuperscript{+}, NO\textsubscript{2}\textsuperscript{-}, and NO\textsubscript{3}\textsuperscript{-}), $J$ draws upon the \textsuperscript{15}N and \textsuperscript{14}N substrate pools according to the atom fractions of \textsuperscript{15}N in each
substrate:

\[ J_i^{15} = J_i \cdot 15 F_{\text{substrate}} \quad \text{and} \quad J_i^{14} = J_i \cdot \left(1 - 15 F_{\text{substrate}}\right), \]  

where \( J_i^{15} \) and \( J_i^{14} \) are the rates of consumption of the \( 15^N \) and \( 14^N \) substrate pools by \( N_2O \)-producing process \( i \), \( J_i \) is the rate in nM N d\(^{-1}\) calculated in Eq. (13) for \( N_2O \) production process \( i \), and \( 15 F_{\text{substrate}} \) is the atom fraction of \( 15^N \) in the given substrate pool (\( NH_3^+, NO_2^-, \) and \( NO_3^- \)). Essentially, Eq. (15) relates how each rate \( J_i \) draws from the \( 15^N \) and \( 14^N \) substrate pools, while Eqs. (9)–(12) determine the \( 15^N \) and \( 14^N \) distribution in the product \( N_2O \). For example, the rate of \( 15^N\)NH\(^+\) consumption by hybrid \( N_2O \) production was represented as

\[ J_{\text{hybrid}}^{15} = \left(k_{\text{hybrid}} \left[ NH_4^+ \right] \left[ NO_2^- \right] \right) \left(15 F_{\text{NH}_4^+}\right), \]  

where \( J_{\text{hybrid}}^{15} \) is the rate of \( 15^N\)–\( NH_4^+ \) consumption via hybrid production in nM N d\(^{-1}\). Equation (16) does not contain the factor of 1/2 in Eq. (14) because the units are nM N d\(^{-1}\), not nM \( N_2O \) d\(^{-1}\). Rates of \( 15^N \) and \( 14^N \) transfer between substrate pools via \( NH_3 \) oxidation, \( NO_2^- \) oxidation, and \( NO_3^- \) reduction were also included in the model. The model solves for \( N_2O \) production rates, given a set of \( NH_3 \) oxidation, \( NO_2^- \) oxidation, and \( NO_3^- \) reduction rates calculated in Sect. 2.5, Eq. (7) (Table S2). These rates were represented in the model as first order:

\[ J^{15} = k \left[ 15^N \right] \quad \text{and} \quad J^{14} = k \left[ 14^N \right], \]  

where \( J^{15} \) and \( J^{14} \) represent the rates of \( 15^N \) and \( 14^N \) transformation via \( NH_3 \) oxidation, \( NO_2^- \) oxidation, or \( NO_3^- \) reduction; \( k \) is a first-order rate constant derived from measured rates; \( \alpha \) is a fractionation factor (Table S3); \( [15^N] \) is the concentration of the \( 15^N \) species; and \( [14^N] \) is the concentration of the \( 14^N \) species. \( N_2O \) consumption was modeled as first order to the concentration of each isotopologue, based on the [\( O_2 \)]-corrected rates of \( N_2O \) consumption measured on the same cruise (Sun et al., 2021a).

The model was optimized against isotopologue data at each time step in each tracer experiment (Fig. S4). The parameters being optimized (inputs to the cost function) were the second-order rate constants \( k_i \) for \( N_2O \) production solely from \( NH_3^+ \), \( N_2O \) production from \( NO_2^- \) via denitrification or nitrifier denitrification, \( N_2O \) production from \( NO_3^- \) via denitrification, hybrid \( N_2O \) production using extracellular \( NO_2^- \), and \( f \) (Fig. 2). In the model, these are all separate processes that operate independently. The model was optimized using the Nelder–Mead simplex algorithm (Nelder and Mead, 1965), implemented in the SciPy optimization library (Virtanen et al., 2020), which has been used successfully for natural abundance \( N_2O \) isotopologue models (Monreal et al., 2022). Model error was estimated by optimizing the model at each station and depth with 100 combinations of model parameters, randomly varying the initial concentrations of each

\( 15^N \) and \( 14^N \) substrate and rate constants for \( NH_3 \) oxidation, \( NO_2^- \) oxidation, and \( NO_3^- \) oxidation by up to 25 %.

To ground-truth the model, rates of \( N_2O \) production obtained from the model were compared to the measured net rates of \( 46^N_2O \) production (Fig. S5). For processes drawing both nitrogen atoms from the same substrate pool (i.e., not hybrid production), the modeled rates of \( N_2O \) production from each substrate should correspond roughly to the net rate of \( 46^N_2O \) production from the same \( 15^N \)-labeled substrate. Higher modeled rates of \( N_2O \) production solely from \( NH_3^+ \) generally corresponded to higher net rates of \( 46^N_2O \) production from \( 15^N\)–\( NH_4^+ \) (Fig. S5a). Since the model cannot produce negative rates, negative net rates of \( 46^N_2O \) production from \( 15^N\)–\( NH_4^+ \) corresponded to modeled \( N_2O \) production rates equal to zero (Fig. S5a). Modeled rates of \( N_2O \) production from \( NO_2^- \) and \( NO_3^- \) via denitrification also corresponded to higher measured rates of \( 46^N_2O \) production from \( 15^N\)–\( NO_2^- \) and \( 15^N\)–\( NO_3^- \), respectively (Fig. S5b, c).

3 Results

3.1 Depth distributions of oxygen, nitrite, and nitrous oxide

Station PS1, which was at the edge of the ODZ, represented a “background” station with no secondary \( NO_2^- \) maximum and a less-pronounced minimum in \( [N_2O] \) below the oxycline (Fig. S6; Kelly et al., 2021). At station PS1, the oxic–anoxic interface, defined in this study as the depth just above the ODZ, occurred at the base of the mixed layer at 100 m depth (Fig. S6). Station PS2 was near the geographic center of the oxygen-deficient region and had a secondary \( NO_2^- \) maximum of 2.2 µM, indicating functional anoxia (Fig. S6). The oxic–anoxic interface at station PS2 occurred at 92 m depth (Fig. S6). Below the oxic–anoxic interface, \( [N_2O] \) declined to 4.5 ± 0.3 nM before increasing again at the base of the secondary \( NO_2^- \) maximum and reaching a local maximum around 800 m depth. Station PS3 was approximately 19 km from the coast of Mexico and had a shallow oxic–anoxic interface that moved up and down on timescales of days: on 10 April, the oxic–anoxic interface occurred at 40 m depth; 2 d later, the oxic–anoxic interface had deepened to 62 m depth. Experiments were performed at the oxic–anoxic interface on both days and are designated with the abbreviations “Interface” and “Interface2” in the experimental metadata (Table S1). The chemical profiles from 11 April (Fig. S6), on which the near-surface \( [N_2O] \) maximum occurred at 61 m (Kelly et al., 2021), are displayed along with the rate data in this study. Station PS3 had a pronounced secondary \( NO_2^- \) maximum of 2.8 µM at 161 m depth (Fig. S6) and an \( NH_3^+ \) maximum of 400 nM at 15 m depth (not shown). On 11 April, \( [N_2O] \) reached a maximum of 195 ± 13 nM at the oxic–anoxic interface and declined below this depth. Below 600 m depth, \( [N_2O] \) began to increase again to 44 ± 3 nM. At ev-
Secondary chlorophyll a maximum was observed near the oxic–anoxic interface, where photosynthetically active radiation was much reduced and [NO$_3^-$] was abundant (Travis et al., 2023). This secondary chlorophyll a maximum tended to develop between the depths of the oxic–anoxic interface and the secondary NO$_2^-$ maximum (Travis et al., 2023).

### 3.2 Nitrification and nitrate reduction rates

NO$_3^-$ reduction to NO$_2^-$ occurred at rates ranging from 0.54 ± 0.04 to 33.2 ± 0.1 nM N d$^{-1}$ (Table S2). There was a small, significant rate of NO$_3^-$ reduction to NO$_2^-$ in apparently aerobic waters near the surface at station PS1 (Fig. 3a). The highest rates of NO$_3^-$ reduction to NO$_2^-$ occurred in the deep, anoxic waters at station PS2 (33.2 ± 0.01 nM N d$^{-1}$; Fig. 3d) and in the secondary chlorophyll a maximum at station PS3 (19.2 ± 0.1 nM N d$^{-1}$; Fig. 3g).

Nitrate oxidation rates ranged from 13.05 ± 0.08 to 465 ± 86 nM N d$^{-1}$ (Table S2). The highest rates of NO$_2^-$ oxidation occurred within apparently oxygen-deficient waters at 81.0 ± 0.2 nM N d$^{-1}$ in the secondary chlorophyll a maximum at station PS2 and at 465 ± 86 N N d$^{-1}$ in the secondary NO$_2^-$ maximum at station PS3 (Fig. 3e, h; Table S2). Note that these are potential rates, since the $^{15}$N addition was generally much greater than the ambient concentration (Lipschultz, 2008). In some cases, NO$_2^-$ oxidation rates appeared negative due to a decrease in $^{15}$N–NO$_3^-$ vs. incubation time (Fig. 3b, h), which was likely an artifact of the elevated $\delta^{15}$N values in some of our $^{15}$N–NO$_3^-$ treatments (discussed above). We chose, however, not to left-censor the data.

NH$_3$ oxidation to NO$_2^-$ occurred at small but significant rates ranging from 0.19 ± 0.0004 to 4.68 ± 0.07 nM N d$^{-1}$ (Table S2). At every station, rates of NH$_3$ oxidation peaked near the base of the mixed layer at the same depth as the near-surface [N$_2$O] maximum (Fig. 3c, f, i). At station PS2, NH$_3$ oxidation showed a secondary peak at the same depth as the deep [N$_2$O] maximum (Fig. 3f). At station PS3, there was also a small, significant rate of NH$_3$ oxidation (0.303 ± 0.005 N N d$^{-1}$) at 898 m, which was close to the bottom depth (Fig. 3i). Rates of NH$_3$ oxidation were generally lower than NO$_2^-$ oxidation and undetectable in oxygen-deficient waters (Fig. 3c, f, i).

### 3.3 Net production rates of $^{45}$N$_2$O$^\alpha$, $^{45}$N$_2$O$^\beta$, and $^{46}$N$_2$O (measured net rates)

At each station, the observed rates of net $^{46}$N$_2$O (Fig. 4), $^{45}$N$_2$O$^\alpha$, and $^{45}$N$_2$O$^\beta$ (Fig. 5) production from $^{15}$N–NH$_3^+$, $^{15}$N–NO$_3^-$, and $^{15}$N–NO$_2^-$ all peaked at or just below the oxic–anoxic interface, where the near-surface [N$_2$O] maximum was found. There were also relatively higher rates of net $^{46}$N$_2$O production from $^{15}$N–NO$_3^-$ and $^{15}$N–NO$_2^-$ within the secondary NO$_2^-$ maximum (253 m) at station PS2 (Fig. 4d–e). Relatively high rates of net $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ production also occurred in the secondary NO$_2^-$ maximum at stations PS2 (253 m; Fig. 5d–e) and PS3 (182 m; Fig. 5g–h). The net rates of $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ production varied in concert at almost every station and depth, with a few exceptions (Fig. 5).

For example, in the secondary NO$_2^-$ maximum (182 m) at station PS3, in the $^{15}$N–NO$_2^-$ experiment, the production of $^{45}$N$_2$O$^\alpha$ was 60 ± 30 pM N d$^{-1}$ ($p = 0.09$) and there was no significant production of $^{45}$N$_2$O$^\beta$ (Fig. 5h). In the parallel $^{15}$N–NH$_3^+$ experiment, the production of $^{45}$N$_2$O$^\beta$ was 0.7 ± 0.3 pM N d$^{-1}$ ($p = 0.06$) and there was no significant production of $^{45}$N$_2$O$^\alpha$. At this station and depth, $j$ (the proportion of N$^\alpha$ derived from NO$_2^-$) was equal to 0.9 ± 0.2 (Table S4). The second experiment in which labeling was unequal occurred at the oxic–anoxic interface (92 m) at station PS2, where, in the $^{15}$N–NH$_3^+$ experiment, the production of $^{45}$N$_2$O$^\alpha$ was 5 ± 2 pM N d$^{-1}$ ($p = 0.02$) and there was

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no significant production of $^{45}$N$_2$O$^\beta$ (Fig. 5f). Here, $f$ was equal to 0.2 ± 0.1. Finally, at the mid-oxygen depth (25 m) at station PS3, in the $^{15}$N–NH$_4^+$ experiment, the production of $^{45}$N$_2$O$^\alpha$ was 0.23 ± 0.8 pM N$_2$O d$^{-1}$ ($p = 0.02$) and there was no significant production of $^{45}$N$_2$O$^\beta$. Here, $f$ was statistically indistinguishable from zero.

At many stations and depths, the net production of $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ exceeded the values expected from $^{46}$N$_2$O production for a process that draws both nitrogen atoms from the same substrate pool (Fig. 5f). This expected value is calculated from the atom fraction of $^{15}$N in the substrate and from a binomial distribution of the isotopocules of N$_2$O during N$_2$O production (Trimmer et al., 2016):

$$p_{\text{expected}}^{45} = \frac{p^{46}}{15F} - \frac{p^{46}}{15F} \left( \frac{15F}{1} \right) \left( 1 - 15F \right) = \frac{p^{46}}{15F} \left( 1 - 15F \right) .$$

where $p_{\text{expected}}^{45}$ is the expected production of $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ from a process that draws both nitrogen atoms from the same substrate pool, $p^{46}$ is the net production rate of $^{46}$N$_2$O, and $F$ is the atom fraction of $^{15}$N in the substrate pool (for example, NO$_3^-$ in a $^{15}$N–NO$_3^-$ experiment). Then, excess production of $^{45}$N$_2$O is any $^{45}$N$_2$O production above and beyond this expected rate:

$$p_{\text{excess}}^{45} = p^{45} - p_{\text{expected}}^{45} = p^{45} - \frac{p^{46}}{15F} \left( 1 - 15F \right) .$$

Figure 4. Net $^{46}$N$_2$O production from $^{15}$N–NO$_3^-$ (a, d, g; indigo), $^{15}$N–NO$_2^-$ (b, e, h; blue), and $^{15}$N–NH$_4^+$ (c, f, i; yellow) at stations PS1 (a–c), PS2 (d–f), and PS3 (g–i). N$_2$O production rates are plotted over depth profiles of dissolved [O$_2$] (dashed lines) and [N$_2$O] (solid lines; from Kelly et al., 2021). Error bars are calculated from the linear regression slope error of $^{46}$N$_2$O vs. incubation time. Note the different x-axis scales for $^{46}$N$_2$O production (top x axes) and [O$_2$] and [N$_2$O] (bottom x axes).

Figure 5. Net $^{45}$N$_2$O$^\alpha$ (open symbols) and $^{45}$N$_2$O$^\beta$ (closed symbols) production from $^{15}$N–NO$_3^-$ (a, d, g; indigo), $^{15}$N–NO$_2^-$ (b, e, h; blue), and $^{15}$N–NH$_4^+$ (c, f, i; yellow) at stations PS1 (a–c), PS2 (d–f), and PS3 (g–i). N$_2$O production rates are plotted over depth profiles of dissolved [O$_2$] (dashed lines) and [N$_2$O] (solid lines; from Kelly et al., 2021). Error bars are calculated from the linear regression slope error of $^{45}$N$_2$O vs. incubation time. Note the different x-axis scales for $^{45}$N$_2$O production (top x axes) and [O$_2$] and [N$_2$O] (bottom x axes).
significant excess $^{45}\text{N}_2\text{O}^\alpha$ and $^{45}\text{N}_2\text{O}^\beta$ production in many of the $^{15}\text{N}−\text{NO}_3^-$ experiments, although this was harder to discern due to the wider range of atom fractions in these experiments (Fig. S7b). In a few experiments, excess $^{45}\text{N}_2\text{O}^\alpha$ and $^{45}\text{N}_2\text{O}^\beta$ production diverged.

### 3.4 N$_2$O production mechanisms and yields (model results)

Based on model results, the rates of N$_2$O production from NO$_3^-$ (denitrification using cellular NO$_2^-$; Fig. 2) were the highest among the N$_2$O production processes measured in this study. At suboxic to anoxic depths, the rates of N$_2$O production from NO$_3^-$ were orders of magnitude higher than all the other N$_2$O production rates (Fig. 6). N$_2$O production from NO$_3^-$ reached its maximum value (1600 ± 400 pM N$_2$O d$^{-1}$; Table S4) at the depth of the near-surface [N$_2$O] maximum at every station (Fig. 6a, e, i), where there were also high rates of NO$_3^-$ reduction to NO$_2^-$ at stations PS2 and PS3 (Fig. 6e, i). N$_2$O production from NO$_3^-$ (denitrification using extracellular NO$_2^-$; Fig. 2) exhibited lower rates, with a maximum of 510 ± 30 pM N$_2$O d$^{-1}$ (Table S4). At stations PS1 and PS3, N$_2$O production from NO$_3^-$ peaked at the depth of the near-surface [N$_2$O] maximum (Fig. 6b, j); at station PS2, N$_2$O production from NO$_3^-$ was observed in the near-surface [N$_2$O] maximum but peaked in the secondary NO$_2^-$ maximum (253 m; Fig. 6f).

Hybrid N$_2$O production occurred at a similar rate to N$_2$O production from NO$_3^-$, ranging from 0.061 ± 0.005 to 230 ± 80 pM N$_2$O d$^{-1}$. Hybrid N$_2$O production peaked within the near-surface [N$_2$O] maximum at all stations (Fig. 6c, g, k). At station PS2, hybrid N$_2$O production exhibited the highest rates at the same depths as NH$_3$ oxidation, with a secondary peak in the deep [N$_2$O] maximum (Fig. 6g). At station PS3, hybrid N$_2$O production, like NH$_3$ oxidation, exhibited a small, significant rate at 898 m, which was very close to the bottom depth at station PS3 (Table S4).

N$_2$O production solely from NH$_4^+$ occurred at the smallest rates overall, ranging from 0.010 ± 0.004 to 8 ± 2 pM N$_2$O d$^{-1}$ (Table S4). N$_2$O production solely from NH$_4^+$ peaked around the near-surface [N$_2$O] maximum at each station (Fig. 6d, h, l) and in the secondary NO$_2^-$ maximum at station PS2 (Fig. 6h).

The percentage of N$_2$O production from NH$_4^+$ comprised of hybrid N$_2$O was calculated as

$$\text{% hybrid} = \frac{\text{hybrid N}_2\text{O (nM N}_2\text{O d}^{-1})}{\text{N}_2\text{O from hydroxylamine (nM N}_2\text{O d}^{-1})}.$$  \hspace{1cm} (20)

On average, hybrid N$_2$O production was 86 ± 28 % of N$_2$O production from NH$_4^+$. Hybrid N$_2$O production was > 75 % of the total N$_2$O production from NH$_4^+$ at all stations and depths except for the top of the oxycline at station PS1 (Fig. 7a), the middle of the oxycline at station PS2 (Fig. 7b), and the top of the oxycline at station PS3 (Fig. 7c), where it comprised 0 %, 68 %, and 19 % of N$_2$O production from NH$_4^+$, respectively. Hybrid production as a percentage of total N$_2$O production from NH$_4^+$ declined with increasing dissolved oxygen (Fig. S8), although more measurements are needed to fully evaluate this trend.

The percentage of hybrid N$_2$O production as a proportion of total N$_2$O production was more variable and tended to decline with decreasing dissolved oxygen as production from NO$_3^-$ increased (Fig. 7). Hybrid N$_2$O production was greater than 75 % of total N$_2$O production only at the surface at station PS1 (Fig. 7a), at the top of the oxycline and in the deep [N$_2$O] maximum at station PS2 (Fig. 7b), and in the deep [N$_2$O] maximum at station PS3 (Fig. 7c).

N$_2$O production from NO$_3^-$ comprised a much greater proportion of total N$_2$O production overall (Fig. 7). In the near-surface [N$_2$O] maximum at station PS1, N$_2$O production was predominantly (95.4 %) from NO$_3^-$, with smaller contributions from hybrid production (4.0 %) and denitrification from NO$_3^-$ (0.6 %; Fig. 7a). In the near-surface [N$_2$O] maximum at station PS2, N$_2$O production was 60.2 % from NO$_3^-$, 32.1 % from hybrid production, 7.3 % from NO$_3^-$, and 0.4 % solely from NH$_4^+$ (Fig. 7b). In the near-surface [N$_2$O] maximum at station PS3, N$_2$O production was 87.0 % from NO$_3^-$, 12.4 % from hybrid production, 0.5 % from NO$_3^-$, and 0.1 % solely from NH$_4^+$ (Fig. 7c).

### 3.5 Oxygen dependence of N$_2$O production

The oxygen dependencies of N$_2$O production pathways were determined by fitting model-derived N$_2$O production pathways vs. [O$_2$] using the following rate law:

$$\text{rate} = ae^{-b[O_2]}.$$  \hspace{1cm} (21)

In this analysis, both ambient [O$_2$] measured by the Sea-Bird sensor mounted on the rosette (“ambient [O$_2$]”) and [O$_2$] measured by chemiluminescent optodes mounted inside incubation bottles (“incubation [O$_2$]”) were examined. The rate dependencies on ambient and incubation [O$_2$] reflect both preconditioning (i.e., the ambient [O$_2$] in which the microbial community was living before the incubation experiment) and the response to perturbation (i.e., the experimental conditions inside the incubation bottles, if different from the environment). Those incubations that had higher incubation [O$_2$] than the ambient [O$_2$] had received small oxygen perturbations.

N$_2$O production via denitrification exhibited an exponentially declining relationship with dissolved O$_2$, where N$_2$O production from NO$_3^-$ was more inhibited by dissolved O$_2$ than N$_2$O production from NO$_3^-$ (Fig. 8). When looking at the oxygen dependence of denitrification, we found several instances of N$_2$O production from NO$_3^-$ via denitrification with dissolved [O$_2$] greater than 3 μM (Fig. 8a–b). For example, at the oxic–anoxic interface at station PS2, where ambient [O$_2$] was 6.49 μM and incubation [O$_2$] was 6.29 ± 0.07 μM (Table S1), N$_2$O production from NO$_3^-$ was...
Figure 6. 

N$_2$O production from NO$_3^-$ (a, e, i; indigo diamonds), N$_2$O production from NO$_2^-$ (b, f, j; blue diamonds), hybrid N$_2$O production (c, g, k; green diamonds), and N$_2$O production solely from NH$^+_4$ (d, h, l; yellow diamonds) at stations PS1 (a–d), PS2 (e–h), and PS3 (i–l). Panels (a), (e), and (i) also show rates of NO$_3^-$ reduction to NO$_2^-$ (open circles). Panels (b), (f), and (j) show depth profiles of dissolved [O$_2$] (dashed lines) and [N$_2$O] (solid lines; from Kelly et al., 2021). Panels (c), (g), and (k) show rates of NH$_3$ oxidation (gray circles). N$_2$O production rate error bars are calculated from 100 model optimizations, varying key parameters by up to 25%. Note the different x-axis scales for NO$_3^-$ reduction to NO$_2^-$ (a, e, i; bottom), N$_2$O production (top), [O$_2$] and [N$_2$O] (b, f, j; bottom), and NH$_3$ oxidation (c, g, k; bottom).

70 ± 10 pM N$_2$O d$^{-1}$ (Fig. 6e, Table S4). N$_2$O production from NO$_3^-$ at the same station and depth was 8.9 ± 0.2 pM N$_2$O d$^{-1}$ (Fig. 6f, Table S4). Similarly, at the oxic–anoxic interface of station PS3, where ambient [O$_2$] was 12.48 µM and incubation [O$_2$] was 6.64 ± 0.03 µM (Table S1), N$_2$O production from NO$_3^-$ was 120 ± 20 pM N$_2$O d$^{-1}$ (Fig. 6i, Table S4). There were also two anoxic depths at station PS2 that were not sparged with He before tracer addition (“base of ODZ” and “deep ODZ core”), where ambient [O$_2$] was below detection but incubation [O$_2$] was significantly elevated (17.7 ± 0.1 and 19.2 ± 0.8 µM, respectively; Table S1). At these depths, N$_2$O production from NO$_3^-$ was 12 ± 1 and 5.2 ± 0.4 pM N$_2$O d$^{-1}$, respectively (Fig. 6f, Table S4). N$_2$O production from NO$_3^-$ at the “deep ODZ core” depth was 210 ± 40 pM N$_2$O d$^{-1}$ (Table S4).

Hybrid N$_2$O production rates also decreased exponentially with increasing dissolved [O$_2$] (Fig. 9a–b). Fitting hybrid rates vs. ambient [O$_2$] produced a rate (Eq. 21) with $a = 65.83$ and $b = 0.17$ (Fig. 9a); hybrid rates vs. incubation [O$_2$] produced fits with $a = 76.26$ and $b = 0.067$ (Fig. 9b).

The rate of N$_2$O production solely from NH$^+_4$ also decreased exponentially with increasing dissolved [O$_2$]. The highest rates of N$_2$O production solely from NH$^+_4$ occurred in the secondary chlorophyll maximum at station PS3 (Table S4), where dissolved oxygen was below detection. N$_2$O yield during production solely from NH$^+_4$ also exhibited exponentially decreasing relationships with dissolved [O$_2$] (Fig. 9e–f). To ensure mass balance in terms of NH$^+_4$ consumption (Fig. S9), N$_2$O yield (%) during production solely
Figure 7. $\text{N}_2\text{O}$ production solely from $\text{NH}_4^+$ (yellow bars), hybrid $\text{N}_2\text{O}$ production (green bars), $\text{N}_2\text{O}$ production from $\text{NO}_3^-$ (hatched blue bars), and $\text{N}_2\text{O}$ production from $\text{NO}_2^-$ (indigo bars) as proportions of total $\text{N}_2\text{O}$ production at stations PS1 (a), PS2 (b), and PS3 (c). Data are plotted over depth profiles of dissolved $[\text{O}_2]$ (dashed lines) and $[\text{N}_2\text{O}]$ (solid lines; from Kelly et al., 2021). Note broken y axes and different x-axis scales for $[\text{O}_2]$ and $[\text{N}_2\text{O}]$ (top) and for proportions (bottom).

Figure 8. $\text{N}_2\text{O}$ production from $\text{NO}_3^-$ via denitrification (a, b) and from $\text{NO}_2^-$ via denitrification (c, d), measured at a range of $[\text{O}_2]$ measured by a Seabird sensor (a, c) or by chemiluminescent optodes mounted inside incubation bottles (b, d). Curves of form, yield $= ae^{-O_2b}$, are fit through the data (black lines); values of $a$ and $b$ are shown in white boxes in each plot.

The $\text{N}_2\text{O}$ yield from hybrid production was calculated as

$$\text{yield} (\%) = \frac{2 \left[ \text{N}_2\text{O solely from NH}_4^+ \left( \text{nM N}_2\text{O d}^{-1} \right) \right]}{2\left[ \text{N}_2\text{O solely from NH}_4^+ \left( \text{nM N}_2\text{O d}^{-1} \right) \right] + \text{hybrid N}_2\text{O} \left( \text{nM N}_2\text{O d}^{-1} \right) + \text{NH}_3 \text{ oxidation} \left( \text{nM N d}^{-1} \right)}.$$

where $\text{N}_2\text{O}$ production solely from $\text{NH}_4^+$ is in units of nM $\text{N}_2\text{O} \text{ d}^{-1}$, hybrid $\text{N}_2\text{O}$ production is in units of nM $\text{N}_2\text{O} \text{ d}^{-1}$, and $\text{NH}_3$ oxidation to $\text{NO}_2^-$ is in units of nM N d$^{-1}$. This assumes that the formation of $\text{N}_2\text{O}$ solely from $\text{NH}_4^+$ draws two nitrogen atoms from the $\text{NH}_4^+$ pool, while hybrid $\text{N}_2\text{O}$ production and the oxidation of $\text{NH}_4^+$ to $\text{NO}_2^-$ each draw one atom from the $\text{NH}_4^+$ pool (Fig. S9). Following the same convention, $\text{N}_2\text{O}$ yield (%) during hybrid production was calculated as

$$\text{yield} (\%) = \frac{\text{hybrid N}_2\text{O} \left( \text{nM N}_2\text{O d}^{-1} \right)}{2\left[ \text{N}_2\text{O solely from NH}_4^+ \left( \text{nM N}_2\text{O d}^{-1} \right) \right] + \text{hybrid N}_2\text{O} \left( \text{nM N}_2\text{O d}^{-1} \right) + \text{NH}_3 \text{ oxidation} \left( \text{nM N d}^{-1} \right)}.$$

The maximum $\text{N}_2\text{O}$ yield from hybrid production was $21 \pm 7 \%$ (Fig. 9c, d), while the maximum $\text{N}_2\text{O}$ yield during production solely from $\text{NH}_4^+$ was $2.2 \pm 0.7 \%$ (Fig. 9e, f). The $\text{N}_2\text{O}$ yield during production solely from $\text{NH}_4^+$ declined more sharply with increased $\text{O}_2$ than the $\text{N}_2\text{O}$ yield during hybrid production did (Fig. 9c–f).
4 Discussion

In this study, we found that N₂O production from denitrification was the dominant source of N₂O both within the ODZ and in the upper oxycline. Hybrid N₂O production was a smaller but significant contributor to N₂O in the upper oxycline. N₂O production solely from NH₃⁴⁺ (which includes N₂O from hydroxylamine oxidation, hybrid production with cellular NO₃⁻, and nitrifier denitrification with cellular NO₃⁻) was negligible everywhere except surface waters. Our findings of equal formation of ⁴⁵N₂O⁶⁺ and ⁴⁵N₂O⁶⁻ in most experiments indicate that N⁶⁺ retains an equal proportion of NO₃⁻ and NH₄⁺-derived N during hybrid production, which may imply that hybrid N₂O production exhibits a constant δ(¹⁵N⁶⁺). All of the processes measured in this study exhibited a strong dependence on dissolved oxygen, although denitrification was less inhibited by dissolved oxygen than previous work would suggest.

4.1 Rates of N₂O production via denitrification

Based on our rate data, N₂O production from NO₃⁻ is the dominant source of N₂O in both the near-surface [N₂O] maximum and the anoxic ODZ core. This agrees well with natural abundance isotopocule measurements in the ETNP, which indicate that the near-surface [N₂O] maximum is likely to comprise ~80% N₂O produced via denitrification and ~20% N₂O produced via nitrification or archeal N₂O production, producing a local minimum in δ(¹⁵N⁶⁺) (Kelly et al., 2021). Natural abundance isotopomer work has shown that N₂O production from NO₃⁻ could be an important source of N₂O in the anoxic core of ODZs, as long as it has a positive δ(¹⁵N⁶⁺) (Casciotti et al., 2018; Kelly et al., 2021; Monreal et al., 2022). While denitrification is generally accepted to produce N₂O with δ(¹⁵N⁶⁺) = 0% (Sutka et al., 2006; other refs), some strains of denitrifying bacteria can produce N₂O with δ(¹⁵N⁶⁺) = 10%–22% (Toyoda et al., 2005; Wang et al., 2023) and denitrifying fungi produce N₂O with δ(¹⁵N⁶⁺) = 35%–37% (Lazo-Murphy et al., 2022; Rohe et al., 2014; Sutka et al., 2008; Yang et al., 2014). Here, the dominance of N₂O production from ¹⁵N–NO₃⁻, combined with parallel natural abundance isotopomer studies, suggest that strains of denitrifying bacteria and fungi that produce N₂O with a high site preference may be important contributors to N₂O in the core of ODZs. The importance of N₂O production from NO₃⁻ also presents an important exception to the modular view of the microbial nitrogen cycle network, which holds that intermediates are passed externally from one cell to the next, rather than being held internally (Kuyper et al., 2018). N₂O production from NO₃⁻ that utilizes an internal NO₃⁻ pool is currently left out of most biogeochemical models of nitrogen cycling in and around oxygen-deficient zones (Bianchi et al., 2023), and modeling work that includes this as a source of N₂O is needed.

4.2 Pathways of hybrid N₂O production and implications for hybrid δ(¹⁵N⁶⁺)

Hybrid N₂O production peaked at the same depths as NH₃ oxidation (Fig. 6c, g, k), which were also the depths at which ammonia-oxidizing archaea were most abundant (Frey et al., 2023), consistent with N₂O production associated with ammonia-oxidizing archaea. At most stations and depths, the production of ⁴⁵N₂O⁶⁺ and ⁴⁵N₂O⁶⁻ in both the ¹⁵N–NO₃⁻ and ¹⁵N–NH₄⁺ experiments were roughly equal. From this we conclude that, during hybrid formation, N⁶⁺ and N⁶⁻ each retained nitrogen atoms derived from both NH₄⁺ and NO₃⁻. The equal formation of ⁴⁵N₂O⁶⁺ and ⁴⁵N₂O⁶⁻ led to values of f
within an error of 0.5 in most of our experiments (Table S4), and the mean value of $f$ across all stations and depths was 0.5 ± 0.2. This means that, during hybrid N$_2$O production, half of the N$^\alpha$ atoms were derived from NO$_2^-$ and half were derived from NH$_4^+$ (likewise for N$^\beta$).

Although our data do not allow us to comment directly on the enzymatic machinery of hybrid N$_2$O formation, our data can be used to theorize hypothetical pathways for hybrid N$_2$O production. Firstly, we see much higher rates of hybrid production using ambient NO$_2^-$ (Pathway 3 in Wan et al., 2023b) than hybrid production using cellular NO$_2^-$ (Pathway 2 in Wan et al., 2023b). Again, this agrees with the results of Wan et al. (2023b), who see higher rates of hybrid formation from extracellular NO$_2^-$ within the range of $\left[^{15}$N–NH$_4^+\right]/[NO_2^-]$ covered by our experiments. In our model, hybrid N$_2$O production is operationally defined as a 1:1 combination of N derived from NH$_4^+$ and NO$_2^-$, which is generally consistent with previous work (Stieglmeier et al., 2014). Any combination of N derived from NO$_2^-$ with a second N derived from NO$_2^-$ would be included in the modeled quantity of N$_2$O production from NO$_2^-$; likewise, any combination of N derived from NH$_4^+$ with a second N derived from NO$_2^-$ would be included in the N$_2$O production solely from NH$_4^+$. The question, then, is what reaction would be specific enough to have one N derived from each substrate but not specific enough to govern $^{15}$N placement in the resulting N$_2$O? One such reaction could be the combination of NH$_4^+$ and NO$_2^-$ to form a symmetrical intermediate such as hyponitrous acid (HONNOH, or hyponitrite -- ONNO -- in its deprotonated form), which has been discussed as a possible intermediate in hybrid nitrous oxide formation (Wei et al., 2019). Hyponitrous acid may react to form N$_2$O via breakage of one of the N–O bonds, resulting in N$_2$O that contains a 1 : 1 ratio of NH$_4^+$–NO$_2^-$. With a precursor such as hyponitrite or hyponitrous acid, equal formation of $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ could be achieved with non-selective N–O bond breakage.

These findings of equal $^{45}$N$_2$O production have important implications for the natural abundance $\delta$($^{15}$N) of N$_2$O produced by the hybrid N$_2$O process. Assuming that hybrid N$_2$O production proceeds through a symmetrical intermediate in which NH$_4^+$ and NO$_2^-$ are paired in a 1 : 1 ratio, we can model $\delta$($^{15}$N) as

$$
\delta\left(^{15}\text{N}\right) = \delta\left(^{15}\text{N}^\alpha\right) - \delta\left(^{15}\text{N}^\beta\right) = \left[\delta\left(^{15}\text{N} - \text{NO}_2^-\right) + (1 - f)\delta\left(^{15}\text{N} - \text{NH}_4^+\right)\right] - \left[(1 - f)\delta\left(^{15}\text{N} - \text{NO}_2^-\right) + f\delta\left(^{15}\text{N} - \text{NH}_4^+\right) - \epsilon\right],
$$

(24)

where $f$ is the proportion of the $\alpha$ nitrogen derived from NO$_2^-$ and the proportion of the $\beta$ nitrogen derived from NH$_4^+$ and $\epsilon$ is the fractionation factor associated with N$^\beta$–O bond breakage. If $f \neq 1/2$, hybrid $\delta$($^{15}$N) retains a dependence on the $\delta$($^{15}$N) of the substrates – or, more accurately, the difference in $\delta$($^{15}$N) of the two substrates; if the $\delta$($^{15}$N) of the substrates is equal, it will cancel out regardless of $f$.

If $\delta\left(^{15}\text{N} - \text{NH}_4^+\right) > \delta\left(^{15}\text{N} - \text{NO}_2^-\right)$, as is generally the case in the secondary nitrite maximum (Buchwald et al., 2015; Casciotti, 2016), then low values of $f$ should produce high hybrid $\delta$($^{15}$N) and high values of $f$ should produce low hybrid $\delta$($^{15}$N) (Fig. 10). If, however, $f = 1/2$, as was the case for most experimental depths in this study, hybrid $\delta$($^{15}$N) should depend only on $\epsilon$ and not on the isotopic composition of each substrate. This means that a $\delta$($^{15}$N) endmember could potentially be established for hybrid N$_2$O production, even though hybrid N$_2$O production draws from different substrate pools. Wei et al. (2019) discuss possible pathways or endmembers of hybrid N$_2$O formation, i.e., via cis-hyponitrous acid, trans-hyponitrous acid, and nitramide, all leading to N$_2$O with different $\delta$($^{15}$N) values. More studies are needed to determine the $\delta$($^{15}$N) of N$_2$O produced by ammonia-oxidizing archaea under a range of conditions.

The unequal production of $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ observed at certain depths led to values of $f$ significantly different from 0.5 (Table S4). At these depths, N$^\alpha$ retained a different proportion of nitrogen derived from NO$_2^-$ and NH$_4^+$ than N$^\beta$ did, causing $^{45}$N$_2$O$^\alpha$ and $^{45}$N$_2$O$^\beta$ to diverge. The depths with $f \neq 0.5$ anchored significant relationships between $f$ and ambient [O$_2$] ($R^2 = 0.84$, $p < 0.001$; Fig. S10a) and potential density anomaly ($\sigma_0$) ($R^2 = 0.72$, $p < 0.001$; Fig. S10b). The oxygen and potential density gradients may be proxies for changing archaeal community compositions at different depths in the water column, which may exhibit different patterns of incorporation of NO$_2^-$-derived N and NH$_4^+$-derived N into N$^\alpha$ and N$^\beta$. It is also possible that we sampled a different “hybrid” N$_2$O-producing process at these depths, such as fungal co-denitrification (Shoun et al., 2012), which may
proceed via a different pathway from archael hybrid N₂O production.

4.3 Rates of nitrification and N₂O production solely from NH₄⁺

The rates of N₂O production from NH₄⁺ in this study (i.e., the sum of hybrid N₂O production and N₂O production solely from NH₄⁺) peaked at 240 ± 80 pM N₂O d⁻¹ (Table S4). These were similar to those measured on the same cruise by Frey et al. (2023), who measured rates of N₂O production from NH₄⁺ in the oxycline of 28–149 pM N₂O d⁻¹ (Frey et al., 2023). The low rates of NH₃ oxidation to NO₂⁻ in this study (0.05–4.68 nM N d⁻¹) were also similar to those measured by Frey et al. (2023), who measured NH₃ oxidation rates of 1.0–11.7 nM d⁻¹ in the oxycline. NH₃ oxidation rates in this study were smaller than those measured on the same cruise by Travis et al. (2023), who measured NH₃ oxidation rates as high as 90 ± 2 nM d⁻¹ in fully oxygenated incubations at station PS3. The highest rates of NO₂⁻ oxidation we observed occurred at anoxic depths at stations PS2 and PS3 (Fig. 3e, h), which agrees with mounting evidence suggesting the importance of NO₂⁻ oxidation in apparently anoxic regions (Sun et al., 2017, 2021b).

When [O₂] was less than 10 µM, the rates of hybrid N₂O production (6–230 pM N₂O d⁻¹) were orders of magnitude greater than the rates of N₂O production solely from NH₄⁺ at the same depths (0–8 pM N₂O d⁻¹) (Fig. 6). Indeed, at the upper oxic–anoxic interface, the rates of hybrid N₂O production were on a similar order of magnitude to N₂O production from NO₂⁻ via denitrification (8–510 pM N₂O d⁻¹). These results agree with previous work showing that hybrid N₂O formation represents a high percentage of total N₂O production from NH₄⁺ in the ETNP and eastern tropical South Pacific (ETSP) (Frey et al., 2020, 2023). The results in this study also agree with recent culture work: the ¹⁵N–NH₄⁺ experiments in this study fell along a range of [¹⁵N–NH₄⁺] / [NO₂⁻] of 0.14–0.5, in which Wan et al. (2023b) found that hybrid N₂O production occurred at a rate 2 to 4 times greater than N₂O production via hydroxylamine oxidation (N derived solely from NH₄⁺) in cultures of Nitrosopumilus maritimus.

We found three depths near the surface where hybrid production comprised a smaller percentage (0 %–68 %) of total N₂O production from NH₄⁺ (Fig. 7a–c). Previous work in the ETNP found that hybrid N₂O production always comprised > 90 % of N₂O production from NH₄⁺ (Frey et al., 2023), and, where our samples overlapped with this previous work, we observed similarly high proportions of hybrid production (Fig. 5). The depths where we observed a smaller proportion of hybrid production had not been sampled previously; it is possible that we sampled different microbial communities there, acclimated to different levels of NH₄⁺, NO₂⁻, and dissolved oxygen. We also found that hybrid N₂O formation generally comprised a small proportion of total N₂O production, which was dominated by N₂O production from NO₃⁻, especially at suboxic depths (Fig. 7d–h). This is similar to previous findings from the ETSP, which showed that hybrid formation comprised 0 %–95 % of total N₂O production from NO₃⁻ along the natural [O₂] gradient (Frey et al., 2020). This large range is due to the large range of rates of N₂O production from NO₃⁻, which can occur at orders of magnitude higher or lower than hybrid N₂O production.

4.4 Oxygen dependence of N₂O production rates and yields

N₂O production from NO₃⁻ and NO₂⁻ exhibited exponential dependence on dissolved oxygen, albeit with smaller maximum rates than those found in the ETSP (Frey et al., 2020; Ji et al., 2015). Most surprising were the significant rates of N₂O production via denitrification at [O₂] > 3 µM (Fig. 8a–d), which has previously been suggested as the threshold above which denitrification ceases (Dalsgaard et al., 2014). These observations are particularly evident in the plots of N₂O production from NO₃⁻ vs. incubation [O₂] (Fig. 8b), where positive, significant rates of N₂O production from NO₃⁻ were evident in incubations containing [O₂] as high as 19.2 ± 0.8 µM (PS2 deep ODZ core experiment). One explanation for N₂O production via denitrification at such high levels of ambient dissolved oxygen is particle-associated denitrification (Bianchi et al., 2018; Smrža et al., 2021; Wan et al., 2023a). Fungal denitrification may also have contributed to these fluxes, since denitrifying fungi can tolerate a higher level of oxygen than their bacterial counterparts. Additionally, denitrifying microbial communities acclimatized to lower ambient [O₂] may be able to continue to produce N₂O when [O₂] is suddenly increased.

These results showed that N₂O production from NO₃⁻ can occur in [O₂] as high as 19.2 ± 0.8 µM, which is similar to results from the ETSP showing that N₂O production from NO₃⁻ in manipulated [O₂] can be as high as 30 µM (Frey et al., 2020). The volume of suboxic water in the ocean has been increasing over the last 50 years and will likely continue to expand over the 21st century (Oschlies et al., 2018; Schmidtko et al., 2017; Stramma et al., 2008), although the extent of this deoxygenation remains uncertain (Bianchi et al., 2018; Busecke et al., 2022; Cabré et al., 2015). Constraining the window of oxygen concentrations under which denitrification leads to N₂O production will be key to understanding how marine deoxygenation and N₂O cycling will interact.

While this study and others have found that hybrid N₂O production represents a consistent percentage of N₂O production from NO₃⁻ along a range of ambient [O₂] (Frey et al., 2020, 2023), the rate of hybrid N₂O production followed a clear exponential dependence on dissolved oxygen (Fig. 9). The differences in ambient and incubation [O₂] resulted in slight differences in the coefficients for each yield curve; nevertheless, hybrid rates plotted along both ambient and incubation [O₂] gradients exhibited remarkably similar [O₂] inhi-
bition curves, with the highest rates at \([O_2] < 7 \mu M\). These results are similar to those of Frey et al. (2023), who showed a decrease in \(N_2O\) production from \(NH_4^+\) with increasing \([O_2]\).

The maximum \(N_2O\) yield for hybrid production (21%; Fig. 8c, d) was 1 order of magnitude higher than previous estimates of \(N_2O\) yields during \(NH_3\) oxidation from ETSP and ETNP, which did not include hybrid \(N_2O\) production (Ji et al., 2018). These high yields occurred at the oxic–anoxic interface at station PS1 and just below the oxic–anoxic interface at station PS3, where ambient \([O_2]\) was below detection but \(NH_3\) oxidation still occurred (Fig. 3). This indicates the potential for extremely high yields of \(N_2O\) from hybrid production where \(NH_3\) oxidation is active in suboxic to anoxic environments.

\(N_2O\) yields during production solely from \(NH_4^+\) also increased with decreasing \([O_2]\) (Fig. 9b), as previously reported (Frey et al., 2020; Goreau et al., 1980; Ji et al., 2018; Nevison et al., 2003). \(N_2O\) yields during production solely from \(NH_3\) increased sharply with decreasing \([O_2]\) along both ambient and incubation \([O_2]\) gradients but were much smaller than the yields from hybrid \(N_2O\) production (Fig. 8c–d). The maximum yields during production solely from \(NH_3\) were similar to the maximum yields found by another study in the ETNP, which were around 3 % (Frey et al., 2023), and much higher than yields from ammonia-oxidizing archaea in soils and culture (up to 0.03 %) (Hink et al., 2017a, b).

4.5 Experimental artifacts

Care was taken to minimize the effects of experimental setup on the microbial communities in each sample. In addition to the steps taken to prevent oxygen contamination (described in Sect. 2: Methods), a relatively short 24 h incubation period was selected to minimize bottle effects and shifts in the microbial community composition over the course of each incubation. Nonetheless, sample collection, preparation, and incubation conditions could have affected the microbial communities in several ways. Firstly, samples were frequently collected from depths where the water temperature was cooler than that of the laboratory, and, while samples were returned to a cool temperature during incubation (12 °C), they were exposed to warmer temperatures (> 20 °C) during the 2 h in which they underwent collection and manipulation prior to incubation. Likewise, during this interval, samples were exposed to higher light levels before being returned to the dark for incubation. While oxygen contamination was minimized during sample collection, it was not eliminated entirely, and a temporary oxygen intrusion before sparging may have poisoned certain anaerobic processes. The 90 min sparge also likely removed carbon dioxide in addition to oxygen and \(N_2O\), increasing the pH of each sample. Finally, the \(NH_4^+\) and \(NO_3^-\) tracer and carrier additions exceeded the ambient concentrations of these substrates, potentially stimulating the rates of processes that rely on these substrates. All of these perturbations, while common among incubation studies, may have affected the microbial community differentially in each sample. Thus, the results presented here represent processes able to withstand these perturbations to ambient environmental conditions. Any abiotic reactions between the HgCl₂ preservative and the NO₃⁻ tracer and carrier would have shifted all three time points equally and thus should not introduce a bias into the slopes of \(^{15}N\)-labeled \(N_2O\) with time and the rates calculated from there.

4.6 Alternative sources of \(N_2O\)

Other processes may have contributed to \(N_2O\) production in our samples. A complementary set of experiments found that fungal denitrification comprised 50 % of total \(N_2O\) production via denitrification at the secondary chlorophyll \(a\) maximum depths discussed here (Peng and Valentine, 2021). Additionally, since our samples were unfiltered, particle-associated \(N_2O\) production and consumption may have occurred in some of our experiments, especially in experiments at the highly productive coastal station. We cannot rule out any of these alternative sources of \(N_2O\) in our samples, so we regard these processes as potential contributors to the bulk denitrifying flux discussed here.

5 Conclusions

We applied \(N_2O\) isotopocule measurements to \(^{15}N\) tracer incubations to measure \(N_2O\) production rates and mechanisms in the ETNP. We found that \(N_2O\) production rates peaked at the oxic–anoxic interface above the ODZ, with the highest rates of \(N_2O\) production from \(NO_3^-\). Hybrid \(N_2O\) production peaked in both the shallow and deep oxic zones, where \(NH_3\) oxidation was also active, and exhibited yields as high as 21 % of ammonia oxidation.

Based on the equal production of \(^{45}N_2O\alpha^\#\) and \(^{45}N_2O\beta^\#\) in the vast majority of our experiments, we posit a two-step process for hybrid \(N_2O\) production involving an initial bond-forming step that draws nitrogen atoms from each substrate to form a symmetric intermediate and a second bond-breaking step that breaks an N–O bond in the symmetric intermediate to form \(N_2O\). From this, we infer that hybrid \(N_2O\) production likely has a consistent \(\delta^{(15}N\text{sp})\), despite drawing from two distinct substrate pools. This has important implications for the interpretation of natural abundance isotopocule measurements, since it implies that it may be possible to define a \(\delta^{(15}N\text{sp})\) endmember for hybrid \(N_2O\) formation. More culture experiments are needed to quantify the \(\delta^{(15}N\text{sp})\) of \(N_2O\) produced by ammonia-oxidizing archaea under different temperatures, oxygen levels, and ratios of \(NH_4^+ / NO_3^-\).

\(N_2O\) production rates and yields of every process examined here were inhibited by dissolved oxygen. The \(N_2O\) yield from hydroxylamine oxidation was most sensitive to
O₂, followed by the rates of N₂O production from NO₂⁻ via denitrification, hybrid N₂O production, and N₂O production from NO₃⁻ via denitrification. Indeed, we measured positive, significant rates of N₂O production from NO₃⁻ at ambient [O₂] as high as 12.5 µM and at manipulated [O₂] as high as 19.2 µM. These denitrifying fluxes may have derived partially from fungal N₂O production, since fungal denitrifiers can tolerate higher oxygen levels than bacteria (Peng and Valentine, 2021) or particle-associated denitrification (Bianchi et al., 2018; Smriga et al., 2021). These results suggest that a broad window of [O₂] could support net N₂O accumulation, and additional studies are needed to further constrain this window and the resulting feedbacks between denitrification and marine deoxygenation.

Appendix A: Estimating uncertainties for nitrate isotope analyses from tracer samples

Since only 2 mL of sample was available for preparation and analysis of nitrate isotopes using the denitrifier method, it was not possible to always achieve consistent peak areas. Instead of discarding low-peak-area samples, however, we wanted to establish a method to estimate the uncertainties associated with individual samples based on their peak area. This uncertainty arises from a correction scheme for δ(N) that assumes constant blank : sample quantity ratios. What follows is a method for estimating this uncertainty, using the slope and intercept of the calibration curve and blank peak area.

In brief, the first step of this method is to calculate the peak area and δ(N) of the blank for an individual run (batch of bacteria) using the slope and intercept of the nitrate isotope calibration curve (Casciotti et al., 2002). Then, a range of theoretical measured δ(N) is calculated for a set of dummy samples based on a range of “actual” δ(N), a range of theoretical peak areas, and the peak area and δ(N) of the blank. Then, we correct each of these theoretical measured δ(N) values with the calibration curve, as one would do normally, to obtain δ(N_corrected) for each dummy sample. We estimate the error for each dummy sample by comparing the δ(N_corrected) we have calculated to the δ(N_sample) we have assigned to it. Then, for each run (and associated blank), we can fit a function through these errors, their corresponding peak areas, and the corresponding δ(N_sample). We can then feed this function the peak area and measured δ(N) of actual samples in that run to estimate their uncertainties.

In practice, we start with a simple mass balance that states that the measured δ(N) is a function of the sample δ(N), sample peak area A_sample, blank δ(N), and blank peak area A_blank:

\[
\delta^{(15)N}_{\text{measured}}(A_{\text{measured}}) = \delta^{(15)N}_{\text{sample}}(A_{\text{sample}}) + \delta^{(15)N}_{\text{blank}}(A_{\text{blank}}).
\]  

where \(\delta^{(15)N}_{\text{measured}}\) is the measured δ(N), \(A_{\text{measured}}\) is the measured peak area, \(\delta^{(15)N}_{\text{sample}}\) is the actual sample δ(N), \(A_{\text{sample}}\) is the peak area attributable to sample N, \(\delta^{(15)N}_{\text{blank}}\) is the δ(N) of the blank, and \(A_{\text{blank}}\) is the peak area attributable to blank N. Dividing through by \(A_{\text{measured}}\):

\[
\delta^{(15)N}_{\text{measured}} = \delta^{(15)N}_{\text{sample}} \left( \frac{A_{\text{sample}}}{A_{\text{measured}}} \right) + \delta^{(15)N}_{\text{blank}} \left( \frac{A_{\text{blank}}}{A_{\text{measured}}} \right).  \tag{A2}
\]

Equation (A2) can be expressed as a linear equation, \(y = mx + b\), where \(m\) is the slope of \(\delta^{(15)N}_{\text{measured}}\) vs.
\[ \delta^{(15)N_{\text{sample}}} \] and \( b \) is the y intercept. Thus

\[
m = \left( \frac{A_{\text{sample}}}{A_{\text{measured}}} \right)
\]

\[
b = \delta^{(15)N_{\text{blank}}} \left( \frac{A_{\text{blank}}}{A_{\text{measured}}} \right).
\]

We can obtain the mean blank peak area \( A_{\text{blank}} \) from the slope and the mean peak area of the measured reference materials \( (A_{\text{measured}}) \):

\[
\left( \frac{A_{\text{blank}}}{A_{\text{measured}}} \right) = 1 - \left( \frac{A_{\text{sample}}}{A_{\text{measured}}} \right) = 1 - (m)
\]

\[
A_{\text{blank}} = [1 - (m)](A_{\text{measured}}).
\]

Finally, we obtain \( \delta^{(15)N_{\text{blank}}} \) from

\[
\delta^{(15)N_{\text{blank}}} = b/ \left( \frac{A_{\text{blank}}}{A_{\text{measured}}} \right) = \frac{b}{1 - (m)}.
\]

We assign the dummy samples a range of theoretical measured peak areas, \( A_{\text{measured}} \). The ratio of the blank peak area to the measured peak area for a given sample is given by dividing \( A_{\text{blank}} \) (calculated from Eq. A6) by this theoretical peak area to obtain \( \left( \frac{A_{\text{blank}}}{A_{\text{measured}}} \right) \), where \( A_{\text{measured}} \) is the theoretical peak area for that sample. Then, the ratio of sample peak area to measured peak area for a given theoretical sample is given by

\[
\left( \frac{A_{\text{sample}}}{A_{\text{measured},i}} \right) = 1 - \left( \frac{A_{\text{blank}}}{A_{\text{measured},i}} \right).
\]

As a first example, we assign all of the theoretical samples the same \( \delta^{(15)N_{\text{sample}}} \) of 180‰. Then, to obtain a range of theoretical measured \( \delta^{(15)N_{\text{measured}}} \), we plug the \( \delta^{(15)N_{\text{blank}}} \) calculated from Eq. (A7), the range of theoretical peak areas \( A_{\text{measured},i} \), and this \( \delta^{(15)N_{\text{sample}}} \) into Eq. (A2):

\[
\delta^{(15)N_{\text{measured},i}} = 180 \text{‰} \cdot \left( \frac{A_{\text{sample}}}{A_{\text{measured},i}} \right) + \delta^{(15)N_{\text{blank}}} \left( \frac{A_{\text{blank}}}{A_{\text{measured},i}} \right).
\]

We correct the range of \( \delta^{(15)N_{\text{measured}}} \) calculated from Eq. (A9) with the slope and intercept of the calibration curve \( \delta^{(15)N_{\text{sample}}} \) vs. \( \delta^{(15)N_{\text{measured}}} \):

\[
\delta^{(15)N_{\text{corrected},i}} = m \left( \frac{A_{\text{sample}}}{A_{\text{measured},i}} \right) + b.
\]

Then we calculate the error associated with each dummy sample using

\[
\delta^{(15)N_{\text{error}}} = \left| \delta^{(15)N_{\text{corrected},i}} - 180 \text{‰} \right|.
\]

Following this exercise with a range of theoretical peak areas from 0.5 to 10 Vs produces the following curve (Fig. A1).

It shows that these theoretical errors increase as peak area decreases, reflecting the basis of the error.

Repeating this exercise with a range of \( \delta^{(15)N_{\text{sample}}} \) values from \(-20\text{‰}\) to \(180\text{‰}\) produces a 3D version of this curve (Fig. A2). This shows that the estimated uncertainty is highest for samples with \( \delta^{(15)N_{\text{sample}}} \) most divergent from \( \delta^{(15)N_{\text{blank}}} \) and for the peak areas most divergent from the reference materials.

Finally, we fit a function of the following form through these theoretical data:

\[
\delta^{(15)N_{\text{error}}} = a \cdot e^{-A_{\text{sample}}} + d \cdot \delta^{(15)N_{\text{sample}}}.
\]

where \( a, \, c, \) and \( d \) are constants, \( A_{\text{sample}} \) is the measured peak areas of the theoretical samples, and \( \delta^{(15)N_{\text{sample}}} \) is the assigned value for the dummy samples.

This procedure was repeated for each denitrifier run to produce coefficients \( a, \, c, \) and \( d \) specific to that set of analyses. Then, to estimate the uncertainty associated with each measurement, we used the corrected \( \delta^{(15)N} \) for each sample’s \( \delta^{(15)N_{\text{sample}}} \) and its measured peak area for \( A_{\text{sample}} \) in Eq. (A12).

Code and data availability. The data reported in this study can be found in the Stanford Digital Repository (https://doi.org/10.25740/ss974md4840; Kelly and Casciotti, 2023). Forward-running model code is available via Zenodo (https://doi.org/10.5281/zenodo.11475416; Kelly, 2024). pyisotopomer, which was used for \( N_2O \) isotopocule data corrections, is available for installation from the Python Package Index and Zenodo (https://doi.org/10.5281/zenodo.7552724; Kelly, 2023).

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References


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