

# Investigating heterogeneity in nitrous oxide cycling of the Eastern Tropical North Pacific through isotopocules

Patrick Joseph Monreal<sup>1,1</sup>, Colette LaMonica Kelly<sup>2,2</sup>, Nicole Mayu Travis<sup>2,2</sup>, and Karen L Casciotti<sup>3,3</sup>

<sup>1</sup>Stanford University

<sup>2</sup>Stanford University, Department of Earth System Science

<sup>3</sup>Stanford University

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## Abstract

Nitrous oxide (N<sub>2</sub>O) is a powerful greenhouse gas, and oceanic sources account for up to one third of total flux to the atmosphere. In oxygen-deficient zones (ODZs) like the Eastern Tropical North Pacific (ETNP), N<sub>2</sub>O can be produced and consumed by several biological processes that are controlled by a variety of oceanographic conditions. In this study, the concentration and isotopocule ratios of N<sub>2</sub>O from a 2016 cruise to the ETNP were analyzed to examine heterogeneity in N<sub>2</sub>O cycling across the region. Along the north-south transect, three distinct biogeochemical regimes were identified: background, core-ODZ, and high-N<sub>2</sub>O stations. Background stations were characterized by less dynamic N<sub>2</sub>O cycling. Core-ODZ stations were characterized by co-occurring N<sub>2</sub>O production and consumption at anoxic depths, indicated by high  $\delta^{18}\text{O}$  (> 90‰), and confirmed by a time-dependent model, which indicated that N<sub>2</sub>O production via denitrification was significant and may occur with a non-zero site preference. High-N<sub>2</sub>O stations were defined by [N<sub>2</sub>O] reaching  $126.07 \pm 12.6$  nM, low oxygen concentrations expanding into near-surface isopycnals, and the presence of a mesoscale eddy. At these stations, model results indicated significant N<sub>2</sub>O production from ammonia-oxidizing archaea and denitrification from nitrate in the near-surface N<sub>2</sub>O maximum, while bacterial nitrification and denitrification from nitrite were insignificant. This study also represents the first in the ETNP to link N<sub>2</sub>O isotopocule measurements to a mesoscale eddy, suggesting the importance of eddies to the spatiotemporal variability in N<sub>2</sub>O cycling in this region.

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Patrick J. Monreal<sup>1</sup>, Colette L. Kelly<sup>1</sup>, Nicole M. Travis<sup>1</sup>, and Karen L. Casciotti<sup>1</sup>

<sup>1</sup>Stanford University, Department of Earth System Science, Stanford, CA

Corresponding author: Patrick J. Monreal ([pmonreal@stanford.edu](mailto:pmonreal@stanford.edu))

**Key words:** Nitrogen cycling, Greenhouse gases, Anoxic environments, Stable isotopes, Climate and interannual variability

## Key Points:

- Nitrous oxide isotopocules revealed distinct cycling regions throughout the Eastern Tropical North Pacific's oxygen-deficient zone
- Archaeal nitrification and bacterial denitrification from nitrate were important sources to near-surface nitrous oxide accumulations
- A mesoscale eddy at the site of high nitrous oxide concentrations suggests the importance of eddies to the area's spatiotemporal variability

## Abstract

Nitrous oxide (N<sub>2</sub>O) is a powerful greenhouse gas, and oceanic sources account for up to one third of total flux to the atmosphere. In oxygen-deficient zones (ODZs) like the Eastern Tropical North Pacific (ETNP), N<sub>2</sub>O can be produced and consumed by several biological processes that are controlled by a variety of oceanographic conditions. In this study, the concentration and isotopic ratios of N<sub>2</sub>O from a 2016 cruise to the ETNP were analyzed to examine heterogeneity in N<sub>2</sub>O cycling across the region. Along the north-south transect, three distinct biogeochemical regimes were identified: background, core-ODZ, and high-N<sub>2</sub>O stations. Background stations were characterized by less dynamic N<sub>2</sub>O cycling. Core-ODZ stations were characterized by co-occurring N<sub>2</sub>O production and consumption at anoxic depths, indicated by high  $\delta^{18}\text{O}$  (> 90‰) and low  $\delta^{15}\text{N}^{\beta}$  (< -10‰) values, and confirmed by a time-dependent model, which indicated that N<sub>2</sub>O production via denitrification was significant and may occur with a non-zero site preference. High-N<sub>2</sub>O stations were defined by [N<sub>2</sub>O] reaching 126.07±12.6 nM, low oxygen concentrations expanding into near-surface isopycnals, and the presence of a mesoscale eddy. At these stations, model results indicated significant N<sub>2</sub>O production from ammonia-oxidizing archaea and denitrification from nitrate in the near-surface N<sub>2</sub>O maximum, while bacterial nitrification and denitrification from nitrite were insignificant. This study also represents the first in the ETNP to link N<sub>2</sub>O isotopic measurements to a mesoscale eddy, suggesting the importance of eddies to the spatiotemporal variability in N<sub>2</sub>O cycling in this region.

## Plain Language Summary

Nitrous oxide (N<sub>2</sub>O) — commonly known as laughing gas — is a greenhouse gas nearly 300 times more potent than carbon dioxide, and microbes in the ocean produce and consume N<sub>2</sub>O through different processes called nitrification and denitrification. Regions of the ocean with little to no oxygen near the surface, like the Eastern Tropical North Pacific, are hot spots of marine N<sub>2</sub>O emissions. By measuring and modeling the isotopes of N<sub>2</sub>O and related molecules in water samples from the region, we were able to better understand what processes are at play and where. N<sub>2</sub>O production and consumption through denitrification appeared to both be important at depths with no oxygen. N<sub>2</sub>O production from denitrification and nitrifying archaea were important sources to high N<sub>2</sub>O concentrations. Untangling the various processes in the marine N<sub>2</sub>O cycle helps researchers predict how the cycle will respond to future climate trends.

## 1 Introduction

Nitrous oxide (N<sub>2</sub>O) is a powerful greenhouse gas with a per-molecule warming potential nearly 300 times greater than that of carbon dioxide (Smith et al., 2021). It is also projected to remain the most important stratospheric ozone-depleting substance throughout the 21st century (Ravishankara et al., 2009; Wuebbles, 2009). Marine sources constitute up to one-third of the total flux of N<sub>2</sub>O into the atmosphere (Ciais et al., 2013; Denman et al., 2007; Freing et al., 2012; Hirsch et al., 2006; Ji et al., 2018), and the three major oxygen-deficient zones (ODZs) — the Eastern Tropical North Pacific (ETNP), Eastern Tropical South Pacific (ETSP), and the Arabian Sea — are dominant sites of N<sub>2</sub>O cycling (Yang et al., 2020). The sharp redox gradients and nanomolar levels of oxygen (O<sub>2</sub>) in the core of the ODZ (Revsbech et al., 2009; Thamdrup et al., 2012; Tiano et al., 2014) promote conditions for several processes that produce and consume N<sub>2</sub>O (Codispoti & Christensen, 1985; Codispoti, 2010; Cohen & Gordon, 1979; Elkins et al., 1978; Law & Owens, 1990; Suntharalingam et al., 2000). With the greenhouse effects of N<sub>2</sub>O in

mind, constraining the sources and sinks of N<sub>2</sub>O in ODZs is of interest and important to predicting how the marine N<sub>2</sub>O cycle will react to climate trends, such as the expansion of ODZs and deoxygenation of the global ocean (Frölicher et al., 2020; Horak et al., 2016; R. F. Keeling et al., 2010).

Nitrous oxide is produced and consumed by a number of biological processes. In oxygenated waters like the surface ocean, ammonia-oxidizing bacteria (AOB) produce N<sub>2</sub>O from ammonia via side-reactions of the nitrification processes that produce nitrite (NO<sub>2</sub><sup>-</sup>; Cohen & Gordon, 1979; Dore et al., 1998; Nevison et al., 2003), and from nitrite via nitrifier-denitrification (Stein & Yung, 2003; Wrage et al., 2001). Ammonia-oxidizing archaea (AOA) produce N<sub>2</sub>O from ammonia (and possibly also nitrite) via one or more mechanisms whose enzymology remains unknown (Kozłowski et al., 2016; Santoro et al., 2011; Stein, 2019; Stieglmeier et al., 2014; Trimmer et al., 2016). In low-oxygen waters like the core of the ODZ, denitrifying organisms sequentially reduce NO<sub>3</sub><sup>-</sup> and nitrite (NO<sub>2</sub><sup>-</sup>) to nitric oxide (NO), N<sub>2</sub>O, and dinitrogen gas (N<sub>2</sub>; Babbin et al., 2014; Bourbonnais et al., 2017; L. A. Codispoti & Christensen, 1985). While N<sub>2</sub>O can be both produced and consumed under anoxic conditions, the enzyme involved in N<sub>2</sub>O consumption via denitrification is more sensitive to O<sub>2</sub> poisoning than that which produces N<sub>2</sub>O, leading to a decoupling of the processes and net production at the oxic-suboxic interface of the oxycline (Babbin et al., 2015; Farías et al., 2009; Körner & Zumft, 1989). Together, these ODZ processes typically lead to N<sub>2</sub>O concentrations ([N<sub>2</sub>O]) that are at or below saturation in the anoxic core but high in near-surface waters.

Given the complex interactions of pathways and substrates, the stable isotopes of nitrogen and oxygen — <sup>15</sup>N and <sup>18</sup>O — in N<sub>2</sub>O are vital to understanding the contribution of each process to a particular pool of N<sub>2</sub>O (Farías et al., 2009; Kim & Craig, 1990; Rahn & Wahlen, 2000; Toyoda & Yoshida, 1999; Yamagishi et al., 2007). Because the two nitrogen atoms are in unique chemical environments within the N<sub>2</sub>O molecule, site-specific isotope ratios of the inner (α) and outer (β) nitrogen atoms may differ in isotopic composition and provide additional information about the sources and cycling of N<sub>2</sub>O in the environment (see Toyoda et al., 2017). Isotopic values are expressed in terms of delta notation, defined as  $\delta^{15}\text{N}$  or  $\delta^{18}\text{O} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$ , where  $R_{\text{sample}}$  is the ratio of <sup>15</sup>N/<sup>14</sup>N or <sup>18</sup>O/<sup>16</sup>O in each sample and  $R_{\text{standard}}$  is that of air or Vienna Standard Mean Ocean Water (VSMOW), respectively (Kim & Craig, 1990; Rahn & Wahlen, 2000; Toyoda & Yoshida, 1999). In this study, bulk nitrogen and oxygen isotope ratios are represented by  $\delta^{15}\text{N}_{2\text{O}^{\text{bulk}}}$  and  $\delta^{18}\text{O}-\text{N}_2\text{O}$ , respectively, while the site-specific ratios of nitrogen are represented by  $\delta^{15}\text{N}_{2\text{O}^{\alpha}}$  and  $\delta^{15}\text{N}_{2\text{O}^{\beta}}$ .

The difference between  $\delta^{15}\text{N}_{2\text{O}^{\alpha}}$  and  $\delta^{15}\text{N}_{2\text{O}^{\beta}}$ , referred to as site preference (SP), is an important diagnostic tool. Preferential breakage of the O-N<sup>α</sup> bond during N<sub>2</sub>O consumption has predictable effects on  $\delta^{15}\text{N}_{2\text{O}^{\alpha}}$  versus  $\delta^{18}\text{O}-\text{N}_2\text{O}$  (Ostrom et al., 2007). Furthermore, due to differences in biochemical mechanism, each N<sub>2</sub>O production process is thought to have a specific SP independent of the isotopic values of the substrate. Thus, SP provides insight into how an N<sub>2</sub>O pool was formed (Frame & Casciotti, 2010; Schmidt et al., 2004; Sutka et al., 2003, 2006; Toyoda et al., 2002, 2005, 2019; Yoshida & Toyoda, 2000). For example, preferential breakage of the <sup>14</sup>N-O bond in the hyponitrite (O-N=N-O) intermediate of bacterial and fungal nitrification gives it a high SP of 30-36‰ (Frame & Casciotti, 2010; Sutka et al., 2006, 2008). On the other hand, a low SP of -1-11‰ is observed in N<sub>2</sub>O produced by denitrification and nitrifier-denitrification (Frame & Casciotti, 2010; Sutka et al., 2006), although the exact mechanism remains unclear (Schmidt et al., 2004; Toyoda et al., 2005).

Indeed, while SP remains a useful measurement in constraining the source and sinks of N<sub>2</sub>O cycling, a few studies have challenged its ability to serve as a clear diagnostic marker. The potential hybrid nature of archaeal nitrification (Kozłowski et al., 2016; Stein, 2019; Stieglmeier et al., 2014) may undermine the notion of an expected SP for this particular process (Casciotti et al., 2018; Kelly et al., 2021), even though N<sub>2</sub>O produced by AOA has been recorded to have an SP of about 30‰ (Löscher et al., 2012; Santoro et al., 2011). Additionally, one strain of denitrifying bacteria has been found to produce N<sub>2</sub>O with SP = 23.3±4.2‰ (Toyoda et al., 2005), and recent studies have suggested that a nonzero SP for denitrification could explain observed isotopic trends in the ETSP (Casciotti et al., 2018), ETNP (Kelly et al., 2021), and the bottom waters of Baffin Bay (Lehmann et al., 2019). Because of these complicating factors, in this study we model and interpret  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{15}\text{N}_2\text{O}^\beta$  as distinct tracers, which provides more information than interpreting site preference alone.

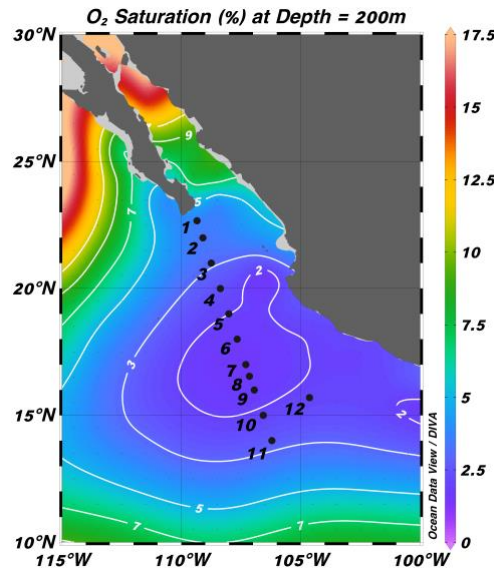
This study addresses core questions surrounding N<sub>2</sub>O cycling in ODZs: how does the mix of N<sub>2</sub>O cycling processes change throughout the water column, what factors contribute to this heterogeneity, and how can isotopic measurements help constrain these processes? We measured concentration and isotope ratios of N<sub>2</sub>O, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> in the ETNP — the geographically broadest ODZ — and leveraged these measurements in time-dependent models of denitrification and nitrification. A Nelder-Mead optimization was applied to determine the rates of N<sub>2</sub>O production and consumption that best explain N<sub>2</sub>O observations for a given set of isotope effects. A nonzero SP for denitrification was tested in some model runs to determine if isotopic trends can be recreated with this process. Finally, and for the first time in this region, N<sub>2</sub>O observations were connected to a mesoscale eddy.

## 2 Methods

### 2.1 Shipboard Sampling

Sampling was conducted in December 2016 – January 2017 on a cruise to the ETNP aboard *R/V Sikuliaq* (SKQ201617S). The sampled cruise track began near the tip of the Baja California Peninsula (22.67°N, 109.34°W) and followed a southeast line, ending at (14.00°N, 106.21°W; Fig. 1). Eleven stations were sampled for N<sub>2</sub>O along this transect, before the cruise

turned toward the coast of Mexico. A 12th station (15.70°N, 104.63°W) was sampled closer to the coast and included in analyses (Fig. 1).



**Figure 1: Station map.** Stations plotted over historically averaged O<sub>2</sub> saturation at a depth of 200 meters from the World Ocean Atlas 2018 product, using all years available (Garcia et al., 2019).

Salinity, potential temperature, pressure and dissolved oxygen were recorded by sensors aboard a conductivity, temperature, depth (CTD) rosette (Sea-Bird SBE-911+). Chlorophyll-*a* (Chl-*a*) was estimated using a correction factor of 3.03 µg/L/V on the package's WETLabs ECO Chlorophyll Fluorometer. This correction factor was obtained from the cruise data deposited in BCO-DMO (Rocap et al., 2017). Seawater samples were collected from a Niskin rosette for NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, and N<sub>2</sub>O concentration and isotopic analysis. NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> samples were first collected in 500-mL Nalgene polypropylene bottles after three rinses of the bottle. NO<sub>3</sub><sup>-</sup> samples were filtered through 0.2 µm pore size filters and stored frozen in 60 mL HDPE bottles until analysis. NO<sub>2</sub><sup>-</sup> concentrations were determined onboard via spectrophotometry (Grasshoff et al., 1999). NO<sub>2</sub><sup>-</sup> samples and reference materials for isotopic analysis were preserved shipboard, immediately after collection according to the "azide method" (McIlvin & Altabet, 2005) and stored as N<sub>2</sub>O gas-tight vials at room temperature until analysis.

N<sub>2</sub>O samples were collected in Wheaton 160-mL glass serum bottles following standard gas-sampling procedures. Briefly, tygon tubing was used to transfer seawater from the Niskin to serum bottles, which were allowed to overflow three times before filling. After filling, ~1 mL of seawater sample was removed, 100 µL of saturated mercuric chloride (HgCl<sub>2</sub>) was added by

pipette, and the serum bottle was then capped with a gray butyl septum, sealed with an aluminum crimp seal, and stored at room temperature for shore-based analysis.

## 2.2 Concentration and Isotope Analyses

Samples were analyzed for  $\text{NO}_x$  concentration via cadmium reduction and spectrophotometric analysis using a Westco discrete analyzer system at Stanford University and then prepared for isotopic analysis using the “denitrifier method” (Casciotti et al., 2002; McIlvin & Casciotti, 2011; Sigman et al., 2001). In the denitrifier method, seawater  $\text{NO}_3^-$  is converted to  $\text{N}_2\text{O}$  for isotopic analysis using a strain of denitrifying bacteria that lacks the terminal  $\text{N}_2\text{O}$  reductase. Sulfamic acid was used to remove nitrite from samples (Granger & Sigman, 2009) prior to analysis via the denitrifier method. Nitrite  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  analyses were conducted using the “azide method”, whereby  $\text{NO}_2^-$  is converted to  $\text{N}_2\text{O}$  via reaction with sodium azide (McIlvin & Altabet, 2005). The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{N}_2\text{O}$  produced from  $\text{NO}_3^-$  or  $\text{NO}_2^-$  samples were measured by running samples on a custom-built purge-and-trap system coupled to a Thermo Finnigan DELTA V isotope ratio mass spectrometer (IRMS) in continuous flow mode (McIlvin & Casciotti, 2011). Nitrate isotope analyses were calibrated to USGS32, USGS34, and USGS35 reference materials (Böhlke et al., 2003) analyzed in parallel with the samples, and nitrite isotope analyses were calibrated to RSIL-N23, RSIL-N7373, and RSIL-N10219 (Casciotti et al., 2007) preserved and analyzed in parallel. Drift corrections and offsets to denitrifier samples were applied as described in McIlvin & Casciotti, 2011, while drift and size corrections were applied to azide samples as described in Kelly et al., 2021. The analytical precision of these isotopic measurements, represented by the pooled standard deviations of reference materials for  $\text{NO}_3^-$  and pooled standard deviations of duplicates for  $\text{NO}_2^-$ , were  $\pm 0.24\text{‰}$ ,  $\pm 0.31\text{‰}$ ,  $\pm 0.69\text{‰}$ , and  $\pm 0.64\text{‰}$  for  $\delta^{15}\text{NO}_3^-$ ,  $\delta^{18}\text{O-NO}_3^-$ ,  $\delta^{15}\text{NO}_2^-$ , and  $\delta^{18}\text{O-NO}_2^-$ , respectively.

For  $\text{N}_2\text{O}$  isotopocule analysis, samples were run against a tank of pure  $\text{N}_2\text{O}$  calibrated by S. Toyoda (Tokyo Tech) on a custom-built purge-and-trap system coupled to a Finnigan DELTA V IRMS (McIlvin & Casciotti, 2010). Ions with mass/charge ( $m/z$ ) ratios of 30, 31, 44, 45, and 46 were measured simultaneously to capture isotopocules of  $\text{N}_2\text{O}$  as well as those of fragment  $\text{NO}^+$  ion (McIlvin & Casciotti, 2010). Reference gases “B6” (pure  $\text{N}_2\text{O}$  internal laboratory standard) and “S2” (a mixture of 90 ppm  $\text{N}_2\text{O}$  in  $\text{N}_2$ ; Mohn et al., 2014), along with atmosphere-equilibrated seawater, were also analyzed in each run to correct for scrambling — the phenomenon by which the N atoms switch during fragmentation, causing the  $\text{NO}^+$  fragment to sometimes contain the outer ( $\text{N}^\beta$ ) atom and not the inner ( $\text{N}^\alpha$ ) atom from an  $\text{N}_2\text{O}$  molecule (Toyoda & Yoshida, 1999). Reference materials were prepared by purging 160 mL Wheaton bottles, each containing approximately 155 mL de-ionized water, with helium for 90 min, injecting a fixed amount of reference gas (2 nmoles for S2 and 10 nmoles for B6), and preserving with 100  $\mu\text{L}$  of saturated  $\text{HgCl}_2$  solution. Atmosphere-equilibrated seawater samples consisted of filtered surface seawater that was allowed to equilibrate with outdoor air for 2-3 days, before re-filtration and preservation with 100  $\mu\text{L}$  of  $\text{HgCl}_2$  solution.

Linearity, scrambling, and two-point scale decompression (Mohn et al., 2014) corrections were applied to the data as described in Kelly et al., 2021, using code now available as the Python or MATLAB package “pyisotopomer” (Kelly et al., submitted). Duplicates of 10% of the  $\text{N}_2\text{O}$  samples were additionally analyzed to verify the data, yielding an analytical precision

calculated by pooled standard deviation of  $\pm 1.91\%$ ,  $\pm 2.69\%$ ,  $\pm 3.98\%$ ,  $\pm 1.21\%$ , and  $\pm 3.86\%$  for  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^\beta$ , SP,  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ , and  $\delta^{18}\text{O}\text{-N}_2\text{O}$ , respectively.

### 2.3 Optimization of Time-Dependent Model

A major feature of this study is the expansion and optimization of the time-dependent model of  $\text{N}_2\text{O}$  cycling employed in Kelly et al., 2021 (based on a similar model in Casciotti et al., 2018) to solve for the rates of  $\text{N}_2\text{O}$  production and consumption by nitrification and denitrification processes. Briefly, the model started with an initial  $[\text{N}_2\text{O}]$  and initial isotopic values for  $\text{N}_2\text{O}$  and tracked concentrations of  $^{14}\text{N}$ ,  $^{15}\text{N}^\alpha$ ,  $^{15}\text{N}^\beta$ ,  $^{16}\text{O}$ , and  $^{18}\text{O}$  in  $\text{N}_2\text{O}$  at each time step (0.2 days) based on specified isotope effects and substrate parameters (substrate concentrations and isotopic compositions were held constant). From the isotopic concentrations,  $[\text{N}_2\text{O}]$ ,  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^\beta$ , SP, and  $\delta^{18}\text{O}\text{-N}_2\text{O}$  were calculated at each time step. First-order rate constants for each reaction were found using a Nelder-Mead optimization (Nelder & Mead, 1965) to determine the rate constants that minimized mean-squared error (MSE) between lab measurements and model output over the course of 1000 time steps (200 days). Optimizations were performed for simulations both in the core of the ODZ (Section 4.1) and above the ODZ (Section 4.2). The model equations, modified from Kelly et al. (2021), can be found in the supplemental material.

The “nmkb()” function of R package “dfoptim” (Varadhan & Borchers, 2020) was used to conduct a bounded Nelder-Mead optimization of each experimental setup. The functions in “dfoptim” are based on previously-established MATLAB code for derivative-free optimizations (Kelley, 1999). The Nelder-Mead algorithm is a simplex-based direct search method that can be used to find minima or maxima, and the bounded version was employed to prevent negative rate constants. Error between model output and cruise measurements of the isotopic tracers was quantified by an MSE calculation. With  $[\text{N}_2\text{O}]$  as the x-value and tracers  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^\beta$ , SP, and  $\delta^{18}\text{O}\text{-N}_2\text{O}$  as the y-values, model output was linearly interpolated to  $[\text{N}_2\text{O}]$  from cruise measurements. This allowed for the calculation of MSE between modeled and measured tracers at measured  $[\text{N}_2\text{O}]$  values. MSE was calculated for each of the tracers, and the average of those four values across all data points served as the cost function to be minimized in the optimizations. Penalties in the cost function ensured that the optimized model output spanned cruise observations.

Three unknown rate constants —  $\text{N}_2\text{O}$  production via denitrification from  $\text{NO}_2^-$ ,  $\text{N}_2\text{O}$  production via denitrification from  $\text{NO}_3^-$ , and  $\text{N}_2\text{O}$  consumption via denitrification — were solved for in simulations in the core of the ODZ. For simulations above the ODZ,  $\text{N}_2\text{O}$  production from  $\text{NH}_4^+$  by AOA and AOB were added by including two additional terms in the cost function. Since there is no oxygen atom in  $\text{NH}_4^+$ , the  $\delta^{18}\text{O}$  of the substrate for nitrification was taken to be  $23.5\%$ , the average value for atmospheric oxygen, which is fractionated slightly in surface seawater (Kroopnick & Craig, 1972).  $\text{N}_2\text{O}$  derived from AOB and AOA were distinguished by different isotope effects, derived from fractionation factors in the literature (Frame and Casciotti, 2010; Santoro et al., 2011; Supplementary materials).

The MSE cost function with unknown rate constants, an initial estimate for those rate constants to use as a starting point in the algorithm, and bounds for the rate constants were input into “nmkb()” to perform the optimization. For simulations in the core of the ODZ, the rate



constants used by Kelly et al. (2021) were used as the initial estimate (Table S1). Because the model from Kelly et al. (2021) was not run for conditions above the ODZ, sensitivity tests were performed to determine the starting point for optimizations above the ODZ (Table S2). The lower bound input into each optimization was zero, while the upper bound for each unknown rate constant was  $2.1 \text{ day}^{-1}$ , which is one order of magnitude greater than the maximum rate constant for  $\text{N}_2\text{O}$  consumption via denitrification measured previously in the ETNP (Babbin et al., 2015).

### 3 Results

#### 3.1 Hydrographic Variation in the ETNP

Based on key hydrographic and  $\text{N}_2\text{O}$  isotopic features, stations were grouped into three regimes: “Background” (Stns. 1-3), “Core ODZ” (Stns. 4-7&12), and “High  $\text{N}_2\text{O}$ ” (Stns. 8-11). Considerable differences were observed in the water properties between station groupings along the transect (Table S3). Surface water became less dense from north to south, with minimum potential density anomaly ( $\sigma_\theta$ ) averaging  $23.01 \text{ kg/m}^3$ ,  $21.88 \text{ kg/m}^3$ , and  $20.88 \text{ kg/m}^3$  for background, core-ODZ, and high- $\text{N}_2\text{O}$  stations, respectively. A temperature-salinity diagram plotted with water mass endmembers as defined in Evans et al., 2020, revealed a southward increasing presence of Tropical Surface Water near the surface, while waters between  $24$  and  $26.5 \text{ kg/m}^3$  showed a shift from lower salinity in the core-ODZ stations to higher salinity in the high- $\text{N}_2\text{O}$  stations (Fig. S1). Waters below  $\sigma_\theta = 26.5 \text{ kg/m}^3$  displayed more similar temperatures and salinities across the region (Fig. S1).

As is characteristic of ODZs, dissolved oxygen concentrations ( $[\text{O}_2]$ ) remained below detection at core anoxic depths in the ETNP. The near-surface oxycline, defined by a rapid decrease in  $[\text{O}_2]$  from saturation to below detection, was shallower at the southern end of the transect (Fig. 2b); from CTD data, the average depth (and  $\sigma_\theta$ ) of the threshold at which  $[\text{O}_2]$  dipped below  $5 \text{ } \mu\text{mol/kg}$  was  $206.6 \text{ m}$  ( $26.33 \text{ kg/m}^3$ ),  $130.4 \text{ m}$  ( $25.87 \text{ kg/m}^3$ ), and  $77.5 \text{ m}$  ( $25.23 \text{ kg/m}^3$ ) for background, core-ODZ, and high- $\text{N}_2\text{O}$  stations, respectively. Oxygen recovered slowly with depth, with measurements reaching above  $5 \text{ } \mu\text{mol/kg}$  at  $\sigma_\theta \sim 27.35 \text{ kg/m}^3$  at each station.

The nitracline, defined by the steep increase in dissolved nitrate ( $[\text{NO}_3^-]$ ) with depth, followed the southward shoaling pattern (Fig. 2d). The average depth (and density) of the threshold at which  $[\text{NO}_3^-]$  reached  $20 \text{ } \mu\text{M}$ , calculated by interpolating  $[\text{NO}_3^-]$  at every meter, was calculated to be  $124.7 \text{ m}$  ( $25.59 \text{ kg/m}^3$ ),  $98 \text{ m}$  ( $25.24 \text{ kg/m}^3$ ), and  $72 \text{ m}$  ( $24.59 \text{ kg/m}^3$ ) for background, core-ODZ, and high- $\text{N}_2\text{O}$  stations, respectively. Below the nitracline,  $[\text{NO}_3^-]$  reached a maximum of  $48 \text{ } \mu\text{M}$  (Stn. 11;  $850 \text{ m}$ ;  $\sigma_\theta = 27.28 \text{ kg/m}^3$ ).

The high- $\text{N}_2\text{O}$  stations, where the oxycline was shallowest, had the largest primary nitrite maximum (PNM;  $0.5\text{-}1.25 \text{ } \mu\text{M}$  near the surface; Fig. 2c), and the highest near-surface Chl-*a* estimates, with a maximum of  $5.97 \text{ } \mu\text{g/L}$  (Stn. 10;  $55.7 \text{ m}$ ;  $\sigma_\theta = 22.58 \text{ kg/m}^3$ ; Fig. S2b). Three out of four of the high- $\text{N}_2\text{O}$  stations also had observable deep Chl-*a* maxima. The core-ODZ stations had the most pronounced secondary nitrite maximum (SNM), with maxima of  $1\text{-}4 \text{ } \mu\text{M}$ . The SNM

observed here was smaller than that sampled in 2012 (Babbin et al., 2015) and more in line with measurements made in 2018 (Kelly et al., 2021).

Satellite altimetry revealed the presence of an anticyclonic eddy at high-N<sub>2</sub>O stations, with elevated sea surface topography at the southern end of the transect relative to the other stations (Fig. S4). The pycnoclines at these stations were stronger than the other areas of the transect, and the waters below 100 m at Stn. 10 exhibited downward-bending isopycnals (Fig. S2a). Stn. 7 was positioned at a pronounced density front near the edge of the eddy and had the highest [NO<sub>2</sub><sup>-</sup>], reaching 4.30 μM in suboxic waters (295 m;  $\sigma_\theta = 26.55 \text{ kg/m}^3$ ).

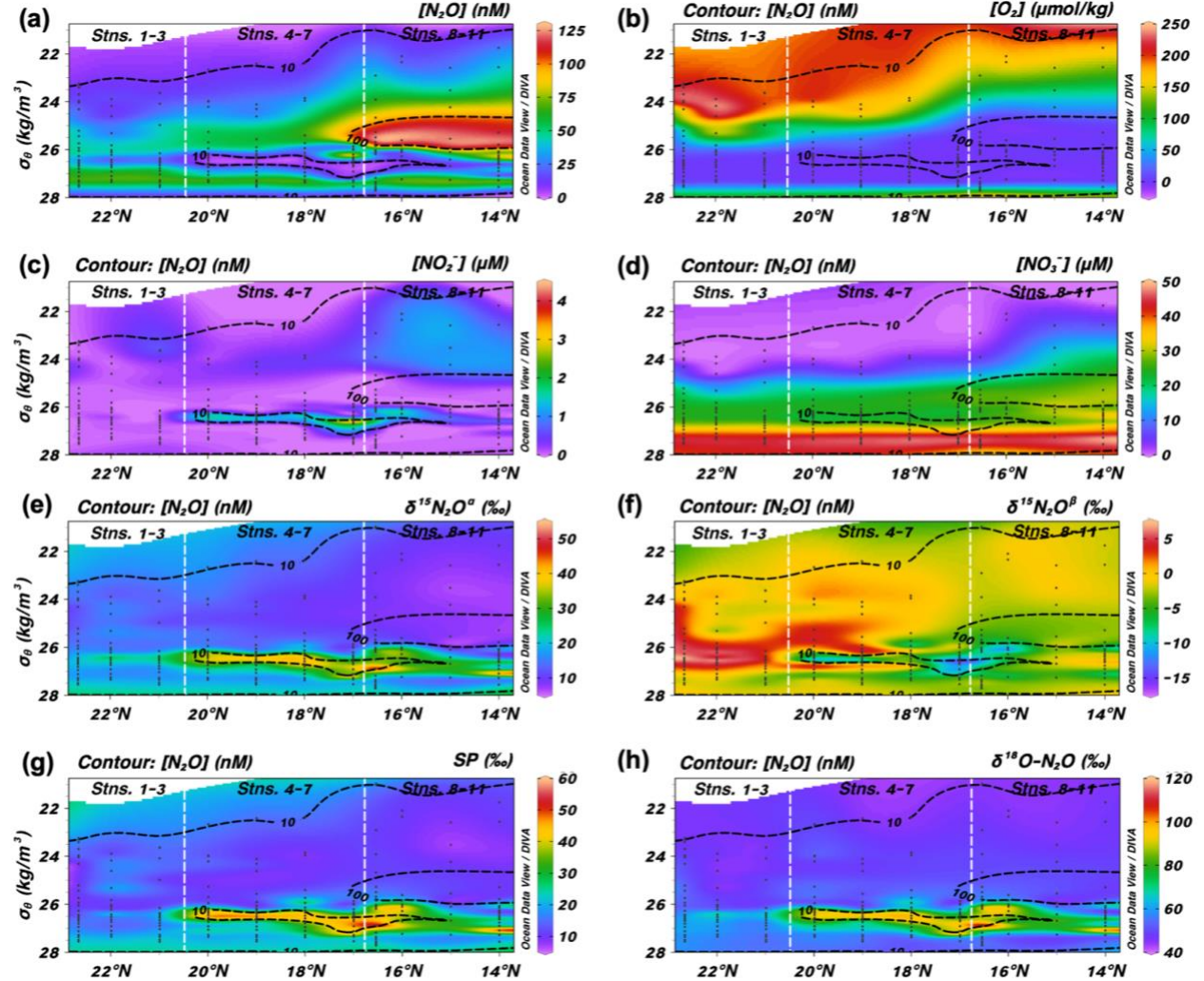
Isotopic ratios for NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were not the focus of this study but were used to initialize the time-step model discussed in Section 4. Local  $\delta^{15}\text{NO}_3^-$  and  $\delta^{18}\text{O-NO}_3^-$  maxima of up to 25‰ were found at core anoxic depths, at densities ranging from 26-27 kg/m<sup>3</sup> (Fig. S3c-d). Isotopologues of NO<sub>2</sub><sup>-</sup> could only be measured at depths where [NO<sub>2</sub><sup>-</sup>] exceeded 0.5 μM, occurring within the PNM and SNM features (Fig. S3).  $\delta^{15}\text{NO}_2^-$  values dropped from near 0‰ at the PNM to minima near -20 to -30‰ in the SNM at core-ODZ stations. Both nitrate and nitrite isotopes showed ranges and patterns similar to those seen in existing measurements from the region (Buchwald et al., 2015; Casciotti & McIlvin, 2007; Kelly et al., 2021).

### 3.2 N<sub>2</sub>O Concentrations

Two [N<sub>2</sub>O] peaks were generally observed at each station — one in the oxycline near the top of the ODZ and one below the ODZ ( $\sigma_\theta > 27 \text{ kg/m}^3$ ; Fig. 2a), consistent with previous ETNP studies (Babbin et al., 2015; Kelly et al., 2021; Yamagishi et al., 2007). Between the two peaks, a local [N<sub>2</sub>O] minimum was observed at anoxic depths (Fig. 2a), which is characteristic of N<sub>2</sub>O consumption in ODZs (Babbin et al., 2015; Bourbonnais et al., 2017; Casciotti et al., 2018; Kelly et al., 2021; McIlvin & Casciotti, 2010; Yamagishi et al., 2007; Yoshinari et al., 1997). This “bite” in the [N<sub>2</sub>O] profiles was most pronounced at core-ODZ stations relative to the other regimes, with [N<sub>2</sub>O] < 5 nM, or below 50% saturation, found between  $\sigma_\theta = 26 \text{ kg/m}^3$  and  $\sigma_\theta = 27 \text{ kg/m}^3$  (Fig. 2a).

The highest near-surface [N<sub>2</sub>O] values were recorded at the high-N<sub>2</sub>O stations, with 119.30-126.07 nM measured at 90 m ( $\sigma_\theta = 25.49\text{-}25.78 \text{ kg/m}^3$ ; Fig. 2a). [N<sub>2</sub>O] reached maximum values between the base of the oxycline (Fig. 2b) and top of the SNM (Fig. 2c). The average near-surface [N<sub>2</sub>O] maxima at core-ODZ and background stations (except Stn. 7, where shallow N<sub>2</sub>O samples were not collected) were 62.64 nM and 44.55 nM, respectively (Fig. 2a). While Stn. 7 displayed a local [N<sub>2</sub>O] minimum between  $\sigma_\theta = 26 \text{ kg/m}^3$  and  $\sigma_\theta = 27 \text{ kg/m}^3$ , characteristic of the ODZ, it also showed a high [N<sub>2</sub>O] value of 95.77 nM near the top of that density layer (145 m;  $\sigma_\theta = 26.21 \text{ kg/m}^3$ ; Fig. 2a). Previous studies have also reported maximal near-surface [N<sub>2</sub>O] of ~100 nM in the offshore ETNP (Babbin et al., 2015; Yamagishi et al., 2007), and up to [N<sub>2</sub>O] > 200 nM near the coast (Kelly et al., 2021). The deep [N<sub>2</sub>O] maximum

was more consistent across the transect, averaging 68.63 nM (~850 m;  $\sigma_\theta = 27.28 \text{ kg/m}^3$ ; Fig. 2a).



**Figure 2: Major chemical and isotopic species along the transect plotted against potential density  $\sigma_\theta$ .** (a)  $[\text{N}_2\text{O}]$  (nM), (b)  $[\text{O}_2]$  ( $\mu\text{mol/kg}$ ), (c)  $[\text{NO}_2^-]$  ( $\mu\text{M}$ ), (d)  $[\text{NO}_3^-]$  ( $\mu\text{M}$ ), (e)  $\delta^{15}\text{N}_2\text{O}^\alpha$  (‰ vs. atmospheric  $\text{N}_2$ ), (f)  $\delta^{15}\text{N}_2\text{O}^\beta$  (‰ vs. atmospheric  $\text{N}_2$ ), (g) site preference (‰ vs. atmospheric  $\text{N}_2$ ), and (h)  $\delta^{18}\text{O}-\text{N}_2\text{O}$  (‰ vs. VSMOW).  $[\text{N}_2\text{O}]$  contours are overlaid on each panel to compare profiles to key  $[\text{N}_2\text{O}]$  features, and white dashed lines separate background (Stns. 1-3), core-ODZ (Stns. 4-7), and high- $\text{N}_2\text{O}$  (Stns. 8-11) stations.

### 3.3 Distribution of $\text{N}_2\text{O}$ Isotopocules

The  $\text{N}_2\text{O}$  isotopocule distributions mirrored the key features of  $[\text{N}_2\text{O}]$  profiles. The highest  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O}-\text{N}_2\text{O}$  on the transect were found in anoxic waters at core-ODZ stations

and some high-N<sub>2</sub>O stations (Fig. 2e and 2h), coinciding with the pronounced [N<sub>2</sub>O] minima of these stations at  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$ . The maximum  $\delta^{15}\text{N}_2\text{O}^\alpha$  found at each station ranged from 39.7‰–49.2‰ and 31.9‰–52.0‰ for the core-ODZ and high-N<sub>2</sub>O stations, respectively. In this same density layer, the  $\delta^{18}\text{O}\text{--N}_2\text{O}$  maxima ranged from 97.2‰–113.7‰ and 83.3‰–117.0‰ for the core-ODZ and high-N<sub>2</sub>O stations, respectively. The increases in  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O}\text{--N}_2\text{O}$  within this layer were less dramatic at background stations (Fig. 2e and 2h).

Minimum  $\delta^{15}\text{N}_2\text{O}^\beta$  values, which reached -16.4‰ (Stn. 7; 295 m;  $\sigma_\theta = 26.56 \text{ kg/m}^3$ ), also accompanied the [N<sub>2</sub>O] minima of core-ODZ and some high-N<sub>2</sub>O stations (Fig. 2f). As described above, this density layer also contained the highest  $\delta^{15}\text{N}_2\text{O}^\alpha$ , leading to SPs peaking at 53.2‰ (850 m;  $\sigma_\theta = 27.27 \text{ kg/m}^3$ ) and 56.5‰ (500 m;  $\sigma_\theta = 26.88 \text{ kg/m}^3$ ) at Stn. 7 and Stn. 8, respectively (Fig. 2g). The background stations exhibited smaller SP gradients and contained the least negative values for  $\delta^{15}\text{N}_2\text{O}^\beta$  (~4‰–7.3‰) along the transect in the same density layer where the other stations contained their minimum (Fig. 2f–2g).

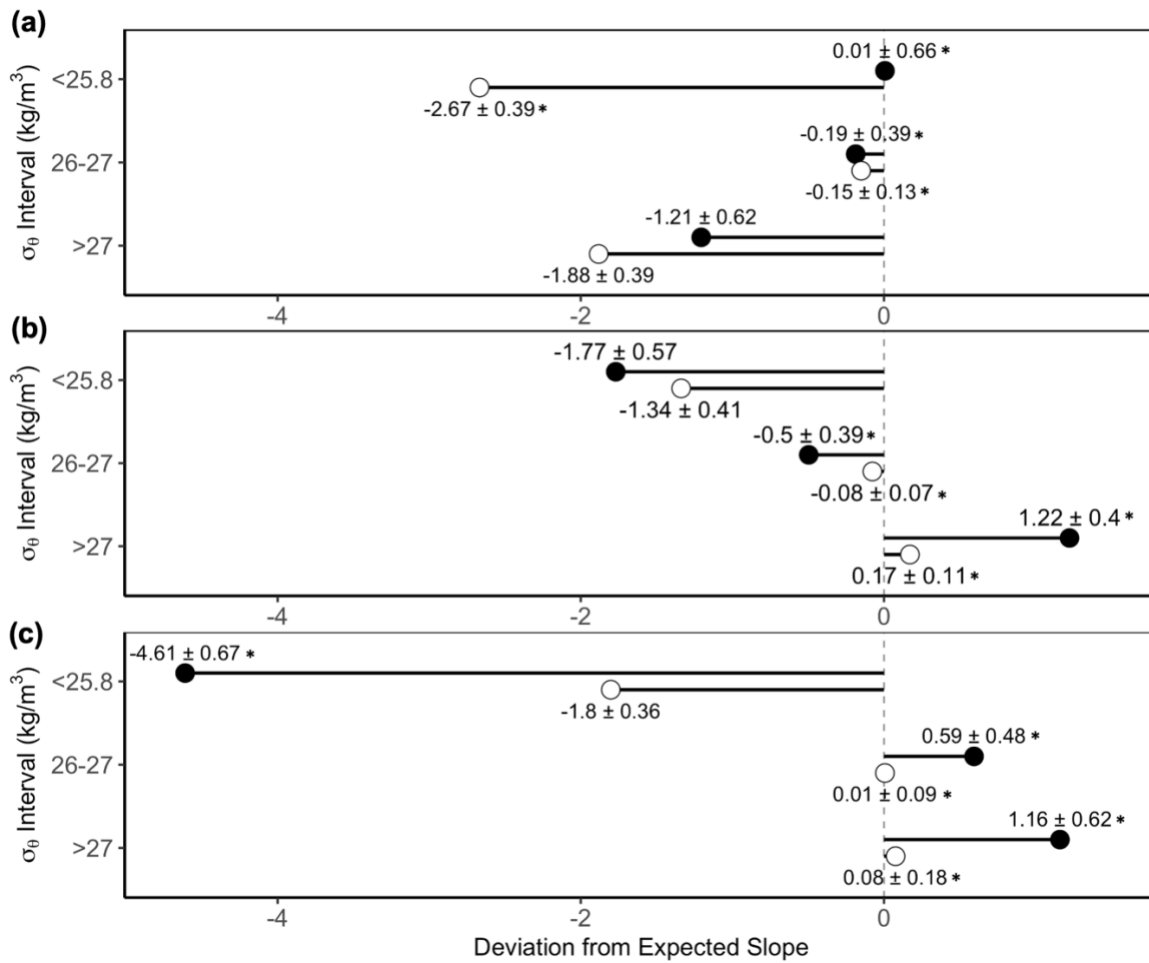
Above the ODZ ( $\sigma_\theta < 25.8 \text{ kg/m}^3$ ),  $\delta^{15}\text{N}_2\text{O}^\alpha$  in near-surface waters decreased toward the southern end of the transect, averaging 14.4‰, 12.6‰, and 9.9‰ at background, core-ODZ, and high-N<sub>2</sub>O stations, respectively.  $\delta^{15}\text{N}_2\text{O}^\beta$  also exhibited this near-surface gradient along the transect, averaging 0.2‰, -0.1‰, and -2.8‰ at background, core-ODZ, and high-N<sub>2</sub>O stations, respectively. Near-surface averages for SP were 14.2‰, 12.7‰, and 12.7‰ and for  $\delta^{18}\text{O}\text{--N}_2\text{O}$  were 48.2‰, 50.3‰, and 48.9‰ at background, core-ODZ, and high-N<sub>2</sub>O stations, respectively (Fig. 2e–2h).

## 4 Discussion

Variations in the distribution of N<sub>2</sub>O and its isotopocules between the three station groupings guided three central research questions: (1) What is the balance of production and consumption of N<sub>2</sub>O in the ODZ core of Stns. 4–7&12? (2) What are the sources and factors contributing to the near-surface N<sub>2</sub>O accumulation at Stns. 8–11? (3) What does the eddy reveal about [N<sub>2</sub>O] variability in the ETNP?

To define regimes for further modeling and analyses, regressions of  $\delta^{18}\text{O}\text{--N}_2\text{O}$  on  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  and  $\delta^{18}\text{O}\text{--N}_2\text{O}$  on  $\delta^{15}\text{N}_2\text{O}^\alpha$  were performed to determine the density layers where isotopic composition was dominated by N<sub>2</sub>O consumption via denitrification (Casciotti et al., 2018; Kelly et al., 2021). Breakage of the O–N <sup>$\alpha$</sup>  bond during N<sub>2</sub>O reduction is associated with isotope effects on both <sup>18</sup>O and <sup>15</sup>N <sup>$\alpha$</sup>  and leads to expected linear relationships between  $\delta^{18}\text{O}\text{--N}_2\text{O}$  and  $\delta^{15}\text{N}_2\text{O}^\alpha$  (Ostrom et al., 2007). In the density layer  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$ , regression slopes between  $\delta^{18}\text{O}\text{--N}_2\text{O}$  and  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  and  $\delta^{18}\text{O}\text{--N}_2\text{O}$  and  $\delta^{15}\text{N}_2\text{O}^\alpha$  closely matched the expected ratios of 2.5 and 1.7, respectively (Ostrom et al., 2007); isotopocule ratios in the other density layers deviated from this relationship (Fig. 3). Thus, we treated  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  as an ODZ cycling regime dominated by N<sub>2</sub>O consumption and  $\sigma_\theta < 25.8 \text{ kg/m}^3$  as a near-surface cycling regime where isotopic composition largely reflects N<sub>2</sub>O production. This framework does not preclude N<sub>2</sub>O production in the consumption-dominated density layer or vice versa, but emphasizes that isotopic composition primarily reflects one or the other (Kelly et al., 2021).  $\sigma_\theta =$

25.8-26 kg/m<sup>3</sup> is taken as a transitional area between the two regimes and is excluded from some analyses specific to the regimes.



**Figure 3: Deviations from reduction line.** Deviations from the slopes of isotopocule regressions found by Ostrom et al. (2007) for N<sub>2</sub>O consumption via denitrification, plotted for chosen density layers at (a) background, (b) core-ODZ, and (c) high-N<sub>2</sub>O stations. Closed circles represent the slope of  $\delta^{18}\text{O}-\text{N}_2\text{O}$  against  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  minus 2.5, and open circles represent the slope of  $\delta^{18}\text{O}-\text{N}_2\text{O}$  against  $\delta^{15}\text{N}_2\text{O}^a$  minus 1.7. Asterisks indicate a p-value < 0.5 for the linear regression.

#### 4.1 Denitrification in the ODZ Core

The anoxic depths of core-ODZ stations were characterized by the lowest [N<sub>2</sub>O] and relatively high  $\delta^{15}\text{N}_2\text{O}^a$  and  $\delta^{18}\text{O}-\text{N}_2\text{O}$  (Fig. 2e and 2h). During N<sub>2</sub>O consumption via denitrification, preferential breakage of light-isotope bonds in the intermediate leads to heavy isotope enrichment in the remaining N<sub>2</sub>O (Popp et al., 2002; Toyoda et al., 2002; Yamagishi et al., 2007). Given that the isotopocule regressions indicated that consumption was a dominant process in the core of the ODZ, the elevated  $\delta^{15}\text{N}_2\text{O}^a$  and  $\delta^{18}\text{O}-\text{N}_2\text{O}$  is expected for  $\sigma_\theta = 26-27$

kg/m<sup>3</sup>. However, this density layer at core-ODZ stations also contained some of the most negative values for  $\delta^{15}\text{N}_2\text{O}^\beta$  (Fig. 2f). Similar signals have been observed in past ODZ studies and interpreted to represent concurrent N<sub>2</sub>O production (Bourbonnais et al., 2017; Casciotti et al., 2018; Farías et al., 2009; Kelly et al., 2021; Westley et al., 2006; Yamagishi et al., 2005, 2007).

Isotopic ratios for  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  were plotted against the natural logarithm of N<sub>2</sub>O concentration to observe how isotopocule ratios change as [N<sub>2</sub>O] varies. As [N<sub>2</sub>O] decreased from its peak concentration to ~ 5 nM, isotopic ratios generally increased, consistent with N<sub>2</sub>O consumption via denitrification. Using a segmented regression model (Muggeo, 2003, 2008), a breakpoint was identified at [N<sub>2</sub>O] ~ 5 nM ( $\ln([\text{N}_2\text{O}]) \sim 1.6$ ), after which  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{18}\text{O}\text{--N}_2\text{O}$ , and SP began to level off or even decrease (Fig. S5). Most of the points after the breakpoint were from core-ODZ stations (Fig. S5), with [N<sub>2</sub>O] extending to lower values. The segmented regression also revealed that  $\delta^{18}\text{O}\text{--N}_2\text{O}$  appeared to decrease at a higher rate than  $\delta^{15}\text{N}_2\text{O}^\alpha$  and SP after the breakpoint (Fig. S5) — a surprising result, given that  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O}\text{--N}_2\text{O}$  would be expected to track each other in the ODZ.

The decreasing  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O}\text{--N}_2\text{O}$  when [N<sub>2</sub>O] < 5 nM, taken together with the negative  $\delta^{15}\text{N}_2\text{O}^\beta$ , suggest that N<sub>2</sub>O production was co-occurring with N<sub>2</sub>O consumption at core-ODZ stations. Babbin et al. (2015) hypothesized that N<sub>2</sub>O in the core of the ODZ is in steady state — that is, N<sub>2</sub>O production is balanced by N<sub>2</sub>O consumption. To test whether isotopic ratios indicated an approach to steady-state conditions, results from a time-dependent model adapted from Kelly et al. (2021) were compared to data collected from  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations and optimized for the rates of N<sub>2</sub>O cycling processes.

The model allowed N<sub>2</sub>O to be produced from NO<sub>2</sub><sup>-</sup>, produced from NO<sub>3</sub><sup>-</sup>, or consumed via denitrification. Experiments were initialized with the mean N<sub>2</sub>O concentration and isotopocules within  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at background stations (at the northern end of the transect), while mean NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations and isotopic content were taken from the core-ODZ stations. This setup simulates the lateral advection of a pool of N<sub>2</sub>O from background to core-ODZ stations, overprinted by local N<sub>2</sub>O production and consumption. Isotope effects for N<sub>2</sub>O production via denitrification were taken from the literature (Table S1), while the isotope effects for N<sub>2</sub>O consumption were calculated in this study with a closed-system Rayleigh model (Fig. S6). The model initialization conditions for this set of experiments are outlined in the supplement (Table S1). Optimizations were performed to find the rates and rate constants for the three denitrification processes (NO<sub>3</sub><sup>-</sup> reduction to N<sub>2</sub>O, NO<sub>2</sub><sup>-</sup> reduction to N<sub>2</sub>O, and N<sub>2</sub>O reduction to N<sub>2</sub>) that best matched observed N<sub>2</sub>O concentration and isotopocule data for  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations. More details about the optimization method can be found in Section 2.3. Starting with N<sub>2</sub>O from background stations, the model attempted to recreate the breakpoint in Fig. S5.

Denitrification is generally thought to produce N<sub>2</sub>O with a low, near-zero SP (Frame & Casciotti, 2010; Sutka et al., 2006). However, one denitrifier has been found to produce N<sub>2</sub>O with a SP of  $23.3 \pm 4.2\%$  (Toyoda et al., 2005), and Kelly et al. (2021) were able to reproduce their observed trends in the ODZ using a SP of 22‰ for denitrification in steady-state model experiments. Because of this, three sets of isotope effects for N<sub>2</sub>O production via denitrification were tested here: one set with no SP and two sets with a SP of 22‰. Introduction of a SP for

denitrification was generated by either changing the isotope effect for production of  $N^a$  ( $^{15}\epsilon_{NOx \rightarrow N_2O}(\alpha)$ ) from 22‰ to 0‰ while keeping the isotope effect for  $N^b$  at 22‰, or by changing the isotope effect for production of  $N^b$  ( $^{15}\epsilon_{NOx \rightarrow N_2O}(\beta)$ ) from 22‰ to 44‰ while keeping the isotope effect for  $N^a$  at 22‰ — the latter of which resembles the case for *Pseudomonas fluorescens* (Toyoda et al., 2005).

Four sets of parameter combinations were tested for each denitrification site preference setting: (1) the default initialization previously described, (2) increasing the initial amount of  $N_2O$  ( $[N_2O]_i$ ), (3) increasing the isotope effects for  $N_2O$  consumption ( $^{15}\epsilon_{cons}$  and  $^{18}\epsilon_{cons}$ ), and (4) combining (2) and (3). With three sets of isotope effects for  $N_2O$  production via denitrification and four parameter combinations, 12 optimizations were performed. The optimized rates and average mean squared error (MSE) for the modeled tracers are listed in Table 1.  $N_2O$  consumption is phased-in nonlinearly in this set of experiments, as described in Section 2.4 of Kelly et al., 2021, and the initial rate of consumption and maximum rate of consumption are given for each optimization (Table 1).

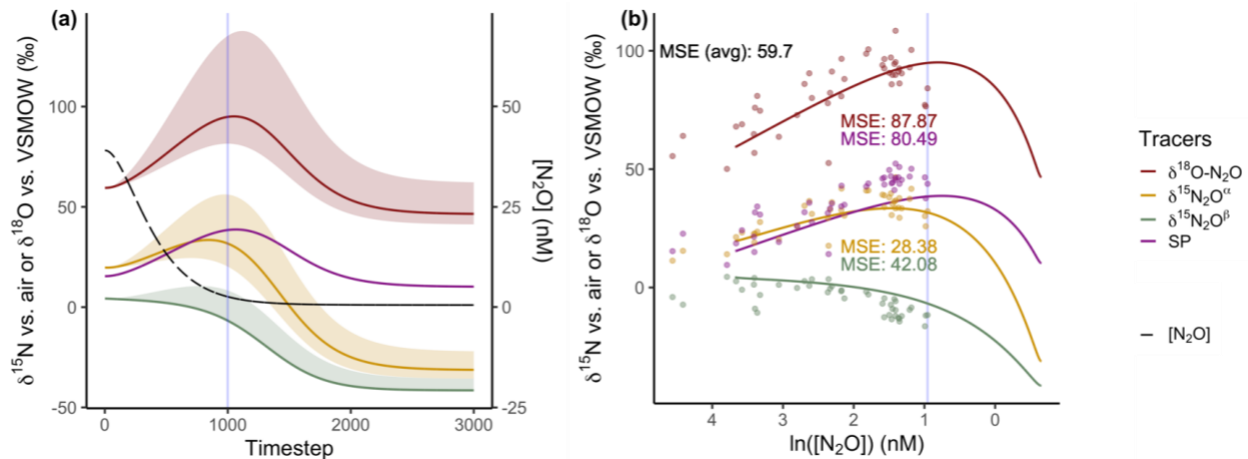
**Table 1: Optimized rates (nM N/day) for  $N_2O$  production and consumption within the ODZ ( $\sigma_0 = 26\text{--}27 \text{ kg/m}^3$ ) at core-ODZ stations.** Mean squared error (MSE) is presented as an average of that of the four tracers. Results in boldface are those where the rate constant for consumption converged at the maximum constraint of  $2.1 \text{ day}^{-1}$ .

|   |                     | Default                                 | $[N_2O]_i = 60 \text{ nM}$              | $^{15,18}\epsilon_{cons} \times 1.5$ | $[N_2O]_i = 60 \text{ nM}$ and $^{15,18}\epsilon_{cons} \times 1.5$ |
|---|---------------------|---|---|--------------------------------------|---|
| SP = 0‰<br><br>$^{15}\epsilon_{NOx \rightarrow N_2O}(\alpha) =$<br>$^{15}\epsilon_{NOx \rightarrow N_2O}(\beta) = 22\text{‰}$ | Prod. from $NO_2^-$ | $1.19 \times 10^{-2}$                   | $3.18 \times 10^{-1}$                   | $2.91 \times 10^{-2}$                | $8.17 \times 10^{-2}$   |
|   | Prod. from $NO_3^-$ | $1.74 \times 10^{-6}$                   | $2.06 \times 10^{-7}$                   | $8.88 \times 10^{-4}$                | $3.81 \times 10^{-2}$   |
|   | Initial Cons.       | $7.77 \times 10^{-3}$                   | <b><math>8.88 \times 10^{-1}</math></b> | $8.99 \times 10^{-3}$                | $4.97 \times 10^{-2}$   |
|   | Max. Cons.          | $6.80 \times 10^{-1}$                   | <b><math>1.37 \times 10^1</math></b>    | $7.67 \times 10^{-1}$                | $2.72 \times 10^0$  |
|   | MSE                 | 59.70                                   | 47.70                                   | 55.84                                | 86.80   |
| SP = 22‰<br><br>$^{15}\epsilon_{NOx \rightarrow N_2O}(\alpha) = 0\text{‰}$  | Prod. from $NO_2^-$ | $2.27 \times 10^{-1}$                   | $2.01 \times 10^{-1}$                   | $3.47 \times 10^{-2}$                | $1.23 \times 10^{-1}$   |
|   | Prod. from $NO_3^-$ | $4.48 \times 10^{-1}$                   | $9.24 \times 10^{-1}$                   | $5.16 \times 10^{-2}$                | $1.78 \times 10^{-1}$   |
|   | Initial Cons.       | <b><math>6.53 \times 10^{-1}</math></b> | $3.18 \times 10^{-1}$                   | $1.43 \times 10^{-2}$                | $9.51 \times 10^{-2}$   |
|   | Max. Cons.          | <b><math>1.02 \times 10^1</math></b>    | $7.96 \times 10^0$                      | $1.09 \times 10^0$                   | $4.18 \times 10^0$  |
|   | MSE                 | 44.57                                   | 37.69                                   | 42.79                                | 77.04   |
| SP = 22‰<br><br>$^{15}\epsilon_{NOx \rightarrow N_2O}(\beta) = 44\text{‰}$  | Prod. from $NO_2^-$ | $3.30 \times 10^{-8}$                   | $1.90 \times 10^{-2}$                   | $3.49 \times 10^{-5}$                | $8.48 \times 10^{-4}$   |
|   | Prod. from $NO_3^-$ | $5.78 \times 10^{-1}$                   | $1.50 \times 10^{-7}$                   | $8.03 \times 10^{-2}$                | $1.17 \times 10^{-1}$   |
|   | Initial Cons.       | <b><math>6.53 \times 10^{-1}</math></b> | $1.41 \times 10^{-2}$                   | $1.36 \times 10^{-2}$                | $2.84 \times 10^{-2}$   |
|   | Max. Cons.          | <b><math>1.00 \times 10^1</math></b>    | $1.17 \times 10^0$                      | $1.05 \times 10^0$                   | $1.95 \times 10^0$  |
|   | MSE                 | 44.90                                   | 41.80                                   | 62.22                                | 105.04  |

For each experiment, the model was generally able to recreate the breakpoint in  $N_2O$  isotopic composition for  $\sigma_0 = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations (Fig. 4). The reported MSE is an average of that of the four tracers ( $\delta^{15}N_2O^a$ ,  $\delta^{15}N_2O^b$ , SP, and  $\delta^{18}O\text{--}N_2O$ ).  $\delta^{18}O\text{--}N_2O$  had the



highest MSE for each experiment, likely because the breakpoint in the isotopic data for  $\delta^{18}\text{O}$ - $\text{N}_2\text{O}$  was the most dramatic (Fig. S5), and thus the model performed most poorly in replicating it. Additionally, there could be a statistical bias in optimizing for the lowest average MSE when the average includes SP, which depends on  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{15}\text{N}_2\text{O}^\beta$ .



**Figure 4: Time-dependent model output from core-ODZ stations.** Model output for the default initialization with SP = 0‰ for  $\text{N}_2\text{O}$  production via denitrification, presented as (a) a time series of  $[\text{N}_2\text{O}]$  and isotopocules and (b) isotopocules against the natural logarithm of  $[\text{N}_2\text{O}]$  for  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations (similar to Fig. S5). The model was optimized at 1000 timesteps (200 days), and then using those optimized rates, ran for 3000 timesteps (600 days) to observe progression after the breakpoint. The maximum and minimum bounds for the time series in (a) are output using the maximum and minimum  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  from literature. The vertical blue line in each panel indicates the timestep with modeled  $[\text{N}_2\text{O}]$  was closest to the smallest measured  $[\text{N}_2\text{O}]$ . In (b), model output is plotted with a series of lines, while observations are plotted with points of matching color.

In most experiments, the rate constant for  $\text{N}_2\text{O}$  consumption ( $k_{\text{cons}}$ ) converged within the range of  $10^{-2}\text{--}10^{-1} \text{ day}^{-1}$ ; however, three runs (bolded in Table 1) converged at the maximum constraint of  $2.1 \text{ day}^{-1}$ , implying that additional  $\text{N}_2\text{O}$  consumption might have improved the model fits in those cases. It is also plausible that  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  calculated with the Rayleigh model were too low, forcing the model to converge at a high  $k_{\text{cons}}$  to reach the high observed isotopic values. The  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  implemented in the model were calculated from high- $\text{N}_2\text{O}$  stations to avoid  $\text{N}_2\text{O}$  production lowering the apparent isotope effects (unpublished data; see Kelly et al., 2021). This approach yielded isotope effects of  $10.54 \pm 0.99\text{‰}$  and  $16.16 \pm 2.28\text{‰}$  for  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$ , respectively. However, given that previous work estimated  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  at  $20 \pm 2\text{‰}$  and  $31 \pm 3\text{‰}$ , respectively (Yamagishi et al., 2007), the values calculated here are at the lower end of the spectrum (see Bourbonnais et al., 2017). Increasing  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  by a factor of 1.5 (to  $15.75\text{‰}$  and  $24\text{‰}$ , respectively) slightly improved MSE in two of the three sets of



isotope effects tested for N<sub>2</sub>O production, and none of the three runs with higher  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  forced  $k_{\text{cons}}$  to converge on the maximum constraint (Table 1).

Initializing the model with a higher [N<sub>2</sub>O]<sub>i</sub> also reduced error (Table 1). This could be a result of the interpolation method for calculating MSE; increasing the starting amount of N<sub>2</sub>O allowed for better interpolation when calculating MSE between model output and observations because three observations had greater [N<sub>2</sub>O] than used in the default initialization (Figure 4). Starting with more N<sub>2</sub>O also allowed for greater first-order consumption rates. In this way, the higher [N<sub>2</sub>O]<sub>i</sub> allowed  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O-N}_2\text{O}$  to reach high values via enhanced N<sub>2</sub>O consumption, whereas increasing  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  values achieved high  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{18}\text{O-N}_2\text{O}$  via higher fractionation at lower extents of consumption. Increasing both [N<sub>2</sub>O]<sub>i</sub> and fractionation, however, led to the worst fits of the 12 model runs (Table 1).

Recently, both tracer experiments and natural abundance isotope ratio measurements have pointed to NO<sub>3</sub><sup>-</sup> as the primary substrate for N<sub>2</sub>O production in ODZs (Casciotti et al., 2018; Frey et al., 2020; Ji et al., 2015, 2018). In this study, production from NO<sub>2</sub><sup>-</sup> was much higher than that from NO<sub>3</sub><sup>-</sup> in optimizations where production from denitrification had a SP of 0‰ (Table 1), meaning that production from NO<sub>2</sub><sup>-</sup> better explained the isotopic composition of N<sub>2</sub>O observed in  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations. This finding is corroborated by the pronounced SNM at these stations (Fig. 2c). When a SP for N<sub>2</sub>O production was added to the model, however, NO<sub>3</sub><sup>-</sup> became the primary substrate in several instances (Table 1). With  $^{15}\epsilon_{\text{NO}_x \rightarrow \text{N}_2\text{O}} (\beta) = 44\text{‰}$ , it is likely that production from NO<sub>2</sub><sup>-</sup> would drive  $\delta^{15}\text{N}_2\text{O}^\beta$  much lower than observed values. Therefore, which substrate best fits the N<sub>2</sub>O data depends on the assumed isotope effects.

Another important takeaway is that adding a SP for N<sub>2</sub>O production lowered MSE in most model experiments (Table 1). Essentially, setting  $^{15}\epsilon_{\text{NO}_x \rightarrow \text{N}_2\text{O}} (\alpha) = 0\text{‰}$  allowed  $\delta^{15}\text{N}_2\text{O}^\alpha$  to reach higher values and more closely match observations, while setting  $^{15}\epsilon_{\text{NO}_x \rightarrow \text{N}_2\text{O}} (\beta) = 44\text{‰}$  and switching the substrate to NO<sub>3</sub><sup>-</sup> allowed  $\delta^{15}\text{N}_2\text{O}^\beta$  to reach slightly lower values and more closely match observations. Both had the effect of improving the fit for SP. This result corroborates prior modeling studies (Kelly et al. 2021; Casciotti et al. 2018) which suggest that N<sub>2</sub>O production via denitrification with a nonzero SP (Schmidt et al., 2004; Toyoda et al., 2005) could explain the isotopic composition of N<sub>2</sub>O in ODZs.

Peng and Valentine (2021) recently suggested a potential suboxic niche for fungal denitrification in the ETNP and found that it contributes significantly to N<sub>2</sub>O production in the water column. Because fungi produce N<sub>2</sub>O with a SP ranging from ~15‰ to ~37‰ (Maeda et al., 2015; Rohe et al., 2014; Sutka et al., 2008), fungal denitrification could partly account for inferred N<sub>2</sub>O production via denitrification with a nonzero SP. This picture complicates current interpretations of N<sub>2</sub>O isotopocules in the marine environment and the ability of SP to serve as a diagnostic tool for N<sub>2</sub>O cycling in the water column, and it highlights the importance of modeling  $\delta^{15}\text{N}_2\text{O}^\alpha$  and for  $\delta^{15}\text{N}_2\text{O}^\beta$  as distinct tracers.

The primary goals of the modeling exercises were to constrain the processes contributing to N<sub>2</sub>O cycling in the core of the ODZ and assess whether the regime was in steady state. In each run, the point at which [N<sub>2</sub>O] in the model most closely matched the lowest [N<sub>2</sub>O] observation from core-ODZ stations (indicated by blue line in Fig. 4) occurred at the part of the time series in

which [N<sub>2</sub>O] was reaching equilibrium; that is, [N<sub>2</sub>O] was close to leveling off in each run (Fig. 4). The evolution of the isotopocules in the model tell a somewhat different story. At the point of the lowest [N<sub>2</sub>O] observation (indicated by blue line in Fig. 4), the isotopocules of N<sub>2</sub>O were still changing dramatically. Thus, while [N<sub>2</sub>O] was close to steady state in  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at core-ODZ stations, the isotopic composition of the pool was still elevated relative to steady state values. At best, based on the model output, it can be said that this area of the water column was approaching steady state but not yet in equilibrium — especially from a stable isotope perspective. This demonstrates that N<sub>2</sub>O measurements in the ODZ represent snapshots of a time-varying system. An example of the rate of change plots used to investigate the state of equilibrium is provided in the supplement (Fig. S7).

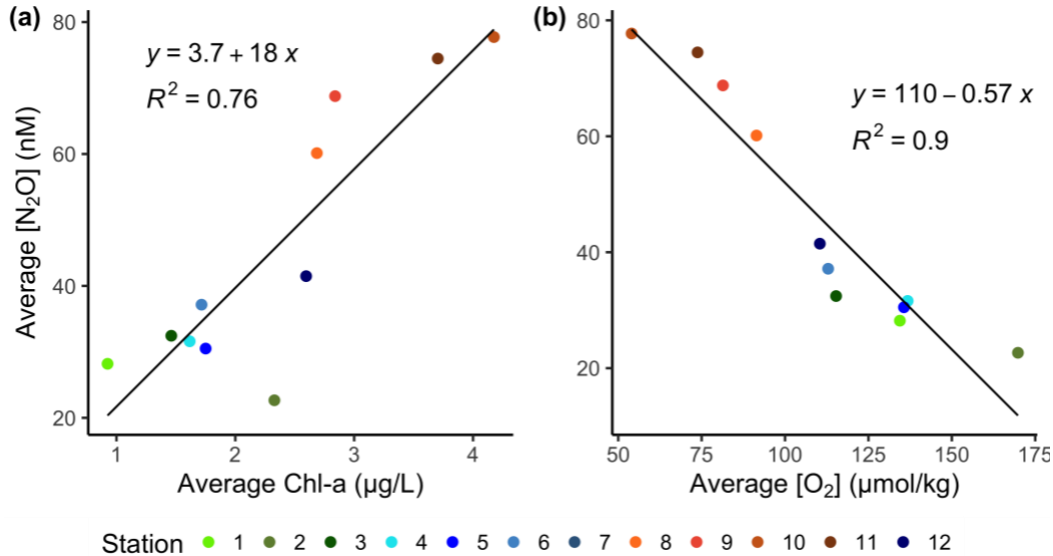
Finally, it is important to acknowledge the variability in the model optimizations presented in Table 1. Adjusting the isotope effects for N<sub>2</sub>O consumption or using a nonzero SP for N<sub>2</sub>O production had large impacts on the model output, including the rate of consumption as well as the primary substrate for N<sub>2</sub>O production (Table 1). To understand the dynamic cycling of N<sub>2</sub>O more fully in ODZs, isotope effects and other environmental conditions need to be constrained.

## 4.2 Contributors to Near-Surface N<sub>2</sub>O Accumulation

The near-surface [N<sub>2</sub>O] maxima, such as the high [N<sub>2</sub>O] at Stns. 8-11, are climatically relevant, as these accumulations may supply N<sub>2</sub>O to the mixed layer and subsequently to the atmosphere via air-sea gas exchange. Based on earlier studies of N<sub>2</sub>O cycling in and around ODZs, is generally thought that nitrification is the primary pathway in the oxygenated surface waters (Cohen & Gordon, 1979; Dore et al., 1998; Nevison et al., 2003), while denitrification is the primary pathway in the anoxic core of the water column (Babbin et al., 2015; Farías et al., 2009), and a combination of nitrification with a high-N<sub>2</sub>O yield and incomplete denitrification drives high concentrations in the oxycline (Babbin et al., 2015; Farías et al., 2009). Recent work also suggests the importance of ammonia-oxidizing archaea to N<sub>2</sub>O production in the ETNP (Trimmer et al., 2016). However, high concentrations of N<sub>2</sub>O are not uniform across the ETNP, as seen in variability across this and other studies (Babbin et al., 2015; Kelly et al., 2021; Yamagishi et al., 2007).

In a global model, Yang et al. (2020) correlated the N<sub>2</sub>O seasonality of the Eastern Tropical Pacific with Chl-*a* and apparent oxygen utilization. In our study, the high-N<sub>2</sub>O stations not only contained the shallowest oxycline and nitracline of the transect (Fig. 2b, 2d) but also the highest estimates of Chl-*a* (Fig. S2b). Additionally, three out of the four high-N<sub>2</sub>O stations contained a deep Chl-*a* maximum. In order to quantify the relationship between [N<sub>2</sub>O], [O<sub>2</sub>], and Chl-*a*, near-surface averages were calculated for each property at each station by linearly interpolating values to every 0.05 kg/m<sup>3</sup> density interval, taking a trapezoidal sum for  $\sigma_\theta < 26 \text{ kg/m}^3$ , and then dividing by the density range the integral was taken over (26 kg/m<sup>3</sup> minus surface density at each station). Regression analysis revealed strong relationships between

average  $[\text{N}_2\text{O}]$  and average  $[\text{O}_2]$  ( $R^2 = 0.90$ ,  $p < 0.001$ ) and average  $[\text{N}_2\text{O}]$  and average Chl-*a* ( $R^2 = 0.76$ ,  $p < 0.001$ ) along the transect (Fig. 5), affirming these two predictors on a local scale.



**Figure 5: Relationships between  $[\text{N}_2\text{O}]$ , Chl-*a*, and  $[\text{O}_2]$ .** Regressions between (a) the average  $[\text{N}_2\text{O}]$  and average Chl-*a* and (b) the average  $[\text{N}_2\text{O}]$  and average  $[\text{O}_2]$  for  $\sigma_\theta < 26 \text{ kg/m}^3$  at each station in the transect. Averages were calculated by linearly interpolating values to every  $0.05 \text{ kg/m}^3$  density interval, taking a trapezoidal sum for  $\sigma_\theta < 26 \text{ kg/m}^3$ , and then dividing by the density range the integral was taken over.

There are likely both biogeochemical and physical mechanisms contributing to the  $\text{N}_2\text{O}$  accumulation in the near-surface maxima. The shoaling of the oxycline brings the suboxic-anoxic interface — the site of incomplete denitrification (Babbin et al., 2015) — up to a portion of the water column with higher primary productivity and thus particulate matter, leading to increased  $\text{N}_2\text{O}$  production. The enhancement of denitrification by particulate organic matter has been observed in ODZs before (Babbin et al., 2014; Chang et al., 2014; Ward et al., 2008), and, in incubations, particulate matter additions have been shown to increase  $\text{N}_2\text{O}$  production via denitrification up to 5-fold (Frey et al., 2020). Similarly, nitrification fueled by enhanced organic matter regeneration could contribute to enhanced  $\text{N}_2\text{O}$  production under these conditions. The high Chl-*a* estimates at high- $\text{N}_2\text{O}$  stations (Fig. S2b) potentially indicate higher levels of particulate organic matter, facilitating higher levels of  $\text{N}_2\text{O}$  production by either mechanism. It is likely that organic matter and a shallow oxycline fuel the  $\text{N}_2\text{O}$  accumulation at these stations, in conjunction with a relatively strong pycnocline (Fig. S2a) preventing the  $\text{N}_2\text{O}$  from escaping to the surface. The isotopic measurements conducted in this study can be used to constrain the sources of  $\text{N}_2\text{O}$  in this near-surface feature.

$\text{N}_2\text{O}$  isotopic ratios in samples with  $\sigma_\theta < 25.8 \text{ kg/m}^3$  were plotted against the inverse of  $\text{N}_2\text{O}$  concentration,  $1/[\text{N}_2\text{O}]$ , to compare near-surface sources at high- $\text{N}_2\text{O}$  stations to those in the

rest of the transect. Under the framework of a background pool of  $\text{N}_2\text{O}$  to which  $\text{N}_2\text{O}$  is added, the y-intercepts of regressions performed on such “Keeling plot” analyses (C. D. Keeling, 1961) yield the isotopic compositions of the  $\text{N}_2\text{O}$  source (Casciotti et al., 2018; Fujii et al., 2013; Ji et al., 2019; Yamagishi et al., 2007). The y-intercepts of  $\delta^{18}\text{O}-\text{N}_2\text{O}$ , SP,  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$ , and  $\delta^{15}\text{N}_2\text{O}^\beta$  on  $1/[\text{N}_2\text{O}]$  for high- $\text{N}_2\text{O}$  stations were 51.4‰, 11.1‰, 8.2‰, 2.6‰, and -2.9‰, respectively, while the y-intercepts for the other eight stations were 53.8‰, 10.0‰, 11.4‰, 6.4‰, and 1.4‰, respectively (Fig. S8). SP was the only tracer that was not significantly different (outside standard error) between the two sets of stations, while intercepts for  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^\beta$ , and  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  were the most different.

Although SP is thought to reflect the  $\text{N}_2\text{O}$  production processes independently of the isotopic composition of substrates (Schmidt et al., 2004; Sutka et al., 2004; Yoshida & Toyoda, 2000), these Keeling analyses show the importance of looking at  $\delta^{15}\text{N}_2\text{O}^\alpha$  and  $\delta^{15}\text{N}_2\text{O}^\beta$  along with SP. While SP as a tracer can distinguish between oxidative (nitrification) and reductive (denitrification)  $\text{N}_2\text{O}$  production, the individual variations in  $\delta^{15}\text{N}_2\text{O}^\alpha$ ,  $\delta^{15}\text{N}_2\text{O}^\beta$ , and  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  help further distinguish between substrate pools ( $\text{NO}_3^-$  vs.  $\text{NO}_2^-$ ) and different reductive processes (denitrification vs. nitrifier-denitrification). With respect to the mix of production processes, the lower  $\delta^{15}\text{N}_2\text{O}$  intercepts at high- $\text{N}_2\text{O}$  stations could indicate a stronger contribution from either denitrification or nitrifier-denitrification, as the low  $\delta^{15}\text{N}_2\text{O}^{\text{bulk}}$  signals are commonly observed during nitrifier-denitrification (Casciotti et al., 2018; Frame & Casciotti, 2010; Sutka et al., 2004; Webster & Hopkins, 1996; Yoshida, 1988).

The intercepts for high- $\text{N}_2\text{O}$  stations were remarkably like those determined in Yamagishi et al. (2007), which concluded that nitrification contributed more to  $\text{N}_2\text{O}$  production than denitrification in the oxycline of the ETNP (Yamagishi et al., 2007). More recent work, however, has attributed denitrification as the dominant source of  $\text{N}_2\text{O}$  in ODZs, especially in the oxycline (Frey et al., 2020; Ji et al., 2015, 2018; Kelly et al., 2020). To better constrain the mix of processes that may contribute to the flux of  $\text{N}_2\text{O}$  to the atmosphere, the time-dependent model and optimization procedure described above for the core-ODZ depths were applied to the near-surface.

For these model experiments,  $\text{N}_2\text{O}$  production and consumption via denitrification were parameterized as they were in the ODZ core. To simulate an area shallower in the water column, nitrification was added as a potential source of  $\text{N}_2\text{O}$ . The isotope systematics for  $\text{N}_2\text{O}$  production from ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were added to the model using values from the literature (Table S2; Frame & Casciotti, 2010; Santoro et al., 2011; Sutka et al., 2006). Rather than starting with a pool of  $\text{N}_2\text{O}$  and simulating the decrease in  $[\text{N}_2\text{O}]$  as in the runs for the anoxic core, the opposite was done for this set of experiments. The smallest  $[\text{N}_2\text{O}]$  measurement of the transect and its associated isotopic value within the model domain  $\sigma_\theta = 23.5\text{--}25.8 \text{ kg/m}^3$  were used to initialize the model, and the model was optimized to match  $\text{N}_2\text{O}$  isotopic values as  $[\text{N}_2\text{O}]$  increased to the maximal value of the transect within the model domain. This density layer was chosen because it contained the highest  $[\text{N}_2\text{O}]$  values at each station, without showing dominant consumption signals. Isotopic values for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were taken from the density layer at the high- $\text{N}_2\text{O}$  stations. Because  $[\text{NO}_2^-]$  was often too low to measure  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , two sets of initializations were tested — one using the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for  $\text{NO}_2^-$  from the PNM and one using the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for  $\text{NO}_2^-$  from the SNM. Neither the PNM nor SNM were exactly in the density layer of interest, but these values were used as two potential

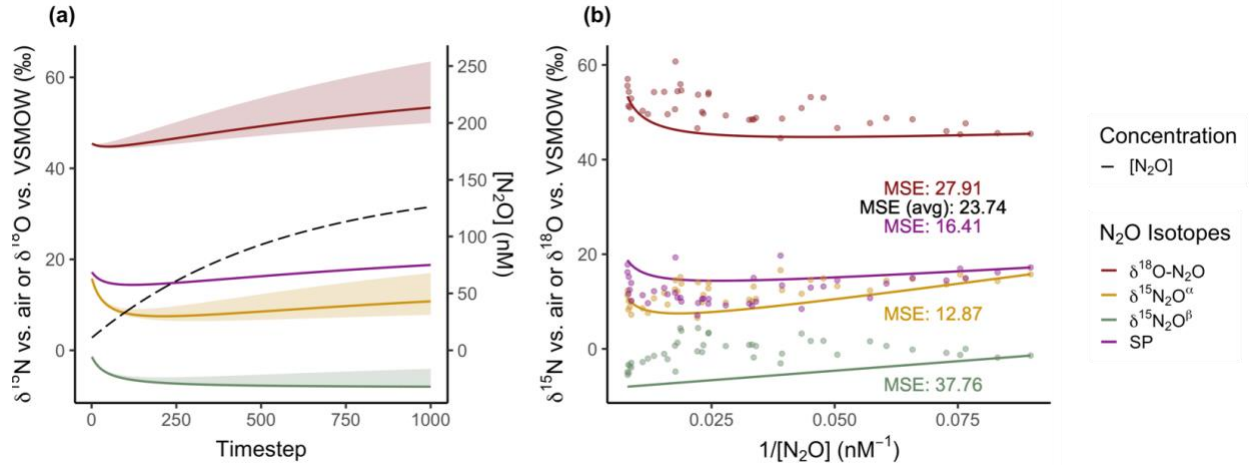
endmembers to evaluate the difference between. In addition, the isotope effects for N<sub>2</sub>O consumption were varied as described for the ODZ formulation. Unlike the previous experiments,  $k_{\text{cons}}$  was held constant and did not evolve nonlinearly. In addition, the isotope effects for N<sub>2</sub>O consumption were varied as described for the ODZ formulation. Unlike the previous experiments,  $k_{\text{cons}}$  was held constant and did not evolve nonlinearly. While  $k_{\text{cons}}$  was constant, the rates (Table 2) still evolved because [N<sub>2</sub>O] changed, so the initial and maximal rates of consumption are reported. The model initializations for this set of experiments are outlined in the supplement (Table S2) and the results in Table 2.

**Table 2: Optimized rates (nM N/day) for N<sub>2</sub>O production and consumption above the ODZ ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ ), using data from all stations.** Mean squared error (MSE) is presented as an average of that of the four tracers.

|   |   | NO <sub>2</sub> <sup>-</sup> PNM | NO <sub>2</sub> <sup>-</sup> SNM | <sup>15,18</sup> ε <sub>cons</sub> × 1.5<br>and NO <sub>2</sub> <sup>-</sup> PNM | <sup>15,18</sup> ε <sub>cons</sub> × 1.5<br>and NO <sub>2</sub> <sup>-</sup> SNM |
|---|---|----------------------------------|----------------------------------|--|--|
| SP = 0‰<br><br><sup>15</sup> ε <sub>NOx→N2O</sub> (α) =<br><sup>15</sup> ε <sub>NOx→N2O</sub> (β) = 22‰ | Prod. from NO <sub>2</sub> <sup>-</sup>       | 0                                | 0                                | 0  | 0  |
|   | Prod. from NO <sub>3</sub> <sup>-</sup>       | 8.33 × 10 <sup>-1</sup>          | 8.33 × 10 <sup>-1</sup>          | 6.20 × 10 <sup>-1</sup>  | 6.20 × 10 <sup>-1</sup>  |
|   | Initial Cons.                                 | 2.01 × 10 <sup>-1</sup>          | 2.01 × 10 <sup>-1</sup>          | 1.17 × 10 <sup>-1</sup>  | 1.17 × 10 <sup>-1</sup>  |
|   | Max. Cons.                                    | 2.28 × 10 <sup>0</sup>           | 2.28 × 10 <sup>0</sup>           | 1.32 × 10 <sup>0</sup>   | 1.32 × 10 <sup>0</sup>   |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOA | 5.10 × 10 <sup>-1</sup>          | 5.10 × 10 <sup>-1</sup>          | 3.65 × 10 <sup>-1</sup>  | 3.65 × 10 <sup>-1</sup>  |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOB | 0                                | 0                                | 0  | 0  |
|   | MSE   | 23.74                            | 23.74                            | 22.88  | 22.88  |
| SP = 22‰<br><br><sup>15</sup> ε <sub>NOx→N2O</sub> (α) = 0‰   | Prod. from NO <sub>2</sub> <sup>-</sup>       | 1.93 × 10 <sup>-6</sup>          | 3.34 × 10 <sup>-9</sup>          | 1.92 × 10 <sup>-7</sup>  | 4.31 × 10 <sup>-3</sup>  |
|   | Prod. from NO <sub>3</sub> <sup>-</sup>       | 5.63 × 10 <sup>1</sup>           | 5.39 × 10 <sup>1</sup>           | 5.63 × 10 <sup>1</sup>   | 5.35 × 10 <sup>1</sup>   |
|   | Initial Cons.                                 | 1.25 × 10 <sup>0</sup>           | 1.28 × 10 <sup>0</sup>           | 1.25 × 10 <sup>0</sup>   | 1.97 × 10 <sup>0</sup>   |
|   | Max. Cons.                                    | 1.13 × 10 <sup>2</sup>           | 1.13 × 10 <sup>2</sup>           | 1.13 × 10 <sup>2</sup>   | 1.13 × 10 <sup>2</sup>   |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOA | 1.74 × 10 <sup>-9</sup>          | 1.75 × 10 <sup>-4</sup>          | 1.52 × 10 <sup>-3</sup>  | 7.14 × 10 <sup>-2</sup>  |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOB | 1.64 × 10 <sup>-12</sup>         | 2.46 × 10 <sup>0</sup>           | 1.88 × 10 <sup>-7</sup>  | 3.10 × 10 <sup>0</sup>   |
|   | MSE   | 42.32                            | 40.69                            | 42.45  | 40.49  |
| SP = 22‰<br><br><sup>15</sup> ε <sub>NOx→N2O</sub> (β) = 44‰  | Prod. from NO <sub>2</sub> <sup>-</sup>       | 8.48 × 10 <sup>-9</sup>          | 8.48 × 10 <sup>-9</sup>          | 0  | 6.24 × 10 <sup>-4</sup>  |
|   | Prod. from NO <sub>3</sub> <sup>-</sup>       | 8.78 × 10 <sup>0</sup>           | 8.78 × 10 <sup>0</sup>           | 6.20 × 10 <sup>-1</sup>  | 1.44 × 10 <sup>-1</sup>  |
|   | Initial Cons.                                 | 1.82 × 10 <sup>0</sup>           | 1.82 × 10 <sup>0</sup>           | 1.17 × 10 <sup>-1</sup>  | 3.69 × 10 <sup>0</sup>   |
|   | Max. Cons.                                    | 1.13 × 10 <sup>2</sup>           | 1.13 × 10 <sup>2</sup>           | 1.32 × 10 <sup>0</sup>   | 4.53 × 10 <sup>1</sup>   |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOA | 3.81 × 10 <sup>1</sup>           | 3.81 × 10 <sup>1</sup>           | 3.65 × 10 <sup>-1</sup>  | 1.55 × 10 <sup>1</sup>   |
|   | Prod. from NH <sub>4</sub> <sup>+</sup> , AOB | 9.73 × 10 <sup>0</sup>           | 9.73 × 10 <sup>0</sup>           | 0  | 6.99 × 10 <sup>0</sup>   |
|   | MSE   | 71.94                            | 71.94                            | 22.88  | 57.44  |

The model was generally able to recreate the Keeling plot for  $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , including the sharp increase in isotopic values at high-N<sub>2</sub>O points (Fig. 6). Unlike in the ODZ, runs with the set of isotope effects for N<sub>2</sub>O production via denitrification with SP = 0‰ performed much better than those with SP = 22‰ (Table 2). In several instances, the optimization converged at the same rates and mechanisms of N<sub>2</sub>O cycling, regardless of whether

$\text{NO}_2^-$  was initialized with  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  from the PNM or SNM (Table 2). This is largely because the rates of  $\text{N}_2\text{O}$  production from  $\text{NO}_2^-$  were negligible in the near-surface maximum, converging to zero in the runs with the lowest MSE (Table 2).



**Figure 6: Time-dependent model output from above-ODZ experiments.** Model output for the default initialization with  $\text{SP} = 0\text{‰}$ , presented as (a) a time series of  $[\text{N}_2\text{O}]$  and isotopocules and (b) a recreated Keeling plot for  $\sigma_\theta = 23.5\text{--}25.8 \text{ kg/m}^3$ . The maximum and minimum bounds for the time series in (a) are output using the maximum and minimum  $^{15}\epsilon_{\text{cons}}$  and  $^{18}\epsilon_{\text{cons}}$  from literature. In (b), model output is plotted with a set of lines, while observations are plotted with color-matched points. While the previous experiment used only data points from core-ODZ stations, this set of runs used data points within the density layer from all stations. Since this set of runs began with a small amount of  $\text{N}_2\text{O}$ , which gradually increased, time progresses on the Keeling plot from right to left.

The model runs with the best fit each included  $\text{N}_2\text{O}$  production via denitrification from  $\text{NO}_3^-$ ,  $\text{N}_2\text{O}$  production via nitrification by AOA, and  $\text{N}_2\text{O}$  consumption via denitrification; rates for denitrification from  $\text{NO}_2^-$  and nitrification via AOB were optimized at or near zero in several of the experiments (Table 2). While the experiments in the anoxic core primarily used  $\text{NO}_2^-$  as the substrate for denitrification (Table 1), these runs at the base of the oxycline primarily used  $\text{NO}_3^-$ , which is in line with tracer experiments and natural abundance isotope ratio measurements (Casciotti et al., 2018; Frey et al., 2020; Ji et al., 2015, 2018). Furthermore, this set of experiments provides support for the idea that AOA and not AOB are largely responsible for the ammonia-derived source of  $\text{N}_2\text{O}$  near the surface, as set forth by Santoro et al. (2011) and Trimmer et al. (2016).

$\text{N}_2\text{O}$  consumption was a significant process across all runs (Table 2), despite the presence of oxygen in part of this area of the water column (Fig. 2b). This agrees with recent nonzero rate measurements of  $\text{N}_2\text{O}$  consumption above the ETNP ODZ (Sun et al., 2021) and model results suggesting that the anoxic interior of particles may allow denitrification to proceed in otherwise

oxygenated waters (Bianchi et al., 2018). In our model, N<sub>2</sub>O consumption in this region allowed the reconstructed Keeling plot to recreate the sharp increase in isotopic values at high-N<sub>2</sub>O points (Fig. 6b). Rates of the two most significant sources of N<sub>2</sub>O — production from NO<sub>3</sub><sup>-</sup> by denitrifiers and production from NH<sub>4</sub><sup>+</sup> by AOA — were the same order of magnitude in the best-fit model runs, with denitrification producing N<sub>2</sub>O at a slightly higher rate (Table 2). These results thus reinforce the importance of incomplete denitrification as a driver of N<sub>2</sub>O accumulations (Babbin et al., 2015; Bourbonnais et al., 2017; Ji et al., 2015, 2018), as well as showing that nitrification is still required to match the observed isotopic trends.

Finally, as in the anoxic core-ODZ experiments (Table 1), these optimizations displayed considerable variability when isotope effects (SP for production via denitrification or <sup>15</sup>ε<sub>cons</sub> and <sup>18</sup>ε<sub>cons</sub>) were varied. In the anoxic core, N<sub>2</sub>O production via denitrification with SP = 22‰ was able to better match isotopic observations (Table 1). Here, however, N<sub>2</sub>O production via denitrification with SP = 22‰ worsened the fit of the optimization (Table 2), highlighting the need to constrain these isotope effects for denitrification throughout the water column. These model results suggest a variable SP for microbial denitrification under different environmental conditions or microbial communities, or variable roles of fungal denitrification throughout the water column.

### 4.3 Eddies and the Variability of N<sub>2</sub>O Cycling in the ETNP

The unique features of the high-N<sub>2</sub>O stations relative to the rest of the transect are made more interesting by the presence of an anticyclonic eddy at these stations (Fig. S4). Previous surveys linking mesoscale eddies to the N<sub>2</sub>O cycle have focused on the ODZ of the Eastern Tropical Southern Pacific (Arévalo-Martínez et al., 2016; Bourbonnais et al., 2015; Cornejo D'Ottone et al., 2016; Frey et al., 2020). There, young eddies are associated with high [N<sub>2</sub>O] and high N<sub>2</sub>O production rates (Frey et al., 2020), while eddy aging generally leads to decreasing [N<sub>2</sub>O] throughout the water column due to the reduced supply of organic matter to fuel denitrification processes (Arévalo-Martínez et al., 2016). The highest N<sub>2</sub>O production rates for denitrification ever measured in the ETSP were in an anticyclonic mode water eddy (Frey et al., 2020). A recent study of an anticyclonic mode water eddy in the eastern tropical North Atlantic — a region where [N<sub>2</sub>O] is typically low relative to ODZs — found N<sub>2</sub>O concentrations up to 115 nM (Grundle et al., 2017). These studies show that environments created by mesoscale eddies are conducive to high N<sub>2</sub>O production and enhanced denitrification in the water column.

This study represents the first in the ETNP to link N<sub>2</sub>O isotopocule measurements to a mesoscale eddy. While relatively high sea surface height anomalies (Fig. S4) and depressed isopycnals in deeper water (Fig. S2a) indicate that the eddy was anticyclonic, several other hydrographic features of the high-N<sub>2</sub>O stations are not typically associated with downwelling eddies. For example, the oxycline was shallower relative to the other stations (Section 3.1), and the water column was more stratified (Supp Fig. 2b). Typically, anticyclonic eddies decrease stratification at their core (Allen et al., 1991). This suggests that the shallow oxycline and stratified column may have been features of this site before the onset of the eddy, which is likely, given that section profiles of similar transects through the ETNP show that the oxycline of waters around 14–16°N historically occurs on lower-density isopycnals relative to the northern

end of the transect (see Horak et al., 2016). Alternatively, the eddy could have imported these features from the water where it formed, particularly if its origin was closer to the coast.

The stations near the anticyclonic eddy also contained the highest biological productivity of the transect (Fig. S2a; Fig. 5). Enhanced Chl-*a* has been previously observed in anticyclonic eddies in other parts of the ocean and could be driven by intense submesoscale upwelling (Brannigan, 2016 and references therein). Additionally, submesoscale processes can operate at the density fronts on the eddy periphery, bringing up nutrients to the surface (Callbeck et al., 2017; Klein & Lapeyre, 2009; Mahadevan, 2016). The salinity maximum at the surface of Stn. 6 (Supp. Fig 2b) could be a signal of this frontal upwelling. Upwelling at the edge of the eddy associated with the density front and subsequent mixing within the eddy could thus explain the enhanced productivity at high-N<sub>2</sub>O stations.

Mesoscale eddies and the submesoscale processes within add new complexity to the spatiotemporal variability of the ETNP N<sub>2</sub>O cycle. Based on biogeochemical and isotopic measurements, this transect alone contained three different regimes of N<sub>2</sub>O cycling (Section 2). The influence of eddies on this variability is unknown, but in this study the highest N<sub>2</sub>O concentrations were associated with the site of an anticyclonic eddy and its shallow oxycline and high primary productivity. Further study on the connection between different types of eddies and N<sub>2</sub>O cycling in the ETNP will be of great importance to modeling the outgassing of N<sub>2</sub>O in this region.

## 5 Conclusion

Three distinct regimes of N<sub>2</sub>O cycling were identified along this north-south transect within the ETNP. The patterns of isotopocule distributions at low [N<sub>2</sub>O] in the core of the ODZ suggested that N<sub>2</sub>O was simultaneously being produced and consumed under anoxia. A time-dependent model of N<sub>2</sub>O isotopocules confirmed that production via denitrification was a significant process and that the continuing evolution of isotopologues past the plateau of [N<sub>2</sub>O] is consistent with a longer time scale for isotopic steady state. Denitrification from NO<sub>3</sub><sup>-</sup> and/or NO<sub>2</sub><sup>-</sup> with a nonzero SP allowed the model to fit the data well, providing evidence that this process — either in the form of fungal denitrification or bacterial denitrification with a variable SP — could occur as previously suggested. Further work could focus on constraining the SP for denitrification throughout the water column, or for different species of denitrifiers.

Implementation of the time-dependent model in the near-surface [N<sub>2</sub>O] maximum led to the conclusion that AOA, and not AOB, were the major nitrifying source of N<sub>2</sub>O; incomplete denitrification of NO<sub>3</sub><sup>-</sup> also remained a significant contributor to the high [N<sub>2</sub>O]. This accumulation of N<sub>2</sub>O coincided with high levels of primary productivity and a shallower oxycline relative to the rest of the transect. A shallower niche for high-yield N<sub>2</sub>O processes and greater levels of organic matter could explain the accumulation. Finally, the presence of a mesoscale eddy at the site of the N<sub>2</sub>O accumulation adds to the heterogeneity of the cycle in the ETNP. By modeling the rates of nitrification and denitrification processes and connecting them to hydrographic parameters, this study adds new insight to the cycling of this important greenhouse gas.

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## Data Availability

The data reported in this study can be found in the Stanford Digital Repository (<https://purl.stanford.edu/hw279tj6064>) and is being submitted to BCO-DMO. CTD and other nutrient data from SKQ201617S is deposited in BCO-DMO (<https://www.bco-dmo.org/dataset/732092>). Code for optimization of the time-dependent model is available on Zenodo (<https://doi.org/10.5281/zenodo.5835562>). RStudio, Ocean Data View, and SeaDAS were the primary software used in this study and are publicly available. Code used to generate figures is available upon request. Sea surface height anomaly data products retrieved from the Copernicus Marine Environmental Monitoring Service (see supplement), and the World Ocean Atlas 2018 product was downloaded as an Ocean Data View collection (<https://odv.awi.de/data/ocean/world-ocean-atlas-2018/>).

## References

- Allen, J. T., Pollard, R. T., & New, A. L. (1991). How do eddies modify the stratification of the thermocline? In J. Potter & A. Warn-Varnas (Eds.), *Ocean Variability & Acoustic Propagation* (pp. 417–431). Springer, Dordrecht.
- Arévalo-Martínez, D. L., Kock, A., Löscher, C. R., Schmitz, R. A., Stramma, L., & Bange, H. W. (2016). Influence of mesoscale eddies on the distribution of nitrous oxide in the eastern tropical South Pacific. *Biogeosciences*, *13*, 1105–1118. <https://doi.org/10.5194/bg-13-1105-2016>
- Babbin, A. R., Keil, R. G., Devol, A. H., & Ward, B. B. (2014). Organic Matter Stoichiometry, Flux, and Oxygen Control Nitrogen Loss in the Ocean. *Science*, *344*(6182), 406–408.

<https://doi.org/10.1126/science.1248364>

Babbin, A. R., Bianchi, D., Jayakumar, A., & Ward, B. B. (2015). Rapid nitrous oxide cycling in the suboxic ocean. *Science*, *348*(6239), 1127–1129. <https://doi.org/10.1126/science.aaa8380>

Bianchi, D., Weber, T. S., Kiko, R., & Deutsch, C. (2018). Global niche of marine anaerobic metabolisms expanded by particle microenvironments. *Nature Geoscience*, *11*, 263–268. <https://doi.org/10.1038/s41561-018-0081-0>

Böhlke, J. K., Mroczkowski, S. J., & Coplen, T. B. (2003). Oxygen isotopes in nitrate: New reference materials for  $^{18}\text{O}$ : $^{17}\text{O}$ : $^{16}\text{O}$  measurements and observations on nitrate-water equilibration. *Rapid Communications in Mass Spectrometry*, *17*, 1835–1846. <https://doi.org/10.1002/rcm.1123>

Bourbonnais, A., Altabet, M. A., Charoenpong, C. N., Larkum, J., Hu, H., Bange, H. W., & Stramma, L. (2015). N-loss isotope effects in the Peru oxygen minimum zone studied using a mesoscale eddy as a natural tracer experiment. *Global Biogeochemical Cycles*, *29*, 793–811. <https://doi.org/10.1002/2014GB005001>.Received

Bourbonnais, A., Letscher, R. T., Bange, H. W., Échevin, V., Larkum, J., Mohn, J., et al. (2017).  $\text{N}_2\text{O}$  production and consumption from stable isotopic and concentration data in the Peruvian coastal upwelling system. *Global Biogeochemical Cycles*, *31*(3), 678–698. <https://doi.org/10.1002/2016GB005567>

Brannigan, L. (2016). Intense submesoscale upwelling in anticyclonic eddies. *Geophysical Research Letters*, *43*, 3360–3369. <https://doi.org/10.1002/2016GL067926>.1.

Buchwald, C., Santoro, A. E., Stanley, R. H. R., & Casciotti, K. L. (2015). Nitrogen cycling in the secondary nitrite maximum of the eastern tropical North Pacific off Costa Rica. *Global Biogeochemical Cycles*, *29*, 2061–2081. <https://doi.org/10.1002/2015GB005187>

- Callbeck, C. M., Lavik, G., Stramma, L., Kuypers, M. M. M., & Bristow, L. A. (2017). Enhanced Nitrogen Loss by Eddy-Induced Vertical Transport in the Offshore Peruvian Oxygen Minimum Zone. *PLoS ONE*, 12(1). <https://doi.org/10.1371/journal.pone.0170059>
- Casciotti, K. L., & McIlvin, M. R. (2007). Isotopic analyses of nitrate and nitrite from reference mixtures and application to Eastern Tropical North Pacific waters. *Marine Chemistry*, 107, 184–201. <https://doi.org/10.1016/j.marchem.2007.06.021>
- Casciotti, K. L., Sigman, D. M., Hastings, M. G., Bohlke, J. K., & Hilkert, A. (2002). Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. *Analytical Chemistry*, 74(19), 4905–4912.
- Casciotti, K. L., Forbes, M., Vedamati, J., Peters, B. D., Martin, T. S., & Mordy, C. W. (2018). Nitrous oxide cycling in the Eastern Tropical South Pacific as inferred from isotopic and isotopomeric data. *Deep-Sea Research Part II*, 156, 155–167. <https://doi.org/10.1016/j.dsr2.2018.07.014>
- Casciotti, Karen L., Bohlke, J. K., Mcilvin, M. R., Mroczkowski, S. J., & Hannon, J. E. (2007). Oxygen Isotopes in Nitrite: Analysis, Calibration, and Equilibration. *Analytical Chemistry*, 79(6), 2427–2436.
- Chang, B. X., Rich, J. R., Jayakumar, A., Naik, H., Pratihary, A. K., Keil, R. G., et al. (2014). The effect of organic carbon on fixed nitrogen loss in the eastern tropical South Pacific and Arabian Sea oxygen deficient zones. *Limnology and Oceanography*, 59(4), 1267–1274. <https://doi.org/10.4319/lo.2014.59.4.1267>
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., et al. (2013). Carbon and Other Biogeochemical Cycles. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental*

*Panel on Climate Change*, 465–570.

Codispoti, L. A., & Christensen, J. P. (1985). Nitrification, Denitrification and Nitrous Oxide

Cycling in the Eastern Tropical South Pacific Ocean. *Marine Chemistry*, 16, 277–300.

Codispoti, Louis A. (2010). Interesting Times for Marine N<sub>2</sub>O. *Science*, 327, 1339–1341.

Cohen, Y., & Gordon, L. I. (1979). Nitrous Oxide Production in the Ocean. *Journal of*

*Geophysical Research*, 84(C1), 347–353.

Cornejo D'Ottone, M., Bravo, L., Ramos, M., Pizarro, O., Karstensen, J., Gallegos, M., et al.

(2016). Biogeochemical characteristics of a long-lived anticyclonic eddy in the eastern

South Pacific Ocean. *Biogeosciences*, 13, 2971–2979. [https://doi.org/10.5194/bg-13-2971-](https://doi.org/10.5194/bg-13-2971-2016)

2016

Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., et al.

(2007). Couplings Between Changes in the Climate System and Biogeochemistry. *In:*

*Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the*

*Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, 499–587.

Dore, J. E., Popp, B. N., Karl, D. M., & Sansone, F. J. (1998). A large source of atmospheric

nitrous oxide from subtropical North Pacific surface waters. *Letters to Nature*, 396, 63–66.

Elkins, J. W., Wofsy, S. C., Mcelroy, M. B., Kolb, C. E., & Kaplan, W. A. (1978). Aquatic

sources and sinks for nitrous oxide. *Nature*, 275, 602–606.

Evans, N., Boles, E., Kwiecinski, J. V., Mullen, S., Wolf, M., Devol, A. H., et al. (2020). The

role of water masses in shaping the distribution of redox active compounds in the Eastern

Tropical North Pacific oxygen deficient zone and influencing low oxygen concentrations in

the eastern Pacific Ocean. *Limnology and Oceanography*, 65, 1688–1705.

<https://doi.org/10.1002/lno.11412>

- Farías, L., Castro-González, M., Cornejo, M., Charpentier, J., Faúndez, J., Boontanon, N., & Yoshida, N. (2009). Denitrification and nitrous oxide cycling within the upper oxycline of the eastern tropical South Pacific oxygen minimum zone. *Limnology and Oceanography*, 54(1), 132–144.
- Frame, C. H., & Casciotti, K. L. (2010). Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences*, 7, 2695–2709. <https://doi.org/10.5194/bg-7-2695-2010>
- Freing, A., Wallace, D. W. R., & Bange, H. W. (2012). Global oceanic production of nitrous oxide. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367, 1245–1255. <https://doi.org/10.1098/rstb.2011.0360>
- Frey, C., Bange, H. W., Achterberg, E. P., Jayakumar, A., Löscher, C. R., Arévalo-Martínez, D. L., et al. (2020). Regulation of nitrous oxide production in low-oxygen waters off the coast of Peru. *Biogeosciences*, 17, 2263–2287.
- Frölicher, T. L., Ashwanden, M. T., Gruber, N., Jaccard, S. L., Dunne, J. P., & Paynter, D. (2020). Contrasting Upper and Deep Ocean Oxygen Response to Protracted Global Warming. *Global Biogeochemical Cycles*, 34. <https://doi.org/10.1029/2020GB006601>
- Fujii, A., Toyoda, S., Yoshida, O., Watanabe, S., Sasaki, K., & Yoshida, N. (2013). Distribution of nitrous oxide dissolved in water masses in the eastern subtropical North Pacific and its origin inferred from isotopomer analysis. *Journal of Oceanography*, 69, 147–157. <https://doi.org/10.1007/s10872-012-0162-4>
- Garcia, H. E., Weathers, K. W., Paver, C. R., Smolyar, I., Boyer, T. P., Locarnini, R. A., et al. (2019). Volume 3: Dissolved Oxygen, Apparent Oxygen Utilization, and Dissolved Oxygen Saturation. In *World Ocean Atlas 2018*. NOAA Atlas NESDIS 83. Retrieved from

<https://www.nodc.noaa.gov/OC5/woa18/pubwoa18.html>

Granger, J., & Sigman, D. M. (2009). Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. *Rapid Communications in Mass Spectrometry*, 23, 3753–3762. <https://doi.org/10.1002/rcm>

Grasshoff, K., Kremling, K., & Ehrhardt, M. (1999). *Methods of Seawater Analysis* (3rd ed.). Weinheim: Wiley-VCH. <https://doi.org/10.1002/9783527613984>

Grundle, D. S., Löscher, C. R., Krahmann, G., Altabet, M. A., Bange, H. W., & Karstensen, J. (2017). Low oxygen eddies in the eastern tropical North Atlantic: Implications for N<sub>2</sub>O cycling. *Scientific Reports*, 7(4806). <https://doi.org/10.1038/s41598-017-04745-y>

Hirsch, A. I., Michalak, A. M., Bruhwiler, L. M., Peters, W., Dlugokencky, E. J., & Tans, P. P. (2006). Inverse modeling estimates of the global nitrous oxide surface flux from 1998–2001. *Global Biogeochemical Cycles*, 20(GB1008). <https://doi.org/10.1029/2004GB002443>

Horak, R. E. A., Ruef, W., Ward, B. B., & Devol, A. H. (2016). Expansion of denitrification and anoxia in the eastern tropical North Pacific. *Geophysical Research Letters*, 43, 5252–5260. <https://doi.org/10.1002/2016GL068871>.Received

Ji, Q., Babbin, A. R., Jayakumar, A., Oleynik, S., & Ward, B. B. (2015). Nitrous oxide production by nitrification and denitrification in the Eastern Tropical South Pacific oxygen minimum zone. *Geophysical Research Letters*, (42), 10,755–10,764. <https://doi.org/10.1002/2015GL066853>

Ji, Q., Buitenhuis, E., Suntharalingam, P., Sarmiento, J. L., & Ward, B. B. (2018). Global Nitrous Oxide Production Determined by Oxygen Sensitivity of Nitrification and Denitrification. *Global Biogeochemical Cycles*, 32, 1790–1802. <https://doi.org/10.1029/2018GB005887>

- 906 Ji, Q., Altabet, M. A., Bange, H. W., Graco, M. I., Ma, X., Arévalo-Martínez, D. L., & Grundle,  
907 D. S. (2019). Investigating the effect of El Niño on nitrous oxide distribution in the eastern  
908 tropical South Pacific. *Biogeosciences*, *16*, 2079–2093.
- 909 Keeling, C. D. (1961). The concentration and isotopic abundances of carbon dioxide in rural and  
910 marine air. *Geochimica et Cosmochimica Acta*, *24*, 277–298.
- 911 Keeling, R. F., Körtzinger, A., & Gruber, N. (2010). Ocean deoxygenation in a warming world.  
912 *Annual Review of Marine Science*, *2*(1), 199–229.  
913 <https://doi.org/10.1146/annurev.marine.010908.163855>
- 914 Kelley, C. T. (1999). *Iterative Methods for Optimization*. Philadelphia: Society for Industrial and  
915 Applied Mathematics.
- 916 Kelly, C. L., Manning, C., Frey, C., Gluschkoff, N., & Casciotti, K. L. (n.d.). pyisotopomer: A  
917 Python package for obtaining nitrous oxide isotopocules from isotope ratio mass  
918 spectrometry. *Rapid Communications in Mass Spectrometry*. Pre-print:  
919 <https://doi.org/10.31223/X58S63>
- 920 Kelly, C. L., Travis, N. M., Baya, P. A., & Casciotti, K. L. (2021). Quantifying Nitrous Oxide  
921 Cycling Regimes in the Eastern Tropical North Pacific Ocean With Isotopomer Analysis.  
922 *Global Biogeochemical Cycles*, *35*(e2020GB006637).  
923 <https://doi.org/10.1029/2020GB006637>
- 924 Kim, K.-R., & Craig, H. (1990). Two-isotope characterization of N<sub>2</sub>O in the Pacific Ocean and  
925 constraints on its origin in deep water. *Letters to Nature*, *347*, 58–61.
- 926 Klein, P., & Lapeyre, G. (2009). The Oceanic Vertical Pump Induced by Mesoscale and  
927 Submesoscale Turbulence. *Annual Review of Marine Science*, *1*, 351–375.  
928 <https://doi.org/10.1146/annurev.marine.010908.163704>

- 929 Körner, H., & Zumft, W. G. (1989). Expression of Denitrification Enzymes in Response to the  
930 Dissolved Oxygen Level and Respiratory Substrate in Continuous Culture of *Pseudomonas*  
931 *stutzeri*. *Applied and Environmental Microbiology*, 55(7), 1670–1676.
- 932 Kozlowski, J. A., Stieglmeier, M., Schleper, C., Klotz, M. G., & Stein, L. Y. (2016). Pathways  
933 and key intermediates required for obligate aerobic ammonia-dependent chemolithotrophy  
934 in bacteria and Thaumarchaeota. *Nature Publishing Group*, 1836–1845.  
935 <https://doi.org/10.1038/ismej.2016.2>
- 936 Kroopnick, P., & Craig, H. (1972). Atmospheric Oxygen: Isotopic Composition and Solubility  
937 Fractionation. *Science*, 175(4017), 54–55.
- 938 Law, C. S., & Owens, N. J. P. (1990). Significant flux of atmospheric nitrous oxide from the  
939 northwest Indian Ocean. *Letters to Nature*, 346, 826–828.
- 940 Lehmann, N., Kienast, M., Granger, J., Bourbonnais, A., Altabet, M. A., & Tremblay, J.-É.  
941 (2019). Remote Western Arctic Nutrients Fuel Remineralization in Deep Baffin Bay.  
942 *Global Biogeochemical Cycles*, 33, 649–667. <https://doi.org/10.1029/2018GB006134>
- 943 Löscher, C. R., Kock, A., Könneke, M., LaRoche, J., Bange, H. W., & Schmitz, R. A. (2012).  
944 Production of oceanic nitrous oxide by ammonia-oxidizing archaea. *Biogeosciences*, 9,  
945 2419–2429. <https://doi.org/10.5194/bg-9-2419-2012>
- 946 Maeda, K., Spor, A., Edel-Hermann, V., Heraud, C., Breuil, M.-C., Bizouard, F., et al. (2015).  
947 N<sub>2</sub>O production, a widespread trait in fungi. *Scientific Reports*, 5(9697).  
948 <https://doi.org/10.1038/srep09697>
- 949 Mahadevan, A. (2016). The Impact of Submesoscale Physics on Primary Productivity of  
950 Plankton. *Annual Review of Marine Science*, 8, 161–184. [https://doi.org/10.1146/annurev-](https://doi.org/10.1146/annurev-marine-010814-015912)  
951 [marine-010814-015912](https://doi.org/10.1146/annurev-marine-010814-015912)



- 952 McIlvin, M. R., & Altabet, M. A. (2005). Chemical Conversion of Nitrate and Nitrite to Nitrous  
953 Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. *Analytical*  
954 *Chemistry*, 77, 5589–5595.
- 955 McIlvin, M. R., & Casciotti, K. L. (2010). Fully automated system for stable isotopic analyses of  
956 dissolved nitrous oxide at natural abundance levels. *Limnology and Oceanography*:  
957 *Methods*, 8, 54–66. <https://doi.org/10.4319/lom.2010.8.54>
- 958 McIlvin, M. R., & Casciotti, K. L. (2011). Technical Updates to the Bacterial Method for Nitrate  
959 Isotopic Analyses. *Analytical Chemistry*, 83, 1850–1856.
- 960 Mohn, J., Wolf, B., Toyoda, S., Lin, C.-T., Liang, M.-C., Brüggemann, N., et al. (2014).  
961 Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass  
962 spectrometry and laser spectroscopy: current status and perspectives. *Rapid*  
963 *Communications in Mass Spectrometry*, 28, 1995–2007. <https://doi.org/10.1002/rcm.6982>
- 964 Muggeo, V. M. R. (2003). Estimating regression models with unknown break-points. *Statistics in*  
965 *Medicine*, 22, 3055–3071. <https://doi.org/10.1002/sim.1545>
- 966 Muggeo, V. M. R. (2008). Segmented: an R package to fit regression models with broken-line  
967 relationships. *R News*, 8/1, 20–25.
- 968 Nelder, J. A., & Mead, R. (1965). A simplex method for function minimization. *The Computer*  
969 *Journal*, 7(4), 308–313.
- 970 Nevison, C., Butler, J. H., & Elkins, J. W. (2003). Global distribution of N<sub>2</sub>O and the DN<sub>2</sub>O-  
971 AOU yield in the subsurface ocean. *Global Biogeochemical Cycles*, 17(1119).  
972 <https://doi.org/10.1029/2003GB002068>
- 973 Ostrom, N. E., Pitt, A., Sutka, R., Ostrom, P. H., Grandy, A. S., Huizinga, K. M., & Robertson,  
974 G. P. (2007). Isotopologue effects during N<sub>2</sub>O reduction in soils and in pure cultures of

denitrifiers. *Journal of Geophysical Research*, 112(G02005).

<https://doi.org/10.1029/2006JG000287>

Peng, X., & Valentine, D. L. (2021). Diversity and N<sub>2</sub>O Production Potential of Fungi in an Oceanic Oxygen Minimum Zone. *Journal of Fungi*, 7(218).

<https://doi.org/10.3390/jof7030218>

Popp, B. N., Westley, M. B., Toyoda, S., Miwa, T., Dore, J. E., Yoshida, N., et al. (2002).

Nitrogen and oxygen isotopomeric constraints on the origins and sea-to-air flux of N<sub>2</sub>O in the oligotrophic subtropical North Pacific gyre. *Global Biogeochemical Cycles*, 16(1064).

<https://doi.org/10.1029/2001GB001806>

Rahn, T., & Wahlen, M. (2000). A reassessment of the global isotopic budget of atmospheric nitrous oxide. *Global Biogeochemical Cycles*, 14(2), 537–543.

Ravishankara, A. R., Daniel, J. S., & Portmann, R. W. (2009). Nitrous Oxide (N<sub>2</sub>O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science*, 326, 123–125.

Revsbech, N. P., Larsen, L. H., Gundersen, J., Dalsgaard, T., Ulloa, O., & Thamdrup, B. (2009). Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. *Limnology and Oceanography: Methods*, 7, 371–381.

Rocap, G., Keil, R., Devol, A., & Deutsch, C. (2017). Water temperature, salinity, and other data from CTD taken from the RV Sikuliaq in the Pacific Ocean between San Diego, California and Manzanillo, Mexico from 2016-12-21 to 2017-01-13. *NOAA National Centers for*

*Environmental Information, NCEI Acces*. Retrieved from

<https://www.ncei.noaa.gov/access/metadata/landing->

[page/bin/iso?id=gov.noaa.nodc:0164968](https://www.ncei.noaa.gov/access/metadata/landing-page/bin/iso?id=gov.noaa.nodc:0164968)

Rohe, L., Anderson, T.-H., Braker, G., Flessa, H., Giesemann, A., Lewicka-Szczebak, D., et al.

- (2014). Dual isotope and isotopomer signatures of nitrous oxide from fungal denitrification – a pure culture study. *Rapid Communications in Mass Spectrometry*, 28, 1893–1903. <https://doi.org/10.1002/rcm.6975>
- Santoro, A. E., Buchwald, C., McIlvin, M. R., & Casciotti, K. L. (2011). Isotopic Signature of N<sub>2</sub>O Produced by Marine Ammonia-Oxidizing Archaea. *Science*, 333, 1282–1285.
- Schmidt, H.-L., Werner, R. A., Yoshida, N., & Well, R. (2004). Is the isotopic composition of nitrous oxide an indicator for its origin from nitrification or denitrification? A theoretical approach from referred data and microbiological and enzyme kinetic aspects. *Rapid Communications in Mass Spectrometry*, 18, 2036–2040. <https://doi.org/10.1002/rcm.1586>
- Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., & Bohlke, J. K. (2001). A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Analytical Chemistry*, 73, 4145–4153.
- Smith, C., Nicholls, Z. R. J., Armour, K., Collins, W., Forster, P., Meinshausen, M., et al. (2021). The Earth’s Energy Budget, Climate Feedbacks, and Climate Sensitivity Supplementary Material. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Retrieved from <https://ipcc.ch/static/ar6/wg1>
- Stein, L. Y. (2019). Insights into the physiology of ammonia-oxidizing microorganisms. *Current Opinion in Chemical Biology*, 49, 9–15. <https://doi.org/10.1016/j.cbpa.2018.09.003>
- Stein, L. Y., & Yung, Y. L. (2003). Production, Isotopic Composition, and Atmospheric Fate of Biologically Produced Nitrous Oxide. *Annual Review of Earth and Planetary Sciences*, 31, 329–356. <https://doi.org/10.1146/annurev.earth.31.110502.080901>
- Stieglmeier, M., Mooshammer, M., Kitzler, B., Wanek, W., Zechmeister-Boltenstern, S.,

- 1021 Richter, A., & Schleper, C. (2014). Aerobic nitrous oxide production through N-nitrosating  
1022 hybrid formation in ammonia-oxidizing archaea. *The ISME Journal*, 8, 1135–1146.  
1023 <https://doi.org/10.1038/ismej.2013.220>
- 1024 Sun, X., Jayakumar, A., Tracey, J. C., Wallace, E., Kelly, C. L., Casciotti, K. L., & Ward, B. B.  
1025 (2021). Microbial N<sub>2</sub>O consumption in and above marine N<sub>2</sub>O production hotspots. *The*  
1026 *ISME Journal*, 15, 1434–1444. <https://doi.org/10.1038/s41396-020-00861-2>
- 1027 Suntharalingam, P., Sarmiento, J. L., & Toggweiler, J. R. (2000). Global significance of nitrous-  
1028 oxide production and transport from oceanic low-oxygen zones: A modeling study. *Global*  
1029 *Biogeochemical Cycles*, 14(4), 1353–1370.
- 1030 Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Gandhi, H., & Breznak, J. A. (2003). Nitrogen  
1031 isotopomer site preference of N<sub>2</sub>O produced by *Nitrosomonas europaea* and *Methylococcus*  
1032 *capsulatus* Bath. *Rapid Communications in Mass Spectrometry*, 17, 738–745.  
1033 <https://doi.org/10.1002/rcm.968>
- 1034 Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Gandhi, H., & Breznak, J. A. (2004). Erratum  
1035 Nitrogen isotopomer site preference of N<sub>2</sub>O produced by *Nitrosomonas europaea* and  
1036 *Methylococcus capsulatus* Bath. *Rapid Communications in Mass Spectrometry*, 18, 1411–  
1037 1412. <https://doi.org/10.1002/rcm.1482>
- 1038 Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Breznak, J. A., Gandhi, H., Pitt, A. J., & Li, F.  
1039 (2006). Distinguishing Nitrous Oxide Production from Nitrification and Denitrification on  
1040 the Basis of Isotopomer Abundances. *Applied and Environmental Microbiology*, 72(1),  
1041 638–644. <https://doi.org/10.1128/AEM.72.1.638>
- 1042 Sutka, Robin L., Adams, G. C., Ostrom, N. E., & Ostrom, P. H. (2008). Isotopologue  
1043 fractionation during N<sub>2</sub>O production by fungal denitrification. *Rapid Communications in*

- 1044 *Mass Spectrometry*, 22, 3989–3996. <https://doi.org/10.1002/rcm>
- 1045 Thamdrup, B., Dalsgaard, T., & Revsbech, N. P. (2012). Widespread functional anoxia in the
- 1046 oxygen minimum zone of the Eastern South Pacific. *Deep-Sea Research Part I*, 65, 36–45.
- 1047 <https://doi.org/10.1016/j.dsr.2012.03.001>
- 1048 Tiano, L., Garcia-Robledo, E., Dalsgaard, T., Devol, A. H., Ward, B. B., Ulloa, O., et al. (2014).
- 1049 Oxygen distribution and aerobic respiration in the north and south eastern tropical Pacific
- 1050 oxygen minimum zones. *Deep-Sea Research Part I*, 94, 173–183.
- 1051 <https://doi.org/10.1016/j.dsr.2014.10.001>
- 1052 Toyoda, S., & Yoshida, N. (1999). Determination of Nitrogen Isotopomers of Nitrous Oxide on a
- 1053 Modified Isotope Ratio Mass Spectrometer. *Analytical Chemistry*, 71, 4711–4718.
- 1054 Toyoda, S., Yoshida, N., Miwa, T., Matsui, Y., Yamagishi, H., Tsunogai, U., et al. (2002).
- 1055 Production mechanism and global budget of N<sub>2</sub>O inferred from its isotopomers in the
- 1056 western North Pacific. *Geophysical Research Letters*, 29(1037).
- 1057 Toyoda, S., Muto, H., Yamagishi, H., Yoshida, N., & Tanji, Y. (2005). Fractionation of N<sub>2</sub>O
- 1058 isotopomers during production by denitrifier. *Soil Biology & Biochemistry*, 37, 1535–1545.
- 1059 <https://doi.org/10.1016/j.soilbio.2005.01.009>
- 1060 Toyoda, S., Yoshida, N., & Koba, K. (2017). Isotopocule Analysis of Biologically Produced
- 1061 Nitrous Oxide in Various Environments. *Mass Spectrometry Reviews*, 36, 135–160.
- 1062 <https://doi.org/10.1002/mas.21459>
- 1063 Toyoda, S., Yoshida, O., Yamagishi, H., Fujii, A., & Yoshida, N. (2019). Identifying the origin
- 1064 of nitrous oxide dissolved in deep ocean by concentration and isotopocule analyses.
- 1065 *Scientific Reports*, (June 2018), 1–9. <https://doi.org/10.1038/s41598-019-44224-0>
- 1066 Trimmer, M., Chronopoulou, P., Maanoja, S. T., Upstill-Goddard, R. C., Kitidis, V., & Purdy, K.

- J. (2016). Nitrous oxide as a function of oxygen and archaeal gene abundance in the North Pacific. *Nature Communications*, 7(13451). <https://doi.org/10.1038/ncomms13451>
- Varadhan, R., & Borchers, H. W. (2020). R Package ‘dfoptim’ (Derivative-Free Optimization).
- Ward, B. B., Tuit, C. B., Jayakumar, A., Rich, J. J., Moffett, J., & Naqvi, S. W. A. (2008). Organic carbon, and not copper, controls denitrification in oxygen minimum zones of the ocean. *Deep-Sea Research Part I*, 55, 1672–1683. <https://doi.org/10.1016/j.dsr.2008.07.005>
- Webster, E. A., & Hopkins, D. W. (1996). Nitrogen and oxygen isotope ratios of nitrous oxide emitted from soil and produced by nitrifying and denitrifying bacteria. *Biology and Fertility of Soils*, 22, 326–330.
- Westley, M. B., Yamagishi, H., Popp, B. N., & Yoshida, N. (2006). Nitrous oxide cycling in the Black Sea inferred from stable isotope and isotopomer distributions. *Deep-Sea Research Part II*, 53, 1802–1816. <https://doi.org/10.1016/j.dsr2.2006.03.012>
- Wrage, N., Velthof, G. L., van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology & Biochemistry*, 33, 1723–1732.
- Wuebbles, D. J. (2009). Nitrous Oxide: No Laughing Matter. *Science*, 326, 56–57.
- Yamagishi, H., Yoshida, N., Toyoda, S., Popp, B. N., Westley, M. B., & Watanabe, S. (2005). Contributions of denitrification and mixing on the distribution of nitrous oxide in the North Pacific. *Geophysical Research Letters*, 32(L04603). <https://doi.org/10.1029/2004GL021458>
- Yamagishi, H., Westley, M. B., Popp, B. N., Toyoda, S., Yoshida, N., Watanabe, S., et al. (2007). Role of nitrification and denitrification on the nitrous oxide cycle in the eastern tropical North Pacific and Gulf of California. *Journal of Geophysical Research*, 112(G02015). <https://doi.org/10.1029/2006JG000227>

- Yang, S., Chang, B. X., Warner, M. J., Weber, T. S., Bourbonnais, A. M., Santoro, A. E., et al. (2020). Global reconstruction reduces the uncertainty of oceanic nitrous oxide emissions and reveals a vigorous seasonal cycle. *PNAS*, *117*(22), 11954–11960. <https://doi.org/10.5281/zenodo.3757194>
- Yoshida, N. (1988).  $^{15}\text{N}$ -depleted  $\text{N}_2\text{O}$  as a product of nitrification. *Letters to Nature*, *335*, 528–529.
- Yoshida, N., & Toyoda, S. (2000). Constraining the atmospheric  $\text{N}_2\text{O}$  budget from intramolecular site preference in  $\text{N}_2\text{O}$  isotopomers. *Letters to Nature*, *405*, 330–334.
- Yoshinari, T., Altabet, M. A., Naqvi, S. W. A., Codispoti, L., Jayakumar, A., Kuhland, M., & Devol, A. (1997). Nitrogen and oxygen isotopic composition of  $\text{N}_2\text{O}$  from suboxic waters of the eastern tropical North Pacific and the Arabian Sea—measurement by continuous-flow isotope-ratio monitoring. *Marine Chemistry*, *56*, 253–264.

**Investigating heterogeneity in nitrous oxide cycling of the Eastern Tropical North Pacific through isotopocules**

Patrick J. Monreal<sup>1</sup>, Colette L. Kelly<sup>1</sup>, Nicole M. Travis<sup>1</sup>, and Karen L. Casciotti<sup>1</sup>

<sup>1</sup>Stanford University, Department of Earth System Science, Stanford, CA

**Contents of this file**

Equations S1 – S5  
Supplementary Tables S1 – S3  
Supplementary Figures S1 – S7

**Introduction**

The following document includes all supplementary material cited in the manuscript, including equations, tables, and figures. First, the equations used in the time-dependent model of N<sub>2</sub>O cycling are described, including how those equations were modified from Kelly et al. (2021). Then, two tables that contain the primary model initializations are provided, followed by a table outlining the key differences between N<sub>2</sub>O cycling regimes. Finally, eight supplementary figures are included: (1) a temperature-salinity diagram for stations sampled in this study, (2) section plots of Chl-*a* and potential density anomaly, (3) density profiles of nitrate and nitrite isotopologues, (4) a map of sea surface height anomaly above geoid, (5) regression of N<sub>2</sub>O isotopic ratios against the natural logarithm of [N<sub>2</sub>O] between  $\sigma_\theta$  26-27 kg/m<sup>3</sup> for all stations, (6) regressions for calculating apparent isotope effects for N<sub>2</sub>O consumption at high N<sub>2</sub>O stations, (7) an illustration of the rate of change of N<sub>2</sub>O concentration and isotopic composition in the ODZ N<sub>2</sub>O model, and (8) near-surface N<sub>2</sub>O Keeling plot analyses. Unless stated otherwise, plots were generated in RStudio.

**1. Equations**

The first-order rate equations used to build the time-step model described in Section 2.3, 4.1, and 4.2 are based on those used in Kelly et al., 2021, modified to include nitrification as a source of N<sub>2</sub>O above the ODZ. Isotopocules of N<sub>2</sub>O were tracked through time as five variables (<sup>14</sup>N, <sup>15</sup>N<sup>*a*</sup>, <sup>15</sup>N<sup>*β*</sup>, <sup>16</sup>O, and <sup>18</sup>O) in N<sub>2</sub>O modified by five processes: N<sub>2</sub>O production from NO<sub>3</sub><sup>-</sup> via denitrification, N<sub>2</sub>O production from NO<sub>2</sub><sup>-</sup> via denitrification, N<sub>2</sub>O consumption via



denitrification, N<sub>2</sub>O production from NH<sub>4</sub><sup>+</sup> via nitrification by ammonia-oxidizing archaea (AOA), and N<sub>2</sub>O production from NH<sub>4</sub><sup>+</sup> via nitrification by ammonia-oxidizing bacteria (AOB). <sup>14</sup>N and <sup>16</sup>O were modeled with the following equations, with new terms shown in bold:

$$[^{14}\text{N}_2\text{O}]_{n+1} = [^{14}\text{N}_2\text{O}]_n + k_{\text{NO}_3 \rightarrow \text{N}_2\text{O}} [^{14}\text{NO}_3]_n + k_{\text{NO}_2 \rightarrow \text{N}_2\text{O}} [^{14}\text{NO}_2]_n - k_{\text{cons}} [^{14}\text{N}_2\text{O}]_n + \mathbf{k_{NH_4 \rightarrow N_2O, AOA} [^{14}NH_4]_n + k_{NH_4 \rightarrow N_2O, AOB} [^{14}NH_4]_n} \quad (S1)$$

$$[\text{N}_2^{16}\text{O}]_{n+1} = [\text{N}_2^{16}\text{O}]_n + \frac{1}{2} k_{\text{NO}_3 \rightarrow \text{N}_2\text{O}} \frac{1}{3} [\text{N}^{16}\text{O}_3]_n + \frac{1}{2} k_{\text{NO}_2 \rightarrow \text{N}_2\text{O}} \frac{1}{2} [\text{N}^{16}\text{O}_2]_n - k_{\text{cons}} [\text{N}_2^{16}\text{O}]_n + \frac{1}{2} \mathbf{k_{NH_4 \rightarrow N_2O, AOA} [^{14}NH_4]_n + k_{NH_4 \rightarrow N_2O, AOB} [^{14}NH_4]_n} \quad (S2)$$

where [<sup>14</sup>N<sub>2</sub>O]<sub>n</sub> is the concentration of <sup>14</sup>N-<sup>14</sup>N-<sup>16</sup>O in micromoles N per liter (μM) at the “n<sup>th</sup>” timestep and [N<sub>2</sub><sup>16</sup>O]<sub>n</sub> is the concentration of <sup>16</sup>O-containing N<sub>2</sub>O (predominantly <sup>14</sup>N-<sup>14</sup>N-<sup>16</sup>O). The ½ factor in front of terms producing [N<sub>2</sub><sup>16</sup>O] represents the 1:2 ratio of oxygen to nitrogen atoms in N<sub>2</sub>O (e.g., for every two nitrogen atoms added to the N<sub>2</sub>O pool, one oxygen atom is added). In line with this, the initial [N<sub>2</sub><sup>16</sup>O] was calculated by multiplying the initial [<sup>14</sup>N<sub>2</sub>O] by ½. Note also that ⅓[N<sup>16</sup>O<sub>3</sub>]<sub>n</sub> (eqn. S2) is approximately equal to [<sup>14</sup>NO<sub>3</sub>]<sub>n</sub> (eqn. S1) and ½[N<sup>16</sup>O<sub>2</sub>]<sub>n</sub> (eqn. S2) is equivalent to [<sup>14</sup>NO<sub>2</sub>]<sub>n</sub> (eqn. S1). The substrate pools were held constant in these experiments, so [<sup>14</sup>NO<sub>3</sub>] was always equal to the initial concentration of NO<sub>3</sub><sup>-</sup> and [N<sup>16</sup>O<sub>3</sub>] was 3 times that (3:1 ratio of oxygen to nitrogen in NO<sub>3</sub><sup>-</sup>). Similarly, [<sup>14</sup>NO<sub>2</sub>] was always equal to the initial concentration of NO<sub>2</sub><sup>-</sup> and [N<sup>16</sup>O<sub>2</sub>] was 2 times that. The factors of ½ for NO<sub>2</sub><sup>-</sup> and ⅓ for NO<sub>3</sub><sup>-</sup> were used to account for the ratio of oxygen to nitrogen in each species of NO<sub>x</sub>. k values represent the first-order reaction rate constants for each reaction.

In nitrification, since the oxygen atom in N<sub>2</sub>O is derived from dissolved O<sub>2</sub>, the model assumed that dissolved oxygen is in excess and reacts in a stoichiometric ratio with NH<sub>4</sub><sup>+</sup>. Thus, [<sup>14</sup>NH<sub>4</sub>] is used in equations both for nitrogen and oxygen. The same framework as above is used for heavy isotopes, except the rate constants are modified by isotope effects (α), equal to k<sup>light</sup>/k<sup>heavy</sup>, or ε/1000 + 1. <sup>15</sup>N<sup>α</sup> and <sup>15</sup>N<sup>β</sup> were modeled separately, as they have different isotope effects:

$$[^{15}\text{N}_2\text{O}^\alpha]_{n+1} = [^{15}\text{N}_2\text{O}^\alpha]_n + \frac{k_{\text{NO}_3 \rightarrow \text{N}_2\text{O}}}{\alpha^{15,\alpha}_{\text{NO}_x \rightarrow \text{N}_2\text{O}}} [^{15}\text{NO}_3]_n + \frac{k_{\text{NO}_2 \rightarrow \text{N}_2\text{O}}}{\alpha^{15,\alpha}_{\text{NO}_x \rightarrow \text{N}_2\text{O}}} [^{15}\text{NO}_2]_n - \frac{k_{\text{cons}}}{\alpha^{15,\alpha}_{\text{cons}}} [^{15}\text{N}_2\text{O}^\alpha]_n + \frac{k_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOA}}}{\alpha^{15,\alpha}_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOA}}} [^{15}\text{NH}_4]_n + \frac{k_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOB}}}{\alpha^{15,\alpha}_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOB}}} [^{15}\text{NH}_4]_n \quad (S3)$$

$$[^{15}\text{N}_2\text{O}^\beta]_{n+1} = [^{15}\text{N}_2\text{O}^\beta]_n + \frac{k_{\text{NO}_3 \rightarrow \text{N}_2\text{O}}}{\alpha^{15,\beta}_{\text{NO}_x \rightarrow \text{N}_2\text{O}}} [^{15}\text{NO}_3]_n + \frac{k_{\text{NO}_2 \rightarrow \text{N}_2\text{O}}}{\alpha^{15,\beta}_{\text{NO}_x \rightarrow \text{N}_2\text{O}}} [^{15}\text{NO}_2]_n - \frac{k_{\text{cons}}}{\alpha^{15,\beta}_{\text{cons}}} [^{15}\text{N}_2\text{O}^\beta]_n + \frac{k_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOA}}}{\alpha^{15,\beta}_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOA}}} [^{15}\text{NH}_4]_n + \frac{k_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOB}}}{\alpha^{15,\beta}_{\text{NH}_4 \rightarrow \text{N}_2\text{O, AOB}}} [^{15}\text{NH}_4]_n \quad (S4)$$

where [<sup>15</sup>N<sub>2</sub>O<sup>α</sup>] and [<sup>15</sup>N<sub>2</sub>O<sup>β</sup>] represent concentrations of <sup>14</sup>N-<sup>15</sup>N-<sup>16</sup>O and <sup>15</sup>N-<sup>14</sup>N-<sup>16</sup>O, respectively. Initial values were calculated using the initialized δ<sup>15</sup>N<sub>2</sub>O<sup>α</sup> and δ<sup>15</sup>N<sub>2</sub>O<sup>β</sup> values. In the same way, <sup>15</sup>N concentrations of substrates were also calculated from their respective initialized isotopic ratios. The equation for <sup>18</sup>O includes branching isotope effects (Casciotti et al., 2007) in addition to kinetic isotope effects:

$$\begin{aligned}
[N_2^{18}O]_{n+1} = & [N_2^{18}O]_n + \frac{1}{2} \frac{k_{NO3 \rightarrow N2O} \alpha^{18}_{NO3 \rightarrow NO2, \text{branching}} \alpha^{18}_{NO2 \rightarrow N2O, \text{branching}}}{\alpha^{18}_{NO2 \rightarrow N2O, \text{kinetic}}} \frac{1}{3} [N^{18}O_3]_n \\
& + \frac{1}{2} \frac{k_{NO2 \rightarrow N2O} \alpha^{18}_{NO2 \rightarrow N2O, \text{branching}}}{\alpha^{18}_{NO2 \rightarrow N2O, \text{kinetic}}} \frac{1}{2} [N^{18}O_2]_n - \frac{k_{\text{cons}}}{\alpha^{18}_{\text{cons}}} [N_2^{18}O]_n \\
& + \frac{1}{2} \frac{k_{NH4 \rightarrow N2O, AOA}}{\alpha^{18}_{NH4 \rightarrow N2O, AOA}} [^{18}O_{NH4 \rightarrow N2O}]_n + \frac{1}{2} \frac{k_{NH4 \rightarrow N2O, AOB}}{\alpha^{18}_{NH4 \rightarrow N2O, AOB}} [^{18}O_{NH4 \rightarrow N2O}]_n
\end{aligned} \tag{S5}$$

where  $[N_2^{18}O]_n$  is the concentration of  $^{18}O$  in  $^{14}N$ - $^{14}N$ - $^{18}O$ , and initial  $^{18}O$  concentrations of substrates were calculated with initialized  $\delta^{18}O$  values. The  $^{18}O$  involved in nitrification — represented by  $[^{18}O_{NH4 \rightarrow N2O}]$  — was a special case, as  $NH_4^+$  does not contain an oxygen atom. Similar to  $^{16}O$ , the model assumed that oxygen reacts stoichiometrically with  $NH_4^+$  to produce hydroxylamine,  $NH_2OH$ , which further reacts to form  $N_2O$ .  $[^{18}O_{NH4 \rightarrow N2O}]$  was thus calculated using the concentration of  $NH_4^+$  as the proxy for oxygen and an isotopic ratio of 23.5‰, the average  $\delta^{18}O$  of dissolved oxygen in seawater, in the following way:  $[^{18}O_{NH4 \rightarrow N2O}] = [^{14}NH_4] * ((23.5‰/1000) + 1) * R_{VSMOW}$ .

As shown in the above equations, the individual isotopologues are modeled as concentrations, which are converted to delta notation, ( $\delta (‰) = R/R_{std} - 1 * 1000$ ), where  $R$  and  $R_{std}$  are the ratios of heavy to light isotope containing isotopologues ( $^{15}N/^{14}N$  or  $^{18}O/^{16}O$ ) in the sample and standard, respectively.

## 2. Tables

**Table S1.** Initialization conditions for the time-step model of N<sub>2</sub>O isotopocules in the core of the ODZ (denitrification processes only), discussed in Section 2.3 and Section 4.1. These are input into equations S1-S5, without the nitrification terms that are bolded.

| <i>Variable</i>   | <i>Value</i>                            | <i>Source</i>   |
|---|---|---|
| $^{15}\epsilon_{NOx \rightarrow N_2O} (\alpha)$               | 22‰                                     | Toyoda et al., 2005   |
| $^{15}\epsilon_{NOx \rightarrow N_2O} (\beta)$                | 22‰                                     | Toyoda et al., 2005   |
| $^{15}\epsilon_{cons} (\alpha)$                               | 10.5‰                                   | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations) |
| $^{15}\epsilon_{cons} (\beta)$                                | 0‰                                      | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations) |
| $^{18}\epsilon_{NO_3 \rightarrow NO_2}$ ,<br><i>branching</i> | 24‰                                     | Casciotti and McIlvin, 2007   |
| $^{18}\epsilon_{NO_2 \rightarrow N_2O}$ ,<br><i>branching</i> | 12‰                                     | Casciotti and McIlvin, 2007   |
| $^{18}\epsilon_{NO_2 \rightarrow N_2O}$ ,<br><i>kinetic</i>   | -2‰                                     | Martin and Casciotti, 2016  |
| $^{18}\epsilon_{cons, kinetic}$                               | 16‰                                     | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations) |
| $[N_2O]_i$  | 39.05 nM                                | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , background stations)            |
| $\delta^{15}N_2O^{\alpha}_i$                                  | 19.73‰                                  | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , background stations)            |
| $\delta^{15}N_2O^{\beta}_i$                                   | 4.29‰                                   | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , background stations)            |
| $\delta^{18}O\text{-}N_2O_i$                                  | 59.48‰                                  | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , background stations)            |
| $[NO_3^-]$  | 25.50 $\mu\text{M}$                     | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $\delta^{15}NO_3^-$   | 18.09‰                                  | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $\delta^{18}O\text{-}NO_3^-$                                  | 16.55‰                                  | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $[NO_2^-]$  | 1.023 $\mu\text{M}$                     | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $\delta^{15}NO_2^-$   | -20.42‰                                 | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $\delta^{18}O\text{-}NO_2^-$                                  | 15.52‰                                  | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , core-ODZ stations)              |
| $k_{NO_3 \rightarrow N_2O}$                                   | $5.25 \times 10^{-5} \text{ day}^{-1}$  | Kelly et al., 2021 (used as starting point of optimization)                             |
| $k_{NO_2 \rightarrow N_2O}$                                   | $3.962 \times 10^{-6} \text{ day}^{-1}$ | Kelly et al., 2021 (used as starting point of optimization)                             |
| $k_{cons}$  | $2.1 \text{ day}^{-1}$                  | Babbin et al., 2015; Kelly et al., 2021 (used as starting point of optimization)        |

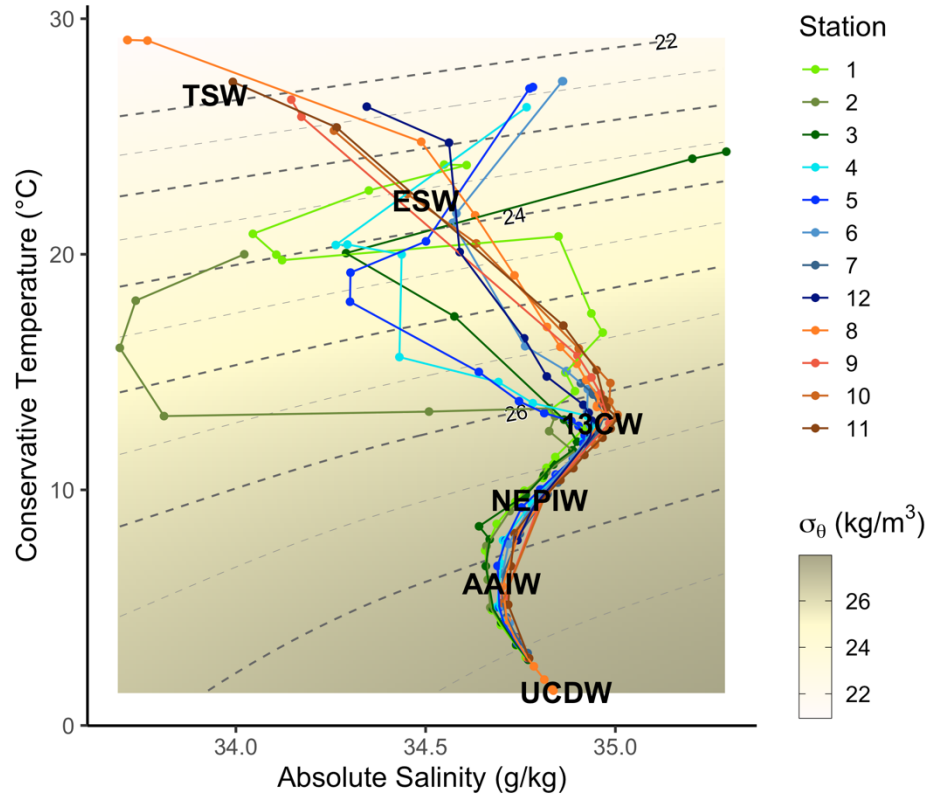
**Table S2.** Initialization conditions for the time-step model of N<sub>2</sub>O isotopocules above the ODZ (denitrification and nitrification processes), discussed in Section 2.3 and Section 4.2. These are input into equations S1-S5.

| <i>Variable</i>  | <i>Value</i>                          | <i>Source</i>   |
|--|---------------------------------------|---|
| $^{15}\epsilon_{NOx \rightarrow N_2O} (\alpha)$                  | 22‰                                   | Toyoda et al., 2005   |
| $^{15}\epsilon_{NOx \rightarrow N_2O} (\beta)$                   | 22‰                                   | Toyoda et al., 2005   |
| $^{15}\epsilon_{cons} (\alpha)$                                  | 10.5‰                                 | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                                   |
| $^{15}\epsilon_{cons} (\beta)$                                   | 0‰                                    | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                                   |
| $^{18}\epsilon_{NO_3 \rightarrow NO_2}$ ,<br><i>branching</i>    | 24‰                                   | Casciotti and McIlvin, 2007   |
| $^{18}\epsilon_{NO_2 \rightarrow N_2O}$ ,<br><i>branching</i>    | 12‰                                   | Casciotti and McIlvin, 2007   |
| $^{18}\epsilon_{NO_2 \rightarrow N_2O}$ ,<br><i>kinetic</i>      | -2‰                                   | Martin and Casciotti, 2016  |
| $^{18}\epsilon_{cons}$ , <i>kinetic</i>                          | 16‰                                   | This study ( $\sigma_0 = 26\text{-}27 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                                   |
| $^{15}\epsilon_{NH_4 \rightarrow N_2O} (\alpha)$ ,<br><i>AOA</i> | -21.3‰                                | Santoro et al., 2011 (adjusted based on $\delta^{15}NH_4^+$ value)  |
| $^{15}\epsilon_{NH_4 \rightarrow N_2O} (\beta)$ ,<br><i>AOA</i>  | 9‰                                    | Santoro et al., 2011 (adjusted based on $\delta^{15}NH_4^+$ value)  |
| $^{18}\epsilon_{NH_4 \rightarrow N_2O}$ ,<br><i>AOA</i>          | -10.5‰                                | Santoro et al., 2011 (adjusted based on $\delta^{18}O_{O_2}$ value)   |
| $^{15}\epsilon_{NH_4 \rightarrow N_2O} (\alpha)$ ,<br><i>AOB</i> | 62.5‰                                 | Sutka et al., 2006  |
| $^{15}\epsilon_{NH_4 \rightarrow N_2O} (\beta)$ ,<br><i>AOB</i>  | 31.5‰                                 | Sutka et al., 2006  |
| $^{18}\epsilon_{NH_4 \rightarrow N_2O}$ ,<br><i>AOB</i>          | 3‰                                    | Frame and Casciotti, 2010   |
| $[N_2O]_i$   | 11.14 nM                              | This study (minimum [N <sub>2</sub> O] data point in $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ )                       |
| $\delta^{15}N_2O^{\alpha}_i$                                     | 15.77‰                                | This study (minimum [N <sub>2</sub> O] data point in $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ )                       |
| $\delta^{15}N_2O^{\beta}_i$                                      | -1.42‰                                | This study (minimum [N <sub>2</sub> O] data point in $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ )                       |
| $\delta^{18}O\text{-}N_2O_i$                                     | 45.45‰                                | This study (minimum [N <sub>2</sub> O] data point in $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ )                       |
| $[NO_3^-]$   | 22.27 $\mu\text{M}$                   | This study ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                               |
| $\delta^{15}NO_3^-$  | 9.91‰                                 | This study ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                               |
| $\delta^{18}O\text{-}NO_3^-$                                     | 8.57‰                                 | This study ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                               |
| $[NO_2^-]$   | 0.286 $\mu\text{M}$                   | This study ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)                               |
| $\delta^{15}NO_2^-_{PNM}$  | 1.58‰                                 | This study (PNM of Stn. 8)  |
| $\delta^{18}O\text{-}NO_2^-_{PNM}$                               | 12.87‰                                | This study (PNM of Stn. 8)  |
| $\delta^{15}NO_2^-_{SNM}$  | -25.64‰                               | This study (SNM of Stn. 8)  |
| $\delta^{18}O\text{-}NO_2^-_{SNM}$                               | 14.49‰                                | This study (SNM of Stn. 8)  |
| $[NH_4^+]$   | 0.263 $\mu\text{M}$                   | Unpublished data from transect ( $\sigma_0 = 23.5\text{-}25.8 \text{ kg/m}^3$ , high-N <sub>2</sub> O stations)           |
| $\delta^{15}NH_4^+$  | 7‰                                    | arbitrary value   |
| $\delta^{18}O_{NH_4 \rightarrow N_2O}$                           | 23.5‰                                 | average $\delta^{18}O$ of dissolved oxygen in seawater, which is incorporated into N <sub>2</sub> O during nitrification. |
| $k_{NO_3 \rightarrow N_2O}$                                      | $3.9 \times 10^{-6} \text{ day}^{-1}$ | starting point that led to best optimization in sensitivity tests   |
| $k_{NO_2 \rightarrow N_2O}$                                      | $1.1 \times 10^{-3} \text{ day}^{-1}$ | starting point that led to best optimization in sensitivity tests   |
| $k_{cons}$   | $2.7 \times 10^{-1} \text{ day}^{-1}$ | starting point that led to best optimization in sensitivity tests   |
| $k_{NH_4 \rightarrow N_2O}$ , <i>AOA</i>                         | $1.4 \times 10^{-1} \text{ day}^{-1}$ | starting point that led to best optimization in sensitivity tests   |
| $k_{NH_4 \rightarrow N_2O}$ , <i>AOB</i>                         | $4.7 \times 10^{-5} \text{ day}^{-1}$ | starting point that led to best optimization in sensitivity tests   |

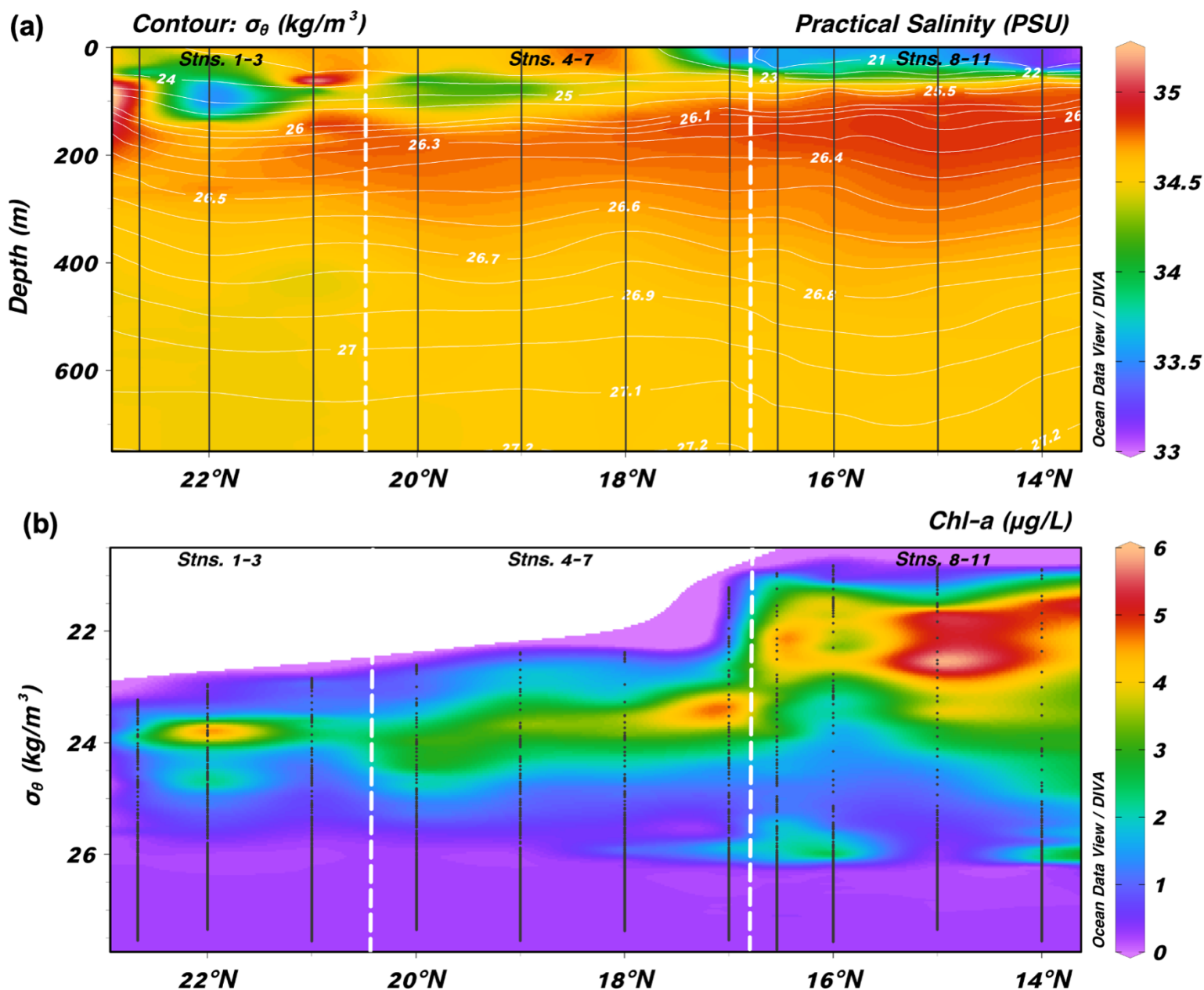
**Table S3.** Summary of the key features of each station grouping (Section 3) provided in table format. The ODZ upper boundary was taken from CTD data as the threshold at which  $[O_2]$  dipped below  $5 \mu\text{mol/kg}$ . Average  $\sigma_\theta$  minimum represents the mean of each station's surface density in a particular regime.

| <i>Regime</i>                        | <i>Stations</i> | <i>General Summary</i>   | <i>ODZ<br/>Upper<br/>Boundary</i>     | <i>Average<br/><math>\sigma_\theta</math><br/>Minimum</i> | <i>Average<br/>ODZ <math>[N_2O]</math><br/>Minimum</i> | <i>Average<br/>Above-ODZ<br/><math>[N_2O]</math><br/>Maximum</i> |
|--------------------------------------|-----------------|--|---------------------------------------|---|--|--|
| <b><i>Back-ground</i></b>            | 1-3             | Closest to Baja Peninsula; no dramatic $[N_2O]$ , $[NO_2^-]$ , $\delta^{15}N_2O^a$ , $\delta^{18}O-N_2O$ , and SP profiles relative to other stations.   | 124.7 m<br>(25.59 kg/m <sup>3</sup> ) | 23.01 kg/m <sup>3</sup>                                   | 23.34 nM   | 44.55 nM   |
| <b><i>Core ODZ</i></b>               | 4-7&12          | Most pronounced $[N_2O]$ minima and secondary nitrite maxima in ODZ; high $\delta^{15}N_2O^a$ , $\delta^{18}O-N_2O$ , and SP in ODZ; low $\delta^{15}N_2O^b$ in ODZ; eddy at Stn. 7  | 98 m<br>(25.24 kg/m <sup>3</sup> )    | 21.88 kg/m <sup>3</sup>                                   | 3.54 nM  | 69.26 nM   |
| <b><i>High <math>N_2O</math></i></b> | 8-11            | Some ODZ properties similar to core-ODZ stations; highest near-surface $[N_2O]$ and Chl- <i>a</i> of the transect; strongest primary nitrite maxima; shallowest oxycline and nitracline relative to other stations; mesoscale eddy | 72 m<br>(24.59 kg/m <sup>3</sup> )    | 20.88 kg/m <sup>3</sup>                                   | 10.72 nM   | 122.58 nM  |

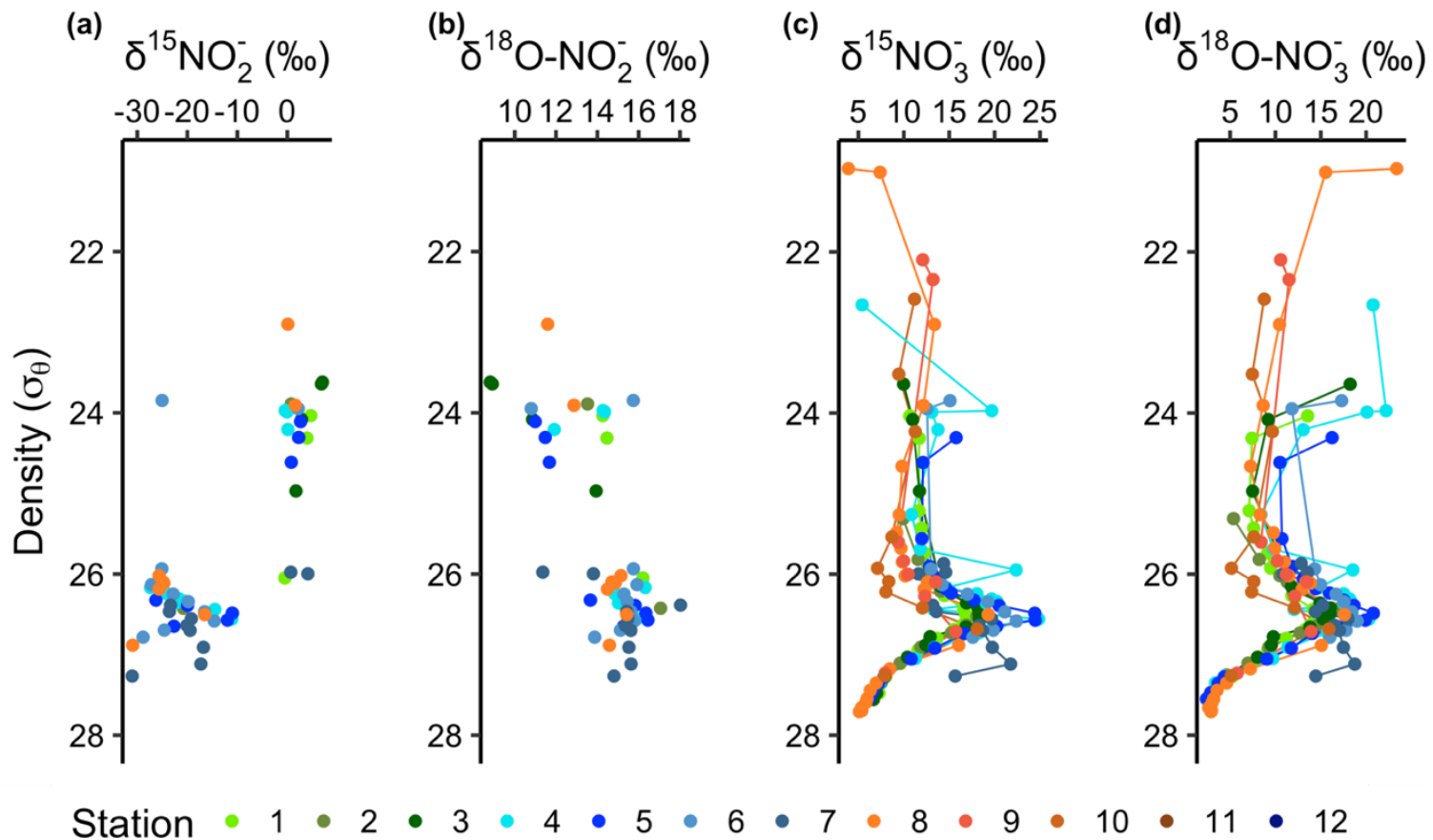
### 3. Figures



**Figure S1.** Temperature-salinity diagram with water mass endmembers from the analysis in Evans et al., 2020: Tropical Surface Water (TSW), Equatorial Surface Water (ESW), 13°C Water (13CW), Northern Equatorial Pacific Intermediate Water (NEPIW), Antarctic Intermediate Water (AAIW), and Upper Circumpolar Deep Water (UCDW). Background, core-ODZ, and high-N<sub>2</sub>O stations are represented by greens, blues, and oranges, respectively.

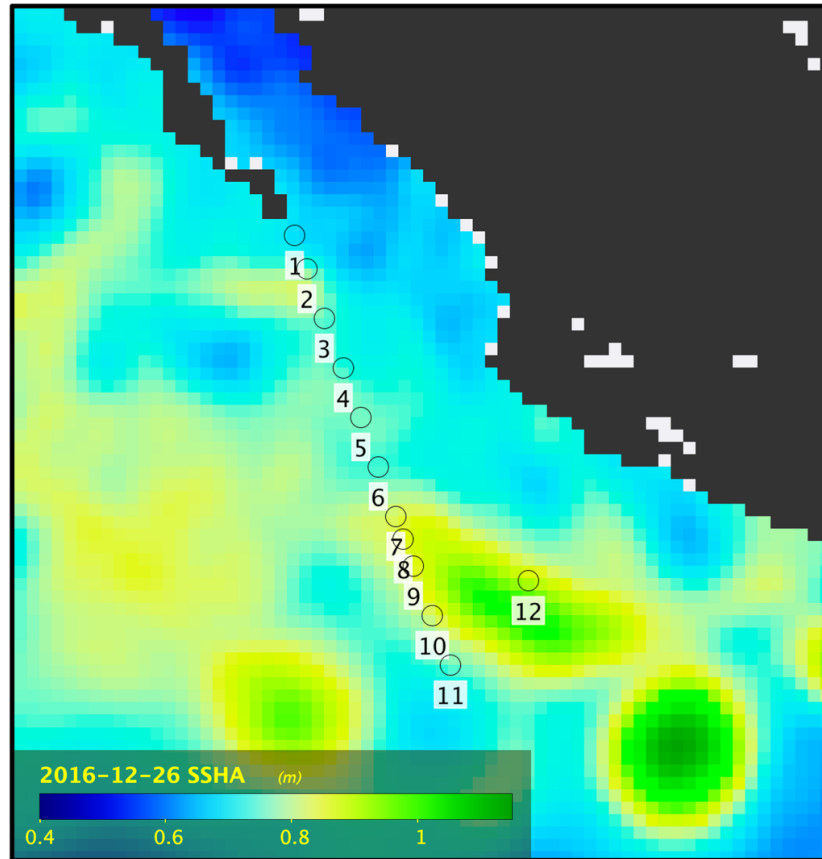


**Figure S2.** (a) Section of practical salinity plotted against depth with contours of potential density anomaly ( $\sigma_\theta$ ) and white dashed lines separating station groupings. Downward-bending isopycnals can be observed at Stn. 10, as well as less dense surface waters at all of the high- $\text{N}_2\text{O}$  stations. (b) Section profile of Chl-*a* (calculated from CTD fluorometer) plotted against  $\sigma_\theta$ , showing the highest surface Chl-*a* at high- $\text{N}_2\text{O}$  stations.

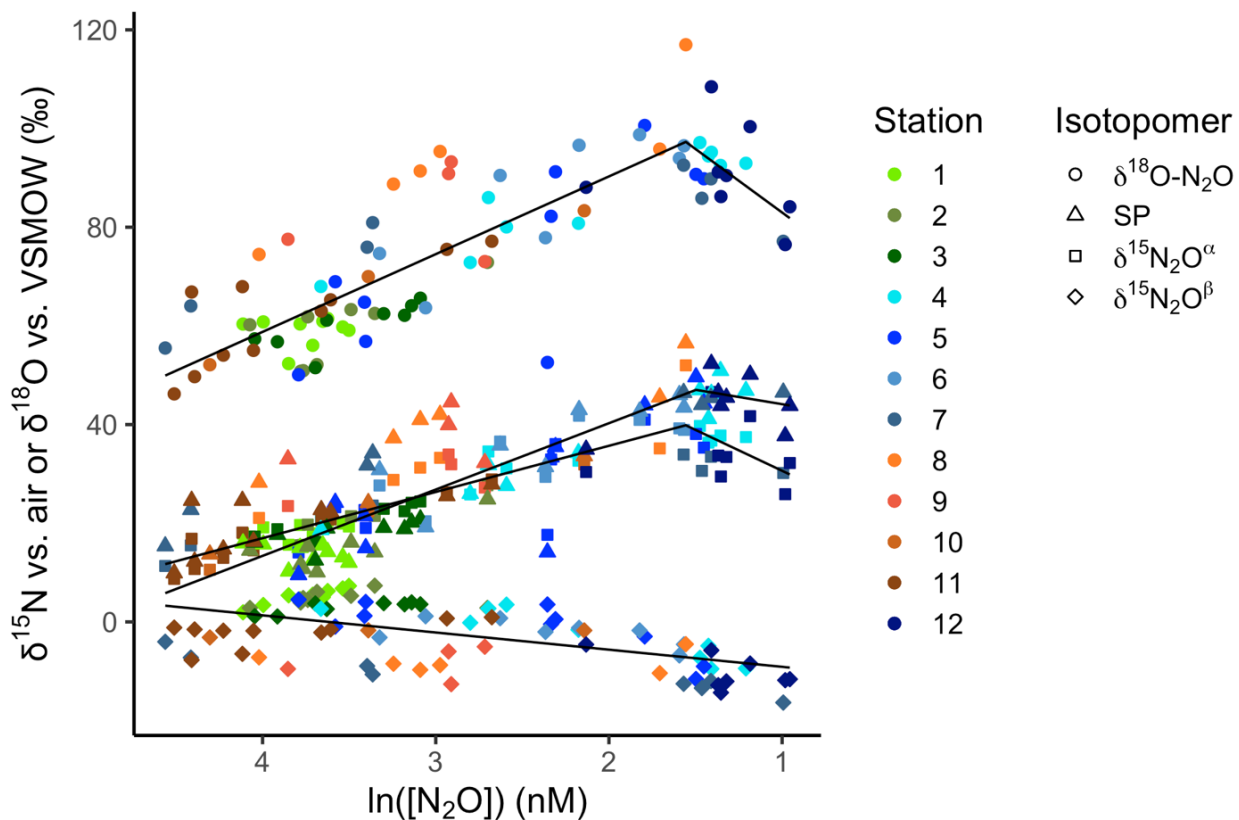


**Figure S3.**  $\text{NO}_2^-$  (a, b) and  $\text{NO}_3^-$  (c, d) isotopologues plotted against  $\sigma_\theta$ . If  $[\text{NO}_2^-]$  is too low ( $< 0.5$   $\mu\text{mol/L}$ ), isotopic measurements cannot be made, which is why the points are not connected in the profiles for (a)  $\delta^{15}\text{NO}_2^-$  and (b)  $\delta^{18}\text{O-NO}_2^-$ . These measurements were primarily used to calculate a starting point for the time-step model runs.

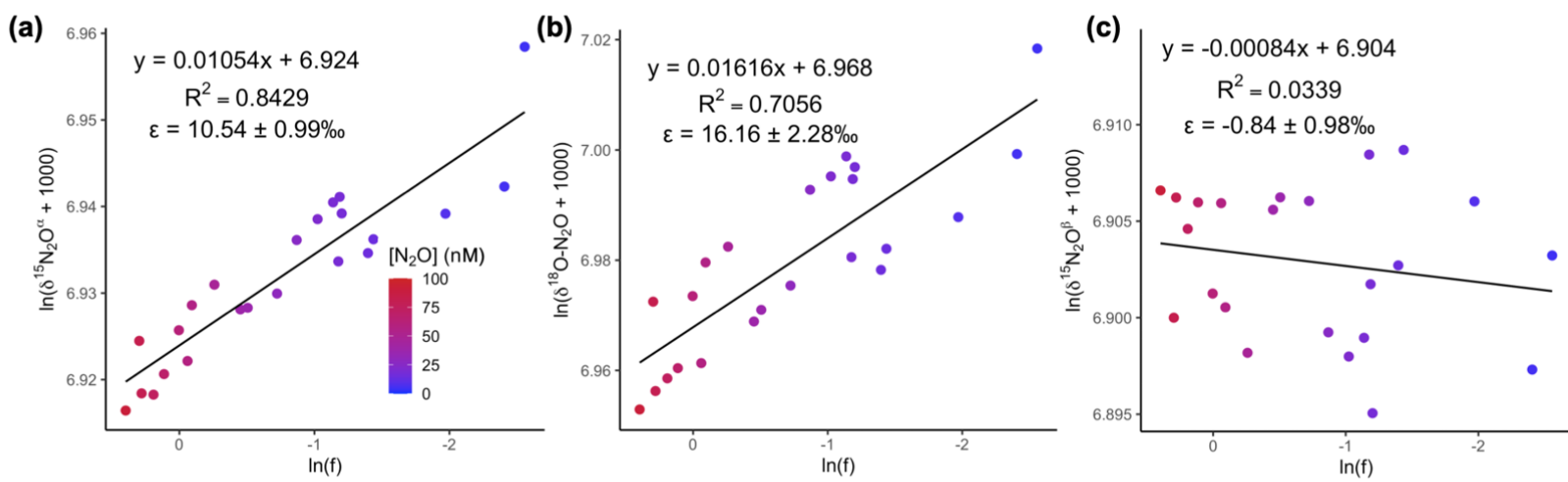




**Figure S4.** Sea surface height anomaly (SSHA) above geoid of the ETNP on the day that Stn. 7 was sampled, retrieved from satellite altimetry. Data product “Global Ocean Gridded L4 Sea Surface Heights and Derived Variables Reprocessed (1993-Ongoing)” with id “SEALEVEL\_GLO\_PHY\_L4\_REP\_OBSERVATIONS\_008\_047” was downloaded for December 26, 2016 from the Copernicus Marine Environmental Monitoring Service website (<http://marine.copernicus.eu/>) and visualized with NASA’s SeaDAS software package. Elevated topography can be observed at the southern end of the transect.



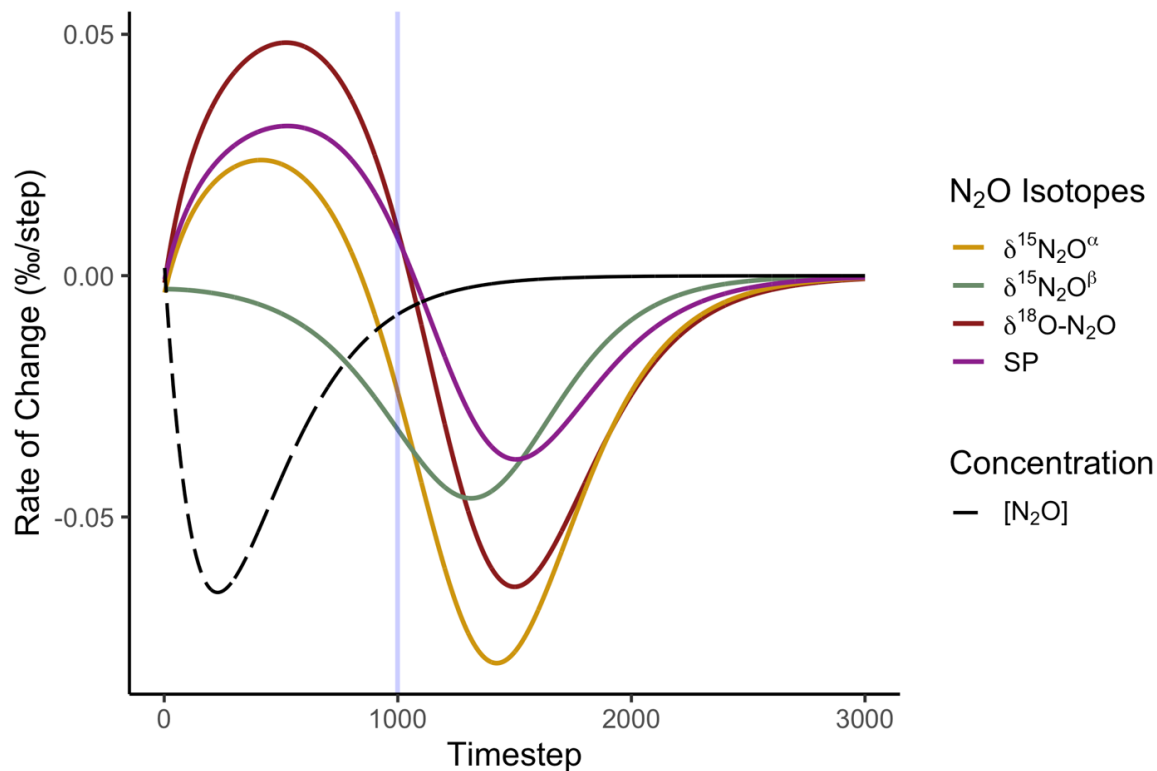
**Figure S5.** Breakpoint analyses for  $N_2O$  isotopic ratios against the natural logarithm of  $[N_2O]$  (where  $[N_2O]$  is given in nM) within  $\sigma_\theta = 26\text{--}27\text{ kg/m}^3$  across all stations. Using a segmented regression model (Muggeo, 2003, 2008), a breakpoint was detected for  $\delta^{18}O-N_2O$ ,  $\delta^{15}N_2O^\alpha$ , and SP at  $\ln([N_2O]) = 1.56 \pm 0.11$ ,  $1.56 \pm 0.07$ , and  $1.50 \pm 0.16$ , respectively. What is more, most of the points after the breakpoint were from core-ODZ stations. These could possibly represent  $N_2O$  production co-occurring with consumption. Adjusted  $R^2$  values for the three segmented regressions were 0.701, 0.805, and 0.748, respectively. The x axis of the plot is reversed to ease visualization of isotopic trends as  $N_2O$  is consumed ( $[N_2O]$  decreases).



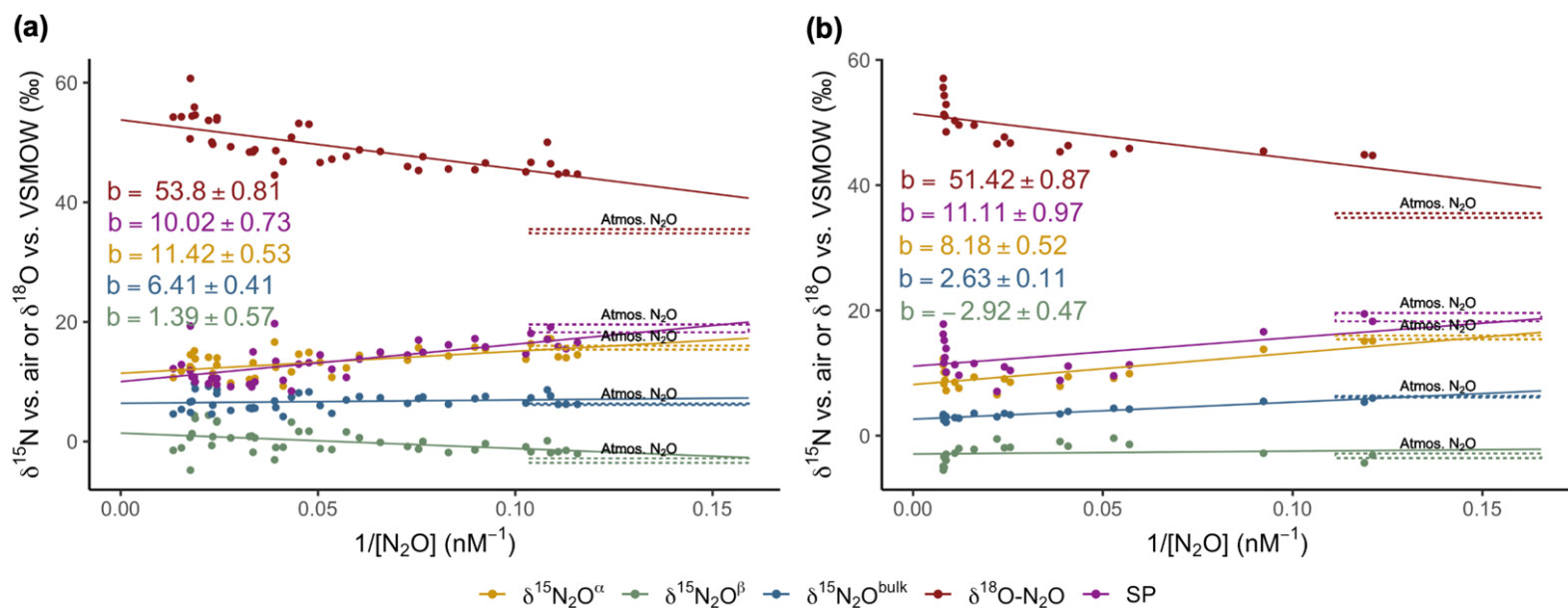
**Figure S6.** Regressions for calculating the apparent isotope effects ( $\epsilon$ ) for N<sub>2</sub>O consumption for (a)  $\delta^{15}\text{N}_2\text{O}^\alpha$ , (b)  $\delta^{18}\text{O-N}_2\text{O}$ , and (c)  $\delta^{15}\text{N}_2\text{O}^\beta$ . Data from  $\sigma_\theta = 26\text{--}27 \text{ kg/m}^3$  at high-N<sub>2</sub>O stations were used for the calculation. Even though the core-ODZ stations were targeted with the timestep model and optimization procedure, these stations also demonstrated a production signal (Fig. S5) that would skew isotope effect calculations (supplement of Kelly et al., 2021). Thus, the data from the ODZ of high-N<sub>2</sub>O were chosen to calculate the isotope effects for N<sub>2</sub>O consumption.

These plots present the data according to the Rayleigh equation  $\ln(\delta_s/1000+1) = \ln(\delta_{s,0}/1000+1) + (\alpha-1)\ln(f)$ , such that the slope of each regression was equal to  $\epsilon/1000$ . “f” represents the fraction of initial N<sub>2</sub>O remaining, and was hence calculated as  $[\text{N}_2\text{O}]_{\text{observed}}$  divided by the maximum N<sub>2</sub>O concentration of the transect in this density layer. See the supplement of Kelly et al. (2021) for a detailed explanation of the same method implemented here.

Values from this Rayleigh model were used as input for the timestep model, except that an isotope effect of 0‰ was used for  $\delta^{15}\text{N}_2\text{O}^\beta$  because the regression slope was insignificant.



**Figure S7.** An example of the rate of change of  $\text{N}_2\text{O}$  concentration and isotopic composition in the ODZ model ( $\sigma_\theta = 26\text{-}27 \text{ kg/m}^3$ ) discussed at the end of Section 4.1. This plot shows the first derivative of Figure 4a. The vertical blue line at timestep 1000 indicates where the modeled  $[\text{N}_2\text{O}]$  was closest to the lowest measured  $[\text{N}_2\text{O}]$  for this density layer and is thus used to gauge whether this area of the water column was in steady state. Note that at this timestep,  $[\text{N}_2\text{O}]$  is approaching equilibrium (low rates of change) while the isotopocule measurements continue to evolve at high rates.



**Figure S8.** Keeling plot for  $\text{N}_2\text{O}$  samples above the ODZ ( $\sigma_\theta < 25.8 \text{ kg/m}^3$ ) at (a) background and core-ODZ stations and (b) high- $\text{N}_2\text{O}$  stations. Each regression was statistically significant. A box representing the atmospheric source of  $\text{N}_2\text{O}$  was also plotted, using values from Mohn et al., 2014.

## References

- Babbin, A. R., Bianchi, D., Jayakumar, A., & Ward, B. B. (2015). Rapid nitrous oxide cycling in the suboxic ocean. *Science*, 348(6239), 1127–1129. <https://doi.org/10.1126/science.aaa8380>
- Casciotti, K. L., & McIlvin, M. R. (2007). Isotopic analyses of nitrate and nitrite from reference mixtures and application to Eastern Tropical North Pacific waters. *Marine Chemistry*, 107, 184–201. <https://doi.org/10.1016/j.marchem.2007.06.021>
- Evans, N., Boles, E., Kwiecinski, J. V., Mullen, S., Wolf, M., Devol, A. H., et al. (2020). The role of water masses in shaping the distribution of redox active compounds in the Eastern Tropical North Pacific oxygen deficient zone and influencing low oxygen concentrations in the eastern Pacific Ocean. *Limnology and Oceanography*, 65, 1688–1705. <https://doi.org/10.1002/lno.11412>
- Frame, C. H., & Casciotti, K. L. (2010). Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences*, 7, 2695–2709. <https://doi.org/10.5194/bg-7-2695-2010>
- Kelly, C. L., Travis, N. M., Baya, P. A., & Casciotti, K. L. (2021). Quantifying Nitrous Oxide Cycling Regimes in the Eastern Tropical North Pacific Ocean With Isotopomer Analysis. *Global Biogeochemical Cycles*, 35(e2020GB006637). <https://doi.org/10.1029/2020GB006637>
- Martin, T. S., & Casciotti, K. L. (2016). Nitrogen and oxygen isotopic fractionation during microbial nitrite reduction. *Limnology and Oceanography*, 61, 1134–1143. <https://doi.org/10.1002/lno.10278>
- Mohn, J., Wolf, B., Toyoda, S., Lin, C.-T., Liang, M.-C., Brüggemann, N., et al. (2014). Interlaboratory assessment of nitrous oxide isotopomer analysis by isotope ratio mass spectrometry and laser spectroscopy: current status and perspectives. *Rapid Communications in Mass Spectrometry*, 28, 1995–2007. <https://doi.org/10.1002/rcm.6982>
- Muggeo, V. M. R. (2003). Estimating regression models with unknown break-points. *Statistics in Medicine*, 22, 3055–3071. <https://doi.org/10.1002/sim.1545>
- Muggeo, V. M. R. (2008). Segmented: an R package to fit regression models with broken-line relationships. *R News*, 8/1, 20–25.
- Ostrom, N. E., Pitt, A., Sutka, R., Ostrom, P. H., Grandy, A. S., Huizinga, K. M., & Robertson, G. P. (2007). Isotopologue effects during N<sub>2</sub>O reduction in soils and in pure cultures of denitrifiers. *Journal of Geophysical Research*, 112(G02005). <https://doi.org/10.1029/2006JG000287>
- Santoro, A. E., Buchwald, C., McIlvin, M. R., & Casciotti, K. L. (2011). Isotopic Signature of N<sub>2</sub>O Produced by Marine Ammonia-Oxidizing Archaea. *Science*, 333, 1282–1285.
- Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Breznak, J. A., Gandhi, H., Pitt, A. J., & Li, F. (2006). Distinguishing Nitrous Oxide Production from Nitrification and Denitrification on the Basis of Isotopomer Abundances. *Applied and Environmental Microbiology*, 72(1), 638–644. <https://doi.org/10.1128/AEM.72.1.638>
- Toyoda, S., Muto, H., Yamagishi, H., Yoshida, N., & Tanji, Y. (2005). Fractionation of N<sub>2</sub>O isotopomers during production by denitrifier. *Soil Biology & Biochemistry*, 37, 1535–1545. <https://doi.org/10.1016/j.soilbio.2005.01.009>