Microbial N$_2$O consumption in and above marine N$_2$O production hotspots

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Abstract

The ocean is a net source of N$_2$O, a potent greenhouse gas and ozone-depleting agent. However, the removal of N$_2$O via microbial N$_2$O consumption is poorly constrained and rate measurements have been restricted to anoxic waters. Here we expand N$_2$O consumption measurements from anoxic zones to the sharp oxygen gradient above them, and experimentally determine kinetic parameters in both oxic and anoxic seawater for the first time. We find that the substrate affinity, O$_2$ tolerance, and community composition of N$_2$O-consuming microbes in oxic waters differ from those in the underlying anoxic layers. Kinetic parameters determined here are used to model in situ N$_2$O production and consumption rates. Estimated in situ rates differ from measured rates, confirming the necessity to consider kinetics when predicting N$_2$O cycling. Microbes from the oxic layer consume N$_2$O under anoxic conditions at a much faster rate than microbes from anoxic zones. These experimental results are in keeping with model results which indicate that N$_2$O consumption likely takes place above the oxygen deficient zone (ODZ). Thus, the dynamic layer with steep O$_2$ and N$_2$O gradients right above the ODZ is a previously ignored potential gatekeeper of N$_2$O and should be accounted for in the marine N$_2$O budget.

Introduction

Nitrous oxide (N$_2$O) is not only a greenhouse gas with about 300 times greater radiative forcing per mole than carbon dioxide, it is also the dominant ozone-depleting agent emitted in the 21st century [1]. The N$_2$O concentration in the atmosphere is increasing [2], and the rate of N$_2$O emission is accelerating [3]. From 2007 to 2016, the ocean contributed 20% of global N$_2$O emissions, and 35% of the natural sources on average [4]. N$_2$O cycling in the ocean thus has the potential to exacerbate climate change, as well as being affected by associated chemical changes, such as ocean acidification [5]. The most intense sources and sinks of N$_2$O in the ocean occur in oxygen minimum zones (OMZs) [6], which are marine regions characterized by a sharp O$_2$ gradient (oxycline) overlying an oxygen deficient zone (ODZ) where O$_2$ concentration is below the detection limit of a switchable trace oxygen (STOX) sensor (10 nM) [7]. There are multiple biological sources of N$_2$O [8–10], but there is only one major biological sink [11] (Fig. S1): the reduction of N$_2$O to N$_2$ by N$_2$O-consuming microbes using the nitrous oxide reductase enzyme (N$_2$OR). The possibility of N$_2$O fixation has been suggested [12], but its mechanism is yet to be determined. N$_2$O consumption in oxic waters, including the oxic layer of OMZs, has been ignored because this process was assumed to be part of the complete denitrification pathway (reduction of nitrate to N$_2$ gas) and to be restricted to suboxic/anoxic environments (such as ODZs) [13]. However, the oxic surface layer and the oxycline of OMZs above the ODZ could be of vital importance in regulating N$_2$O emissions if N$_2$O consumption occurs there. N$_2$O concentration in the oxycline or the anoxic ODZ of OMZs can be ≥10-fold higher than atmospheric saturation at the air–sea interface [11]. If not consumed in situ, this excess N$_2$O could diffuse through the oxycline and the surface layer or be upwelled into the surface where it can exchange with the atmosphere.

Supplementary information

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The functional marker of the operon encoding N₂OR, *nosZ*, has been used as a proxy for the presence of N₂O-consuming microbes. Recent detection of *nosZ* genes and transcripts in oxic seawater [14, 15] implies the potential for N₂O consumption there. The presence of genes and transcripts, however, does not guarantee the successful translation or activity of the enzyme. Direct rate measurements are required to determine whether this microbial potential actually results in N₂O consumption. Here, the abundance and community composition of N₂O-consuming microbes were determined by qPCR and microarray, respectively. N₂O-consuming microbes that contain only *nosZ* (i.e., none of the other genes in the complete denitrification pathway) are of particular interest, because their activity results in net N₂O consumption. Based on the analysis of 652 draft or complete microbial genomes with one or more denitrification genes, *nosZ*-only microbes are overrepresented among these isolates from the ocean compared to other ecosystems [16]. N₂O consumption rates were also measured under a matrix of controlled N₂O and O₂ concentrations in ~3000 samples collected from oxic and anoxic depths in the Eastern Tropical North Pacific (ETNP) OMZ, one of the three major oceanic OMZs. O₂ tolerance and substrate kinetics of N₂O consumption were determined and used to estimate in situ N₂O consumption and production rates, which reflect the in situ conditions more accurately than directly using measured rates from incubation experiments without correcting for substrate additions.

**Results and discussion**

**N₂O consumption rates and N₂O-consuming microbes in the OMZ**

Potential N₂O consumption rates (hereafter “measured rates”) were determined in March and April 2018 at three stations in the ETNP OMZ (Fig. 1a). Anoxic incubations were amended with standard additions of (15N)₂O tracer with a final concentration of 50 nM at stations PS1 (on the west margin of the OMZ), PS2 (the open ocean station) and PS3 (the coastal station). Measured N₂O consumption rates varied from zero to 5.1 nM d⁻¹ at different depths (Fig. 1c, f, i). Measured rates in oxygen deficient waters were on the same order of magnitude (a few nM d⁻¹) as previously measured rates in the ETNP, but lower than rates at one coastal station in that study [6], indicating high variability of N₂O cycling in the coastal regions as previously suggested [17]. Notably, significant N₂O consumption rates were measured in these anoxic incubations, even in samples collected from the oxycline and the oxygenated surface ocean (in situ [O₂] up to 199 µM, Tables S1, S2), where N₂O-consuming microbes were present and the *nosZ* gene was transcribed (Fig. 1d, g, j). In the upper water column, measured N₂O consumption rates were highest in the upper oxycline, above the peak of in situ N₂O concentrations at each station (Fig. 1b, e, h), and the rate maximum at station PS2 was detected in samples collected from 60 m where in situ O₂ was 173.9 µM (Tables S1, S2). The rates of N₂O consumption measured in surface waters stripped of oxygen were similar to or larger than rates measured in the ODZ. The larger consumption rates in the oxic layer given the same N₂O and O₂ concentrations as the ODZ layer might be due to more available dissolved organic matter at the shallower depths compared to the deeper ODZ layer [18] and/or the presence of different microbial communities at these depths.

N₂O-consuming microbes in the oxic surface water and oxycline were at least as abundant (DNA) and transcriptionally active (in terms of *nosZ* RNA abundance) as those in ODZs (Fig. 1d, g, j). Diverse archetypes of N₂O-consuming microbes were detected in the oxic water above the ODZ at three stations in the ETNP, one station in the Arabian Sea, and were previously detected at two stations in the Eastern Tropical South Pacific (ETSP) (Fig. 2a) using a *nosZ* microarray. The microarray is not quantitative, but it can detect diverse, low abundance microbes from environmental microbial assemblages. Even though the microarray cannot represent every *nosZ* variant, the probe set (which includes marine, salt marsh, and terrestrial representatives) allowed us to determine that the community composition of the ETSP N₂O-consuming microbial assemblages differs from the other two OMZs (Fig. 2a). Within the ETNP, the community composition of *nosZ* microbes at the RNA level differed between oxic and ODZ waters (Fig. 2b). The detection of N₂O consumption in samples from the oxycline and the oxygenated surface seawater of the ETNP OMZ, and the presence of N₂O-consuming microbes in all three major OMZs (Fig. 2a), indicate that microbes in the oxic layer above ODZs have the capacity to consume N₂O at least under anoxic incubation conditions.

**O₂ tolerance of N₂O-consuming microbes**

The O₂ tolerance of N₂O-consuming microbes was determined by incubating seawater under a range of O₂ conditions and measuring the O₂ concentration corresponding to each rate using Pyroscience optical O₂ sensors. N₂O consumption rates were highest when O₂ was lowest in almost all incubations (Fig. 3). The highest rates were measured in anoxic incubations with samples from the oxycline and the oxygenated surface seawater of the ETNP OMZ, and the presence of N₂O-consuming microbes in all three major OMZs (Fig. 2a), indicate that microbes in the oxic layer above ODZs have the capacity to consume N₂O at least under anoxic incubation conditions.
than anaerobic organisms when conditions become anoxic (Fig. 1d, g, j). At stations PS1 and PS3, the higher potential of N$_2$O consumption also corresponds to higher nosZ gene copy numbers in the oxic layer (Fig. 1d, j). N$_2$O consumption rates in samples from oxic seawater, but not the ODZ, decreased sharply with increasing O$_2$, indicating that N$_2$O-consuming microbes from oxic seawater (Fig. 3a–c) were more sensitive to O$_2$ than those from anoxic seawater (Fig. 3d–g). Although N$_2$O consumption in samples from the oxic layer did not occur at high O$_2$ concentrations, it started rapidly (<1 day) after transitioning from oxic in situ conditions to anoxic incubation conditions (Fig. S2). The speed of this response might be due to the growth of N$_2$O-consuming microbes, fast enzyme (N$_2$OR) translation, or a response by already translated N$_2$OR in the oxic seawater prior to sampling.

While our rate measurements suggest that N$_2$OR is not active under oxic conditions, molecular data obtained here (Fig. 1d, g, j) and previously [15] show that both nosZ RNA and N$_2$OR can be synthesized under oxic conditions. This phenomenon is seen in other environments as well, for example, an obligate aerobe from soil requires O$_2$ to initiate
nosZ expression, and can use the N$_2$OR enzyme to consume N$_2$O to survive temporary anoxia [19]. Another microbial culture continually makes N$_2$OR and stores the enzymes inside their cells under oxic conditions, which was proposed as a “bet-hedging” strategy by this facultative anaerobe to allow for a rapid transition into anoxic environments [20]. Regardless of the mechanism of the rapid response, our results indicate that microbes from oxic seawater have the genetic potential to consume N$_2$O, that the consumption was not limited by organic matter supply (in situ limitation by organic matter would prevent the observed increase in rate with increasing N$_2$O concentration, Fig. 4a–c) and that they could consume N$_2$O under anoxic conditions. These anoxic conditions could occur at a small scale in otherwise oxic water; for example they could be associated with phytoplankton colonies [14] and other particles [21, 22], especially in the productive and dynamic oxyclines of the OMZ with strong O$_2$ and N$_2$O gradients at shallow depths.

Substrate affinity of N$_2$O-consuming microbes

In incubations without O$_2$, measured $K_m$ values (half-saturation constants of the Michaelis–Menten curve) (Fig. 4) for N$_2$O consumption were in excess of in situ N$_2$O concentrations at every station and depth (Fig. 1b, e, h), indicating that the in situ N$_2$O concentrations were too low to saturate the N$_2$O consumption rate. Notably, the potential maximum rate of N$_2$O consumption in the oxic layer, upon removal of O$_2$, was much higher than that in the ODZ at the same station (Fig. 4), and the substrate affinities of N$_2$O-consuming microbes were distinct between oxic and anoxic depths (Fig. 4). Consistently, the community composition of N$_2$O-consuming microbes at the RNA level was also different between oxic layers and anoxic ODZs of the ETNP (Fig. 2b). These results indicate that different kinetics parameters should be applied to the estimate of the anoxic N$_2$O sink and the newly discovered potential N$_2$O sink in the oxic layer. The difference in substrate affinities is likely due to diverse N$_2$O-consuming microbes occupying different niches, because their affinities for N$_2$O can vary by two orders of magnitude as shown in pure cultures [23]. This difference might be obscured when microbes in different layers are mixed up by physical processes such as upwelling and eddies.

Significant $K_m$ for N$_2$O consumption could be determined for the oxic layer at station PS2 and the oxic–anoxic interface at station PS3 (Fig. 4b, e). The lack of Michaelis–Menten kinetics in samples from anoxic ODZs (Fig. 4f, g) implies that factors (e.g., organic matter) other than the added substrate were limiting N$_2$O consumption. $K_m$ for the oxic layer at station PS3 was likely larger than that of station PS2, because the rate was not saturated even at the maximum N$_2$O concentration (Fig. 4c). As for station PS1, the decreasing rate at the 4th datapoint suggests a mixture of N$_2$O-consuming microbes with different substrate affinities (Fig. 4a). Although not significant, $K_m$ was 110 (±230) nM when only including the first four data-points. When excluding the 4th datapoint, $K_m$ was 1354 (±653) nM for the oxic layer at station PS1. Larger $K_m$ values in samples from the oxic layer indicate that microbes there have lower affinity for N$_2$O.

Notably, nosZ archetypes closely related to A. dehalogenans were among the top five most abundant archetypes at almost all examined depths from the ETNP (Tables S4, S5), ETSP [15], and Arabian Sea (Table S6), implying the importance of A. dehalogenans-like N$_2$O-consuming microbes in both oxic layers and ODZs of all major OMZs. Furthermore, the $K_m$ (1.3 μM) of Anaeromyxobacter dehalogenans determined in cultures [23] was within the range of the $K_m$ values determined in this study (2.8 μM in the oxic layer and 0.3 μM at the top of the ODZ, Fig. 4). Different overall community composition, but similar affiliation of the most abundant archetypes, implies that the difference in community composition between the oxic and ODZ assemblages in the ETNP (Fig. 2b) and the difference between the ETSP and the other two OMZs (Fig. 2a) results

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**Fig. 2** Detrended correspondence analysis (DCA) of nosZ DNA and nosZ RNA transcripts. a N$_2$O-consuming microbes from all three major OMZs (ETNP, ETSP (Eastern Tropical South Pacific) and AS (Arabian Sea)). Arabian Sea samples include two samples from the oxic layer above the ODZ and two inside the ODZ at station 1 (19N, 66E) collected on a previous cruise [34], and ETSP samples include four samples from oxic layers and four inside ODZs. ETSP data were obtained from a previous study [15]. b nosZ data from the ETNP only.
from a diversity of low abundance microbes rather than a few abundant clades. The microarray probes cannot identify exact species, but can differentiate among archetypes representing unknown microbes, such as the A. dehalogenans-like N2O-consuming types that were detected in most of these samples. The vital role of A. dehalogenans-like microbes and other microbes in the same clade has been demonstrated in soils [24, 25]. Because A. dehalogenans possesses nosZ but no other denitrification genes [26], A. dehalogenans-like microbes may decouple N2O consumption from its production, resulting in a net N2O sink at depths where they dominate N2O-consuming assemblages.

In addition to anoxic incubations, we also examined N2O consumption kinetics in oxic incubations. Consistent with the low O2 tolerance of N2O-consuming microbes, especially in the oxic layers (Fig. 3), the kinetics of N2O consumption could not be determined under most incubations with O2 additions because N2O consumption rates were not detected (Fig. 4). Only samples from the oxic–anoxic interface at station PS3 showed Michaelis–Menten kinetics in the oxic incubation (Fig. 4e), likely because the O2 addition in this incubation (4.5 μM, Table S3) was less than all the other oxic incubations (8.1–342.0 μM, Table S3) and N2O-consuming microbes in ODZs had higher tolerance to increasing O2 concentration than those from the oxic layer (Fig. 3e). The O2 concentration (≥4.5 μM) allowing the occurrence of N2O consumption (N2O → N2) here was much higher than the previously determined threshold (0.2–0.3 μM) for denitrification (NO2− → N2O or N2) [27], which might be due to differential O2 sensitivities of microbes possessing different parts of the denitrification pathway. Variable oxygen sensitivities have also been observed in O2 thresholds for N2O production from NO2− and NO3−, the latter showing a higher tolerance to O2 [10, 28]. In addition, unlike the presence of nosZ in the oxic layers, the group of denitrifiers represented by nitrite reductase genes (nirK and nirS) were very rare in the oxic layers [29]. These observations suggest that denitrification can be carried out in a
modular fashion by independent organisms possessing different segments of the pathway [16], rather than one process of coupled reactions that occur without exchange of intermediates. Modular denitrification may have implications for the interpretation of classical isotope pairing experiments.

Estimated in situ N₂O consumption and production rates

Rates of biogeochemical processes inferred from incubation experiments can be biased away from in situ values due to the dependence of rates on substrate concentrations and other environmental factors (e.g., O₂ concentration), which often differ between in situ and incubation conditions. However, the new quantitative information on N₂O consumption kinetics and effects of environmental factors like O₂ derived here can be used to estimate in situ rates. Using these kinetic parameters most in situ N₂O consumption rates were inferred to be zero in oxic layers (Fig. 1c, f, i) based on the high sensitivity of those microbes to O₂ (Fig. 3a–c). The exception was 90 m at station PS2 where the in situ O₂ concentration (4.4 µM, Table S1) was likely to be low enough to allow N₂O consumption based on similar kinetics in anoxic incubations and incubations at PS3 with 4.5 µM O₂ (Fig. 4e). In situ N₂O consumption rates in anoxic ODZs were simulated by the Michaelis–Menten equation using the Km value determined here (Fig. 4e) and the in situ N₂O concentrations (Fig. 1b, e, h). Measured N₂O consumption rates had maxima in the upper oxycline above the N₂O concentration peaks at all three stations, but the maxima in estimated in situ rates at these stations occurred at or below the oxic–anoxic interface, and the highest rate at station PS2 (6.3 nM d⁻¹) was at 850 m, the lower edge of the ODZ (Fig. 1c, f, i). The secondary peak of N₂O consumption
inside the ODZ at station PS2 was greatly reduced after corrections were made using in situ N₂O concentrations due to low in situ N₂O concentrations (Fig. 1e). The peak inside the ODZ at station PS1, however, was larger after correction because the in situ N₂O concentration (~80 nM, Fig. 1b) was higher than that in incubations (50 nM). The persistently high N₂O concentration in the ODZ core at station PS1 reflects the slow N₂O removal by denitrification at the margin of the OMZ. The lack of a SNM, a typical feature for anoxic ODZs, at station PS1 (Fig. S3) is also consistent with its position at the oceanic edge of the OMZ. The difference between measured rates and kinetics-corrected in situ rates indicates the need for more information on the kinetics of N₂O consumption under different environmental conditions and in different OMZ regions.

In situ N₂O production rates were modeled from estimated in situ N₂O consumption rates, N₂O concentrations, advection, and diffusion using a 1-D steady-state framework (Fig. 5), which reflects a weak lateral advection and upwelling scenario as in a previous study [6]. Production and consumption rates were mostly balanced but were decoupled at the sharp N₂O concentration gradient at station PS3. The decoupling of the production and consumption was due to the strong N₂O fluxes from physical processes (i.e., advection and diffusion) in the sharp N₂O gradient coinciding with the sharp O₂ gradient. This decoupling was not reported previously because measurements of N₂O consumption were all below the bottom of the upper oxycline [6]. Notably, the modeled production rates in the oxic layer at stations PS1 and PS3 (Fig. 5) with 199.0 and 89.9 µM in situ O₂ were negative considering advection, diffusion and zero estimated consumption rates (Table S1). Since the production rate cannot be negative, this analysis suggests that N₂O consumption occurs at least sometimes at these depths to balance the N₂O flux from physical processes (assuming steady state). Consistent with the model results, N₂O consumption rates were detected when oxygen is above the 4.5 µM threshold especially at station PS3 (Fig. 3c, e, j). The significant rates under oxic conditions might imply more micro-anoxic sites (e.g., particulate organic matter) at the coastal station. Although we chose 4.5 µM as a conservative oxygen threshold here, different thresholds for N₂O consumption need to be determined for different environmental conditions in future studies. Particles and other microsites might disintegrate during sampling and purging, so the in situ N₂O consumption rates in the oxic layer were potentially underestimated by the incubation experiments.

Implications for the oceanic N₂O budget

The annual N₂O emission rate of the ocean is 3–5 Tg-N yr⁻¹ based on recent estimates [4, 30]. Developing an understanding of what controls the major biological sink of N₂O is vitally important to better constrain estimation of the highly uncertain [17] marine N₂O budget. Using direct rate measurements, we demonstrated the ability of microbial assemblages in the oxic layer above N₂O production hotspots to consume N₂O under anoxic conditions and quantified the rate dependence on N₂O and O₂ concentrations. The potential N₂O consumption rate in oxic seawaters was at least two orders of magnitude faster than that in ODZs under favorable conditions.
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board, and then preserved at −80 °C until DNA and RNA extraction in the lab. Seawater samples for determining N₂O consumption rate were collected into 320 mL ground glass-stoppered glass bottles after overflowing three times to minimize O₂ contamination. Seawater (8 mL) was then aliquoted into 12 mL exetainers inside a N₂ flushed glove bag to leave a 4 mL headspace for purging. After sealing in the glove bag, exetainers were purged with helium for 5 min to reach anoxia for depth profiles shown in Fig. 1 and kinetics determined under anoxic conditions shown in Fig. 4. (¹⁵N)₂O tracer (Cambridge Isotope Laboratories, purity ≥98%) was added as gas into each exetainer using a helium-flushed gas-tight glass syringe to reach a final concentration of 50 nM N₂O (standard additions shown in Figs. 1, 3) or varying from 50 to 1978 nM N₂O for kinetics experiments in Fig. 4. A 50 nM N₂O addition was made to ensure the produced N₂ was detectable. A set of 15 exetainers incubated in a time series (triplicates for each time point, 5 time points in total including three time zero bottles as abiotic controls) was used to determine a single rate. Incubations were sampled approximately every 12 h for 2 days and were terminated by adding 0.05 mL of 50% (w/v) ZnCl₂ following previous procedures [6]. The amount and isotopic composition of N₂ in each exetainer was measured on a mass spectrometer (Europa Scientific 20-20, Crewe, UK), and the rate of N₂O consumption was calculated from the linear regression of the excess of ³⁰N₂ over the incubation time following the previous method [34].

N₂O consumption rates were measured at 10 depths at each station, including oxic surface water, upper and lower oxycline, top of the anoxic ODZ (oxic–anoxic interface), and core of the ODZ. The position of the upper oxycline varies among the three stations (station PS2: 59–96 m; station PS3: 48–84 m; station PS3: 0–35 m). The lower oxycline starts at around 700 m for station PS1, 850 m for station PS2, and 860 m for station PS3. N₂O kinetics and O₂ tolerance experiments were performed in the oxic layer, at the top of the ODZ (oxic–anoxic interface), and in the core of the ODZ. Ambient O₂ concentrations at both the top and the core of the ODZ were below detection limit of the Seabird sensor (Table S3). The kinetics of N₂O consumption were determined by measuring rates with varying added (¹⁵N)₂O concentrations (50, 99, 198, 371, 865, and 1978 nM). Different O₂ concentrations in incubations investigating the O₂ tolerance of N₂O consumption in Figs. 3, 4 were achieved prior to initiation of the experiment by varying flow rates of O₂ and helium gases using a custom-assembled gas flow manifold with two gas flow meters on board. The O₂ concentration for each set of exetainers was monitored by direct measurement using optical oxygen sensors with a detection limit of 0.06 µM (PyroScience GmbH, Aachen, Germany), and is shown on the x-axis in Fig. 3.

**Kinetics models and in situ rate estimation**

Half-saturation constant (kₘ) and the maximum rate (vₘ) were determined by fitting N₂O consumption rate and N₂O concentration data to the Michaelis–Menten equation (Eq. (1)). kₘ is the N₂O concentration at which the rate (V) equals half of Vₘ. Fitting was performed by the curve fitting tool in Matlab. The 95% confidence interval was used to determine whether a parameter is significantly different from zero. The half-inhibition constant (kᵢ), analogous to kₘ, is the O₂ concentration that causes half of the potential maximum inhibition (Iₘ). kᵢ was calculated from fitting an inhibition curve (Eq. (2)). The unit of V or Vₘ is nm d⁻¹, the unit of [N₂O] or kₘ is nM, the unit of [O₂] or kᵢ is µM and Iₘ is unitless.

\[
V = Vₘ \times \frac{[N₂O]}{[N₂O] + kₘ}.
\]

(1)

\[
\frac{V}{Vₘ} = 1 - Iₘ \times \frac{[O₂]}{[O₂] + kᵢ}.
\]

(2)

The in situ N₂O consumption rate was estimated from in situ O₂ concentration, in situ N₂O concentration, and calculated kₘ and Vₘ. First, kₘ in Fig. 4, measured rates (V) and measured [N₂O] were used to calculate Vₘ for each depth based on Eq. (1). kₘ and Vₘ determined in samples from the oxic layer were used to calculate in situ rates in oxic seawaters, and those determined in samples from the ODZ were used in ODZ rate estimation. Then, in situ rates were set to zero if in situ O₂ concentrations were above a threshold level of 4.5 µM. This threshold was chosen because it was the highest oxygen concentration at which N₂O consumption was dependent upon N₂O concentration i.e., N₂O consumption showed similar kinetics in response to N₂O concentration under undetectable O₂ concentration and at 4.5 µM O₂ (Fig. 4e), but not at higher O₂ concentrations. This is a conservative threshold because we did not determine the absolute highest O₂ level that allowed N₂O consumption.

In situ N₂O production rate was estimated by subtracting the advection and diffusion of N₂O from in situ consumption rates assuming a steady state (Eq. (3)). v (1 × 10⁻⁷ m s⁻¹) is advection coefficient and D (2 × 10⁻⁵ m² s⁻¹) is the diffusivity coefficient. Coefficients and the steady-state 1-D model follow (Babbin et al.) [6].

\[
\text{Production rate} = \text{Consumption rate} - v \times (\partial [N₂O]/\partial \text{depth}) - D \times (\partial^2 [N₂O]/\partial \text{depth}^2).
\]

(3)

A mechanistic 1-D biogeochemistry model [6] was updated based on the experimental results in this study to assess the effect of different O₂ thresholds on N₂O predictions (Fig. S6). Briefly, the model was built upon the
balance of physical and biological processes at steady state. Physical processes in the model include advection, diffusion, and gravitational sinking. Biological processes include aerobic respiration (i.e., remineralization fueled by O₂), nitrification, and denitrification. The production of N₂O from both nitrification and denitrification, and the consumption of N₂O from denitrification are considered. The O₂ threshold for N₂O consumption is 0.3 µM in the original model and 4.5 µM in the updated model. The O₂ threshold for N₂O production via denitrification is 1 µM in the original model and 20 µM in the updated model. The only differences between the updated model and the original model are the O₂ threshold values. Model code, detailed evaluation of the model and all the other parameters of the model are available in the previous study [6].

DNA and RNA extraction, quantitative PCR (qPCR) assays, and nosZ microarray

These experiments were performed as previously described [15]. Briefly, DNA and RNA were extracted from Sterivex filters including four filters collected from a previous cruise in the Arabian Sea OMZ [34]. Each DNA or RNA copy number value corresponds to one Sterivex. RNA was reverse transcribed into cDNA. qPCR was used to estimate the abundance of total and transcribed nosZ assemblages using the nosZ1F (5′-WCSYTGTTCMTGACACAGCCAG-3′) and nosZ1R (5′-AGTGTCATCARCTGVKCRTTYTC-3′) primer set [35]. The qPCR products were purified from agarose gels and then used as targets for microarray experiments following a previous protocol [36]. The detection limit of qPCR is 18.1 copies mL⁻¹. The microarray contains 114 nosZ archetype probes and the sequences of all probes are published in the supplementary dataset in a previous study [15]. The fluorescence ratio of each archetype on the microarray is defined as the ratio of Cy3 to Cy5 fluorescence. Normalized fluorescence ratio (FRn) was calculated by dividing the fluorescence ratio of each archetype by the maximum fluorescence ratio on the same microarray. FRn is used as a proxy for the relative abundance of each nosZ archetype. FRn was used to determine the top five most abundant archetypes in each sample. Detrended correspondence analysis was performed on FRn to analyze the community composition of N₂O-consuming assemblages using the vegan package in R (version 3.6.0). Identification of nosZ sequences is limited by the probe selection on the array and the larger database of nosZ sequences now available might help identify the oxic nosZ more precisely. Nonetheless, the limited database represented on the array sufficed to detect significant differences among samples and to identify phylogenetic affinities of OMZ nosZ genes.

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Author contributions XS and BBW conceptualized the research. XS, BBW, and JCT sampled and performed incubation experiments on board. CLK and KLC sampled and measured N₂O concentrations. AJ collected microbial samples, extracted DNA, and performed qPCR and microarray experiments. XS and EW assisted with DNA extraction and qPCR experiments. XS, AJ, and BBW analyzed microbial data. XS measured N₂O consumption rates, analyzed the data, and modeled the rates. XS and BBW wrote the paper with inputs from all authors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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