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

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Abstract

Nitrous oxide (N2O) 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), N2O 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 N2O from a 2016 cruise to the ETNP were analyzed to examine heterogeneity in N2O cycling across the region. Along the north-south transect, three distinct biogeochemical regimes were identified: background, core-ODZ, and high-N2O stations. Background stations were characterized by less dynamic N2O cycling. Core-ODZ stations were characterized by co-occurring N2O production and consumption at anoxic depths, indicated by high $\delta 18O$ (> 90values, and confirmed by a time-dependent model, which indicated that N2O production via denitrification was significant and may occur with a non-zero site preference. High-N2O stations were defined by [N2O] 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 N2O production from ammonia-oxidizing archaea and denitrification from nitrate in the near-surface N2O maximum, while bacterial nitrification and denitrification from nitrite were insignificant. This study also represents the first in the ETNP to link N2O isotopocule measurements to a mesoscale eddy, suggesting the importance of eddies to the spatiotemporal variability in N2O cycling in this region.

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10 11	Key words: Nitrogen cycling, Greenhouse gases, Anoxic environments, Stable isotopes, Climate and interannual variability				
12	Key Points:				
13 14	 Nitrous oxide isotopocules revealed distinct cycling regions throughout the Eastern Tropical North Pacific's oxygen-deficient zone 				
15 16	• Archaeal nitrification and bacterial denitrification from nitrate were important sources to near-surface nitrous oxide accumulations				
17 18	• A mesoscale eddy at the site of high nitrous oxide concentrations suggests the importance of eddies to the area's spatiotemporal variability				

19 Abstract

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- 22 North Pacific (ETNP), N₂O can be produced and consumed by several biological processes that
- are controlled by a variety of oceanographic conditions. In this study, the concentration and
- 24 isotopocule ratios of N₂O from a 2016 cruise to the ETNP were analyzed to examine
- 25 heterogeneity in N₂O cycling across the region. Along the north-south transect, three distinct
- ²⁶ biogeochemical regimes were identified: background, core-ODZ, and high-N₂O stations.
- 27 Background stations were characterized by less dynamic N₂O cycling. Core-ODZ stations were
- 28 characterized by co-occurring N₂O production and consumption at anoxic depths, indicated by
- high $\delta^{18}O$ (> 90‰) and low $\delta^{15}N^{\beta}$ (< -10‰) values, and confirmed by a time-dependent model,
- 30 which indicated that N₂O production via denitrification was significant and may occur with a
- non-zero site preference. High-N₂O stations were defined by [N₂O] reaching 126.07±12.6 nM,
 low oxygen concentrations expanding into near-surface isopycnals, and the presence of a
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- 35 amonia-oxidizing archaea and denitrification from nitrate in the near-surface N₂O maximum,
- while bacterial nitrification and denitrification from nitrite were insignificant. This study also
- $_{36}$ represents the first in the ETNP to link N₂O isotopocule measurements to a mesoscale eddy,
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- 38 region.

39 Plain Language Summary

- 40 Nitrous oxide (N₂O) commonly known as laughing gas is a greenhouse gas nearly 300
- 41 times more potent than carbon dioxide, and microbes in the ocean produce and consume N₂O
- 42 through different processes called nitrification and denitrification. Regions of the ocean with
- 43 little to no oxygen near the surface, like the Eastern Tropical North Pacific, are hot spots of
- 44 marine N_2O emissions. By measuring and modeling the isotopes of N_2O and related molecules in
- 45 water samples from the region, we were able to better understand what processes are at play and
- 46 where. N₂O production and consumption through denitrification appeared to both be important at
- 47 depths with no oxygen. N₂O production from denitrification and nitrifying archaea were
- 48 important sources to high N₂O concentrations. Untangling the various processes in the marine
- 49 N_2O cycle helps researchers predict how the cycle will respond to future climate trends.

50 1 Introduction

Nitrous oxide (N_2O) is a powerful greenhouse gas with a per-molecule warming potential 51 nearly 300 times greater than that of carbon dioxide (Smith et al., 2021). It is also projected to 52 53 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 54 total flux of N₂O into the atmosphere (Ciais et al., 2013; Denman et al., 2007; Freing et al., 2012; 55 56 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 57 58 Sea — are dominant sites of N₂O cycling (Yang et al., 2020). The sharp redox gradients and 59 nanomolar levels of oxygen (O_2) in the core of the ODZ (Revsbech et al., 2009; Thamdrup et al., 60 2012; Tiano et al., 2014) promote conditions for several processes that produce and consume N₂O (Codispoti & Christensen, 1985; Codispoti, 2010; Cohen & Gordon, 1979; Elkins et al., 61

62 1978; Law & Owens, 1990; Suntharalingam et al., 2000). With the greenhouse effects of N₂O in

mind, constraining the sources and sinks of N₂O in ODZs is of interest and important to 63

predicting how the marine N₂O cycle will react to climate trends, such as the expansion of ODZs 64

and deoxygenation of the global ocean (Frölicher et al., 2020; Horak et al., 2016; R. F. Keeling 65 et al., 2010). 66

Nitrous oxide is produced and consumed by a number of biological processes. In 67 oxygenated waters like the surface ocean, ammonia-oxidizing bacteria (AOB) produce N₂O from 68 ammonia via side-reactions of the nitrification processes that produce nitrite (NO₂⁻; Cohen & 69 Gordon, 1979; Dore et al., 1998; Nevison et al., 2003), and from nitrite via nitrifier-70 denitrification (Stein & Yung, 2003; Wrage et al., 2001). Ammonia-oxidizing archaea (AOA) 71 produce N₂O from ammonia (and possibly also nitrite) via one or more mechanisms whose 72 enzymology remains unknown (Kozlowski et al., 2016; Santoro et al., 2011; Stein, 2019; 73 Stieglmeier et al., 2014; Trimmer et al., 2016). In low-oxygen waters like the core of the ODZ, 74 75 denitrifying organisms sequentially reduce NO_3^- and nitrite (NO_2^-) to nitric oxide (NO), N_2O , and dinitrogen gas (N₂; Babbin et al., 2014; Bourbonnais et al., 2017; L. A. Codispoti & Christensen, 76 1985). While N_2O can be both produced and consumed under anoxic conditions, the enzyme 77 involved in N₂O consumption via denitrification is more sensitive to O₂ poisoning than that 78 which produces N₂O, leading to a decoupling of the processes and net production at the oxic-79 80 suboxic interface of the oxycline (Babbin et al., 2015; Farías et al., 2009; Körner & Zumft,

1989). Together, these ODZ processes typically lead to N₂O concentrations ([N₂O]) that are at or 81

below saturation in the anoxic core but high in near-surface waters. 82

83 Given the complex interactions of pathways and substrates, the stable isotopes of nitrogen and oxygen — 15 N and 18 O — in N₂O are vital to understanding the contribution of each 84 process to a particular pool of N₂O (Farías et al., 2009; Kim & Craig, 1990; Rahn & Wahlen, 85 2000; Toyoda & Yoshida, 1999; Yamagishi et al., 2007). Because the two nitrogen atoms are in 86 87 unique chemical environments within the N₂O molecule, site-specific isotope ratios of the inner (α) and outer (β) nitrogen atoms may differ in isotopic composition and provide additional 88 89 information about the sources and cycling of N₂O in the environment (see Toyoda et al., 2017). Isotopic values are expressed in terms of delta notation, defined as δ^{15} N or δ^{18} O = 90 $(R_{sample}/R_{standard} - 1) \times 1,000$, where R_{sample} is the ratio of ${}^{15}N/{}^{14}N$ or ${}^{18}O/{}^{16}O$ in each sample and 91 R_{standard} is that of air or Vienna Standard Mean Ocean Water (VSMOW), respectively (Kim & 92 93 Craig, 1990; Rahn & Wahlen, 2000; Toyoda & Yoshida, 1999). In this study, bulk nitrogen and oxygen isotope ratios are represented by $\delta^{15}N_2O^{\text{bulk}}$ and $\delta^{18}O-N_2O$, respectively, while the site-94

specific ratios of nitrogen are represented by $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$. 95

The difference between $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$, referred to as site preference (SP), is an 96 97 important diagnostic tool. Preferential breakage of the O-N^a bond during N₂O consumption has predictable effects on $\delta^{15}N_2O^{\alpha}$ versus $\delta^{18}O-N_2O$ (Ostrom et al., 2007). Furthermore, due to 98 differences in biochemical mechanism, each N₂O production process is thought to have a 99 specific SP independent of the isotopic values of the substrate. Thus, SP provides insight into 100 how an N₂O pool was formed (Frame & Casciotti, 2010; Schmidt et al., 2004; Sutka et al., 2003, 101 2006; Toyoda et al., 2002, 2005, 2019; Yoshida & Toyoda, 2000). For example, preferential 102 breakage of the ¹⁴N-O bond in the hyponitrite (O-N=N-O) intermediate of bacterial and fungal 103 nitrification gives it a high SP of 30-36‰ (Frame & Casciotti, 2010; Sutka et al., 2006, 2008). 104 On the other hand, a low SP of -1-11‰ is observed in N₂O produced by denitrification and 105 nitrifier-denitrification (Frame & Casciotti, 2010; Sutka et al., 2006), although the exact 106 mechanism remains unclear (Schmidt et al., 2004; Toyoda et al., 2005). 107

Indeed, while SP remains a useful measurement in constraining the source and sinks of 108 N₂O cycling, a few studies have challenged its ability to serve as a clear diagnostic marker. The 109 potential hybrid nature of archaeal nitrification (Kozlowski et al., 2016; Stein, 2019; Stieglmeier 110 et al., 2014) may undermine the notion of an expected SP for this particular process (Casciotti et 111 al., 2018; Kelly et al., 2021), even though N₂O produced by AOA has been recorded to have an 112 SP of about 30‰ (Löscher et al., 2012; Santoro et al., 2011). Additionally, one strain of 113 denitrifying bacteria has been found to produce N₂O with SP = $23.3\pm4.2\%$ (Toyoda et al., 2005), 114 and recent studies have suggested that a nonzero SP for denitrification could explain observed 115 isotopic trends in the ETSP (Casciotti et al., 2018), ETNP (Kelly et al., 2021), and the bottom 116 waters of Baffin Bay (Lehmann et al., 2019). Because of these complicating factors, in this study 117

- we model and interpret $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$ as distinct tracers, which provides more
- 119 information than interpreting site preference alone.

This study addresses core questions surrounding N₂O cycling in ODZs: how does the mix of N₂O cycling processes change throughout the water column, what factors contribute to this

heterogeneity, and how can isotopic measurements help constrain these processes? We measured

123 concentration and isotope ratios of N_2O , NO_2^- , and NO_3^- 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 N₂O

production and consumption that best explain N₂O 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_2O observations were connected to a mesoscale eddy.

130 **2 Methods**

131 2.1 Shipboard Sampling

132Sampling 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,

135 106.21° W; Fig. 1). Eleven stations were sampled for N₂O 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
- 137 the coast and included in analyses (Fig. 1).
- 138



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Figure 1: Station map. Stations plotted over historically averaged O₂ saturation at a depth of
 200 meters from the World Ocean Atlas 2018 product, using all years available (Garcia et al.,
 2019).

143 Salinity, potential temperature, pressure and dissolved oxygen were recorded by sensors aboard a conductivity, temperature, depth (CTD) rosette (Sea-Bird SBE-911+). Chlorophyll-a 144 (Chl-a) was estimated using a correction factor of $3.03 \,\mu g/L/V$ on the package's WETLabs ECO 145 Chlorophyll Fluorometer. This correction factor was obtained from the cruise data deposited in 146 147 BCO-DMO (Rocap et al., 2017). Seawater samples were collected from a Niskin rosette for NO₃⁻ , NO_{2⁻}, and N₂O concentration and isotopocule analysis. NO_{3⁻} and NO_{2⁻} samples were first 148 collected in 500-mL Nalgene polypropylene bottles after three rinses of the bottle. NO₃⁻ samples 149 were filtered through 0.2 um pore size filters and stored frozen in 60 mL HDPE bottles until 150 analysis. NO₂⁻ concentrations were determined onboard via spectrophotometry (Grasshoff et al., 151 1999). NO₂⁻ samples and reference materials for isotopic analysis were preserved shipboard, 152 153 immediately after collection according to the "azide method" (McIlvin & Altabet, 2005) and stored as N₂O gas-tight vials at room temperature until analysis. 154

N₂O 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 uL of saturated mercuric chloride (HgCl₂) 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 anaylsis.

161 2.2 Concentration and Isotope Analyses

Samples were analyzed for NO_x concentration via cadmium reduction and 162 spectrophotometric analysis using a Westco discrete analyzer system at Stanford University and 163 then prepared for isotopic analysis using the "denitrifier method" (Casciotti et al., 2002; McIlvin 164 & Casciotti, 2011; Sigman et al., 2001). In the denitrifier method, seawater NO_3^- is converted to 165 N₂O for isotopic analysis using a strain of denitrifying bacteria that lacks the terminal N₂O 166 167 reductase. Sulfamic acid was used to remove nitrite from samples (Granger & Sigman, 2009) prior to analysis via the denitrifier method. Nitrite δ^{15} N and δ^{18} O analyses were conducted using 168 169 the "azide method", whereby NO2⁻ is converted to N2O via reaction with sodum azide (McIlvin & Altabet, 2005). The δ^{15} N and δ^{18} O of N₂O produced from NO₃⁻ or NO₂⁻ samples were 170 measured by running samples on a custom-built purge-and-trap system coupled to a Thermo 171 Finnigan DELTA V isotope ratio mass spectrometer (IRMS) in continuous flow mode (McIlvin 172 173 & 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 174 analyses were calibrated to RSIL-N23, RSIL-N7373, and RSIL-N10219 (Casciotti et al., 2007) 175 preserved and analyzed in parallel. Drift corrections and offsets to denitrifier samples were 176 applied as described in McIlvin & Casciotti, 2011, while drift and size corrections were applied 177 to azide samples as described in Kelly et al., 2021. The analytical precision of these isotopic 178 179 measurements, represented by the pooled standard deviations of reference materials for NO₃⁻ and pooled standard deviations of duplicates for NO₂⁻, were $\pm 0.24\%$, $\pm 0.31\%$, $\pm 0.69\%$, and $\pm 0.64\%$ 180 for $\delta^{15}NO_3^-$, $\delta^{18}O-NO_3^-$, $\delta^{15}NO_2^-$, and $\delta^{18}O-NO_2^-$, respectively. 181

For N₂O isotopocule analysis, samples were run against a tank of pure N₂O calibrated by 182 S. Tovoda (Tokyo Tech) on a custom-built purge-and-trap system coupled to a Finnigan DELTA 183 V IRMS (McIlvin & Casciotti, 2010). Ions with mass/charge (m/z) ratios of 30, 31, 44, 45, and 184 46 were measured simultaneously to capture isotopocules of N₂O as well as those of fragment 185 NO⁺ ion (McIlvin & Casciotti, 2010). Reference gases "B6" (pure N₂O internal laboratory 186 standard) and "S2" (a mixture of 90 ppm N₂O in N₂; Mohn et al., 2014), along with atmosphere-187 equilibrated seawater, were also analyzed in each run to correct for scrambling — the 188 phenomenon by which the N atoms switch during fragmentation, causing the NO⁺ fragment to 189 sometimes contain the outer (N^{β}) atom and not the inner (N^{α}) atom from an N₂O molecule 190 (Toyoda & Yoshida, 1999). Reference materials were prepared by purging 160 mL Wheaton 191 bottles, each containing approximately 155 mL de-ionized water, with helium for 90 min, 192 injecting a fixed amount of reference gas (2 nmoles for S2 and 10 nmoles for B6), and preserving 193 with 100 µL of saturated HgCl₂ solution. Atmosphere-equilibrated seawater samples consisted of 194 filtered surface seawater that was allowed to equilibrate with outdoor air for 2-3 days, before re-195 196 filtration and preservation with 100 µL of HgCl₂ 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 N₂O 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 $\delta^{15}N_2O^{\alpha}$, $\delta^{15}N_2O^{\beta}$, SP, $\delta^{15}N_2O^{bulk}$, and $\delta^{18}O$ -N₂O, respectively.

203 **2.3 Optimization of Time-Dependent Model**

A major feature of this study is the expansion and optimization of the time-dependent 204 model of N₂O cycling employed in Kelly et al., 2021 (based on a similar model in Casciotti et 205 206 al., 2018) to solve for the rates of N_2O production and consumption by nitrification and denitrification processes. Briefly, the model started with an initial [N₂O] and initial isotopic 207 values for N₂O and tracked concentrations of ${}^{14}N$, ${}^{15}N^{\alpha}$, ${}^{15}N^{\beta}$, ${}^{16}O$, and ${}^{18}O$ in N₂O at each time 208 209 step (0.2 days) based on specified isotope effects and substrate parameters (substrate concentrations and isotopic compositions were held constant). From the isotopic concentrations, 210 211 $[N_2O]$, $\delta^{15}N_2O^{\alpha}$, $\delta^{15}N_2O^{\beta}$, SP, and $\delta^{18}O-N_2O$ were calculated at each time step. First-order rate constants for each reaction were found using a Nelder-Mead optimization (Nelder & Mead, 212 213 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 214 215 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 216 supplemental material. 217

The "nmkb()" function of R package "dfoptim" (Varadhan & Borchers, 2020) was used 218 to conduct a bounded Nelder-Mead optimization of each experimental setup. The functions in 219 "dfoptim" are based on previously-established MATLAB code for derivative-free optimizations 220 (Kelley, 1999). The Nelder-Mead algorithm is a simplex-based direct search method that can be 221 used to find minima or maxima, and the bounded version was employed to prevent negative rate 222 constants. Error between model output and cruise measurements of the isotopic tracers was 223 quantified by an MSE calculation. With [N₂O] as the x-value and tracers $\delta^{15}N_2O^{\alpha}$, $\delta^{15}N_2O^{\beta}$, SP, 224 225 and δ^{18} O-N₂O as the v-values, model output was linearly interpolated to [N₂O] from cruise measurements. This allowed for the calculation of MSE between modeled and measured tracers 226 at measured $[N_2O]$ values. MSE was calculated for each of the tracers, and the average of those 227 four values across all data points served as the cost function to be minimized in the 228 optimizations. Penalties in the cost function ensured that the optimized model output spanned 229 cruise observations. 230

Three unknown rate constants — N_2O production via denitrification from NO_2 , N_2O 231 232 production via denitrification from NO₃⁻, and N₂O consumption via denitrification — were solved for in simulations in the core of the ODZ. For simulations above the ODZ, N₂O 233 production from NH₄⁺ by AOA and AOB were added by including two additional terms in the 234 cost function. Since there is no oxygen atom in NH₄⁺, the δ^{18} O of the substrate for nitrification 235 was taken to be 23.5%, the average value for atmospheric oxygen, which is fractionated slightly 236 in surface seawater (Kroopnick & Craig, 1972). N2O derived from AOB and AOA were 237 distinguished by different isotope effects, derived from fractionation factors in the literature 238 239 (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

245 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 day⁻¹, which is one order of magnitude greater than the maximum rate constant for N₂O consumption via denitrification measured previously in the ETNP (Babbin et al., 2015).
- for N₂O consumption via denitrification measured previously in the ETNP (Babbin et al., 2015).

2493 Results

3.1 Hydrographic Variation in the ETNP

Based on key hydrographic and N₂O isotopic features, stations were grouped into three 251 regimes: "Background" (Stns. 1-3), "Core ODZ" (Stns. 4-7&12), and "High N₂O" (Stns. 8-11). 252 Considerable differences were observed in the water properties between station groupings along 253 the transect (Table S3). Surface water became less dense from north to south, with minimum 254 potential density anomaly (σ_{θ}) averaging 23.01 kg/m³, 21.88 kg/m³, and 20.88 kg/m³ for 255 background, core-ODZ, and high-N2O stations, respectively. A temperature-salinity diagram 256 plotted with water mass endmembers as defined in Evans et al., 2020, revealed a southward 257 258 increasing presence of Tropical Surface Water near the surface, while waters between 24 and 26.5 kg/m^3 showed a shift from lower salinity in the core-ODZ stations to higher salinity in the 259 high-N₂O stations (Fig. S1). Waters below $\sigma_{\theta} = 26.5 \text{ kg/m}^3$ displayed more similar temperatures 260

and salinities across the region (Fig. S1).

262 As is characteristic of ODZs, dissolved oxygen concentrations ([O₂]) remained below detection at core anoxic depths in the ETNP. The near-surface oxycline, defined by a rapid 263 decrease in [O₂] from saturation to below detection, was shallower at the southern end of the 264 265 transect (Fig. 2b); from CTD data, the average depth (and σ_{θ}) of the threshold at which [O₂] dipped below 5 µmol/kg was 206.6 m (26.33 kg/m³), 130.4 m (25.87 kg/m³), and 77.5 m (25.23 266 kg/m³) for background, core-ODZ, and high-N₂O stations, respectively. Oxygen recovered 267 268 slowly with depth, with measurements reaching above 5 μ mol/kg at $\sigma_{\theta} \sim 27.35$ kg/m³ at each station. 269

The nitracline, defined by the steep increase in dissolved nitrate ([NO₃⁻]) with depth, followed the southward shoaling pattern (Fig. 2d). The average depth (and density) of the threshold at which [NO₃⁻] reached 20 μ M, calculated by interpolating [NO₃⁻] at every meter, was calculated to be 124.7 m (25.59 kg/m³), 98 m (25.24 kg/m³), and 72 m (24.59 kg/m³) for background, core-ODZ, and high-N₂O stations, respectively. Below the nitracline, [NO₃⁻] reached a maximum of 48 μ M (Stn. 11; 850 m; $\sigma_{\theta} = 27.28$ kg/m³).

The high-N₂O stations, where the oxycline was shallowest, had the largest primary nitrite maximum (PNM; 0.5-1.25 μ M near the surface; Fig. 2c), and the highest near-surface Chl-*a* estimates, with a maximum of 5.97 μ g/L (Stn. 10; 55.7 m; $\sigma_{\theta} = 22.58$ kg/m³; Fig. S2b). Three out of four of the high-N₂O stations also had observable deep Chl-*a* maxima. The core-ODZ stations had the most pronounced secondary nitrite maximum (SNM), with maxima of 1-4 μ 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-N₂O 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 [NO₂⁻], reaching 4.30 μ M in suboxic waters (295 m; $\sigma_{\theta} = 26.55 \text{ kg/m}^3$).

Isotopic ratios for NO₃⁻ and NO₂⁻ were not the focus of this study but were used to initialize the time-step model discussed in Section 4. Local $\delta^{15}NO_3^-$ and $\delta^{18}O-NO_3^-$ maxima of up to 25‰ were found at core anoxic depths, at densities ranging from 26-27 kg/m³ (Fig. S3c-d). Isotopologues of NO₂⁻ could only be measured at depths where [NO₂⁻] exceeded 0.5 μ M, occurring within the PNM and SNM features (Fig. S3). $\delta^{15}NO_2^-$ 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).

297 **3.2 N₂O Concentrations**

Two [N₂O] peaks were generally observed at each station — one in the oxycline near the 298 top of the ODZ and one below the ODZ ($\sigma_{\theta} > 27 \text{ kg/m}^3$; Fig. 2a), consistent with previous ETNP 299 studies (Babbin et al., 2015; Kelly et al., 2021; Yamagishi et al., 2007). Between the two peaks, a 300 local [N₂O] minimum was observed at anoxic depths (Fig. 2a), which is characteristic of N₂O 301 consumption in ODZs (Babbin et al., 2015; Bourbonnais et al., 2017; Casciotti et al., 2018; Kelly 302 303 et al., 2021; McIlvin & Casciotti, 2010; Yamagishi et al., 2007; Yoshinari et al., 1997). This 304 "bite" in the [N₂O] profiles was most pronounced at core-ODZ stations relative to the other regimes, with [N₂O] < 5 nM, or below 50% saturation, found between $\sigma_{\theta} = 26 \text{ kg/m}^3$ and $\sigma_{\theta} = 27$ 305 kg/m^3 (Fig. 2a). 306

The highest near-surface $[N_2O]$ values were recorded at the high-N₂O stations, with 307 119.30-126.07 nM measured at 90 m ($\sigma_{\theta} = 25.49-25.78 \text{ kg/m}^3$; Fig. 2a). [N₂O] reached 308 maximum values between the base of the oxycline (Fig. 2b) and top of the SNM (Fig. 2c). The 309 average near-surface [N₂O] maxima at core-ODZ and background stations (except Stn. 7, where 310 shallow N₂O samples were not collected) were 62.64 nM and 44.55 nM, respectively (Fig. 2a). 311 While Stn. 7 displayed a local [N₂O] minimum between $\sigma_{\theta} = 26 \text{ kg/m}^3$ and $\sigma_{\theta} = 27 \text{ kg/m}^3$. 312 characteristic of the ODZ, it also showed a high [N₂O] value of 95.77 nM near the top of that 313 density layer (145 m; $\sigma_{\theta} = 26.21 \text{ kg/m}^3$; Fig. 2a). Previous studies have also reported maximal 314 near-surface [N₂O] of ~100 nM in the offshore ETNP (Babbin et al., 2015; Yamagishi et al., 315 2007), and up to $[N_2O] > 200$ nM near the coast (Kelly et al., 2021). The deep $[N_2O]$ maximum 316

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

319



320

Figure 2: Major chemical and isotopic species along the transect plotted against potential

322 **density** σ_θ. (a) [N₂O] (nM), (b) [O₂] (µmol/kg), (c) [NO₂⁻] (µM), (d) [NO₃⁻] (µM), (e) δ^{15} N₂O^α

323 (‰ vs. atmospheric N₂), (f) $\delta^{15}N_2O^{\beta}$ (‰ vs. atmospheric N₂), (g) site preference (‰ vs.

atmospheric N₂), and (h) δ^{18} O-N₂O (‰ vs. VSMOW). [N₂O] contours are overlaid on each panel

to compare profiles to key [N₂O] features, and white dashed lines separate background (Stns. 1-

326 3), core-ODZ (Stns. 4-7), and high- N_2O (Stns. 8-11) stations.

327

328 **3.3 Distribution of N₂O Isotopocules**

The N₂O isotopocule distributions mirrored the key features of [N₂O] profiles. The highest δ^{15} N₂O^{α} and δ^{18} O-N₂O on the transect were found in anoxic waters at core-ODZ stations

- and some high-N₂O stations (Fig. 2e and 2h), coinciding with the pronounced [N₂O] minima of these stations at $\sigma_{\theta} = 26-27 \text{ kg/m}^3$. The maximum $\delta^{15}N_2O^{\alpha}$ found at each station ranged from
- 333 39.7%-49.2‰ and 31.9‰-52.0‰ for the core-ODZ and high-N₂O stations, respectively. In this
- same density layer, the δ^{18} O-N₂O maxima ranged from 97.2‰-113.7‰ and 83.3‰-117.0‰ for
- the core-ODZ and high-N₂O stations, respectively. The increases in $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$
- within this layer were less dramatic at background stations (Fig. 2e and 2h).
- Minimum $\delta^{15}N_2O^{\beta}$ values, which reached -16.4‰ (Stn. 7; 295 m; $\sigma_{\theta} = 26.56$ kg/m³), also accompanied the [N₂O] minima of core-ODZ and some high-N₂O stations (Fig. 2f). As described above, this density layer also contained the highest $\delta^{15}N_2O^{\alpha}$, leading to SPs peaking at 53.2‰ (850 m; $\sigma_{\theta} = 27.27$ kg/m³) and 56.5‰ (500 m; $\sigma_{\theta} = 26.88$ kg/m³) at Stn. 7 and Stn. 8, respectively (Fig. 2g). The background stations exhibited smaller SP gradients and contained the least negative values for $\delta^{15}N_2O^{\beta}$ (~4‰-7.3‰) along the transect in the same density layer where
- the other stations contained their minimum (Fig. 2f-2g).
- Above the ODZ ($\sigma_{\theta} < 25.8 \text{ kg/m}^3$), $\delta^{15}N_2O^{\alpha}$ 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-N₂O stations, respectively. $\delta^{15}N_2O^{\beta}$ also exhibited this near-surface gradient along the transect, averaging 0.2‰, -0.1‰, and -2.8‰ at background, core-ODZ, and high-N₂O stations, respectively. Near-surface averages for SP were 14.2‰, 12.7‰, and 12.7‰ and for $\delta^{18}O$ -N₂O were 48.2‰, 50.3‰, and 48.9‰ at background, core-ODZ, and high-N₂O stations, respectively (Fig. 2e-2h).

351 4 Discussion

Variations in the distribution of N₂O and its isotopocules between the three station groupings guided three central research questions: (1) What is the balance of production and consumption of N₂O in the ODZ core of Stns. 4-7&12? (2) What are the sources and factors contributing to the near-surface N₂O accumulation at Stns. 8-11? (3) What does the eddy reveal about [N₂O] variability in the ETNP?

To define regimes for further modeling and analyses, regressions of δ^{18} O-N₂O on 357 $\delta^{15}N_2O^{\text{bulk}}$ and $\delta^{18}O-N_2O$ on $\delta^{15}N_2O^{\alpha}$ were performed to determine the density layers where 358 isotopic composition was dominated by N₂O consumption via denitrification (Casciotti et al., 359 2018; Kelly et al., 2021). Breakage of the O-N $^{\alpha}$ bond during N₂O reduction is associated with 360 isotope effects on both ¹⁸O and ¹⁵N^{α} and leads to expected linear relationships between δ^{18} O-N₂O 361 and $\delta^{15}N_2O^{\alpha}$ (Ostrom et al., 2007). In the density layer $\sigma_{\theta} = 26-27$ kg/m³, regression slopes 362 between δ^{18} O-N₂O and δ^{15} N₂O^{bulk} and δ^{18} O-N₂O and δ^{15} N₂O^{α} closely matched the expected 363 ratios of 2.5 and 1.7, respectively (Ostrom et al., 2007); isotopocule ratios in the other density 364 layers deviated from this relationship (Fig. 3). Thus, we treated $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ as an ODZ 365 cycling regime dominated by N₂O consumption and $\sigma_{\theta} < 25.8$ kg/m³ as a near-surface cycling 366 regime where isotopic composition largely reflects N₂O production. This framework does not 367 preclude N₂O production in the consumption-dominated density layer or vice versa, but 368 emphasizes that isotopic composition primarily reflects one or the other (Kelly et al., 2021). $\sigma_{\theta} =$ 369

 $25.8-26 \text{ kg/m}^3$ is taken as a transitional area between the two regimes and is excluded from some analyses specific to the regimes.



372

Figure 3: Deviations from reduction line. Deviations from the slopes of isotopolocule regressions found by Ostrom et al. (2007) for N₂O consumption via denitrification, plotted for chosen density layers at (a) background, (b) core-ODZ, and (c) high-N₂O stations. Closed circles represent the slope of δ^{18} O-N₂O against δ^{15} N₂O^{bulk} minus 2.5, and open circles represent the slope of δ^{18} O-N₂O against δ^{15} N₂O^{α} minus 1.7. Asterisks indicate a p-value < 0.5 for the linear regression.

379

380 **4.1 Denitrification in the ODZ Core**

The anoxic depths of core-ODZ stations were characterized by the lowest [N₂O] and relatively high $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$ (Fig. 2e and 2h). During N₂O consumption via denitrification, preferential breakage of light-isotope bonds in the intermediate leads to heavy isotope enrichment in the remaining N₂O (Popp et al., 2002; Toyoda et al., 2002; Yamagishi et al., 2007). Given that the isotopolocule regressions indicated that consumption was a dominant process in the core of the ODZ, the elevated $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$ is expected for $\sigma_{\theta} = 26-27$ kg/m^3 . However, this density layer at core-ODZ stations also contained some of the most negative values for $\delta^{15}N_2O^{\beta}$ (Fig. 2f). Similar signals have been observed in past ODZ studies and interpreted to represent concurrent N₂O 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 $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ were plotted against the natural logarithm of N₂O 392 concentration to observe how isotopolocule ratios change as $[N_2O]$ varies. As $[N_2O]$ decreased 393 394 from its peak concentration to ~ 5 nM, isotopic ratios generally increased, consistent with N₂O consumption via denitrification. Using a segmented regression model (Muggeo, 2003, 2008), a 395 breakpoint was identified at [N₂O] ~ 5 nM (ln([N₂O]) ~ 1.6), after which $\delta^{15}N_2O^{\alpha}$, $\delta^{18}O$ -N₂O. 396 and SP began to level off or even decrease (Fig. S5). Most of the points after the breakpoint were 397 from core-ODZ stations (Fig. S5), with [N₂O] extending to lower values. The segmented 398 regression also revealed that δ^{18} O-N₂O appeared to decrease at a higher rate than δ^{15} N₂O^{α} and SP 399 after the breakpoint (Fig. S5) — a surprising result, given that $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O$ -N₂O would be 400 expected to track each other in the ODZ. 401

The decreasing $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$ when $[N_2O] < 5$ nM, taken together with the negative $\delta^{15}N_2O^{\beta}$, suggest that N₂O production was co-occurring with N₂O consumption at core-ODZ stations. Babbin et al. (2015) hypothesized that N₂O in the core of the ODZ is in steady state — that is, N₂O production is balanced by N₂O 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 $\sigma_{\theta} = 26-27$ kg/m³ at core-ODZ stations and optimized for the rates of N₂O cycling processes.

The model allowed N₂O to be produced from NO₂⁻, produced from NO₃⁻, or consumed 409 via denitrification. Experiments were initialized with the mean N₂O concentration and 410 isotopocules within $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ at background stations (at the northern end of the transect). 411 while mean NO_3^- and NO_2^- concentrations and isotopic content were taken from the core-ODZ 412 stations. This setup simulates the lateral advection of a pool of N₂O from background to core-413 ODZ stations, overprinted by local N₂O production and consumption. Isotope effects for N₂O 414 production via denitrification were taken from the literature (Table S1), while the isotope effects 415 for N₂O consumption were calculated in this study with a closed-system Rayleigh model (Fig. 416 S6). The model initialization conditions for this set of experiments are outlined in the supplement 417 (Table S1). Optimizations were performed to find the rates and rate constants for the three 418 denitrification processes (NO₃⁻ reduction to N₂O, NO₂⁻ reduction to N₂O, and N₂O reduction to 419 N₂) that best matched observed N₂O concentration and isotopocule data for $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ at 420 core-ODZ stations. More details about the optimization method can be found in Section 2.3. 421 Starting with N₂O from background stations, the model attempted to recreate the breakpoint in 422 Fig. S5. 423

Denitrification is generally thought to produce N₂O with a low, near-zero SP (Frame & Casciotti, 2010; Sutka et al., 2006). However, one denitrifier has been found to produce N₂O 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 N₂O production via denitrification were tested here: one set with no SP and two sets with a SP of 22‰. Introduction of a SP for 430 denitrification was generated by either changing the isotope effect for production of N^{α}

431 $(^{15}\varepsilon_{NOx\to N2O}(\alpha))$ from 22‰ to 0‰ while keeping the isotope effect for N^b at 22‰, or by

432 changing the isotope effect for production of N^{β} (¹⁵ $\varepsilon_{NOx \rightarrow N2O}$ (β)) from 22‰ to 44‰ while

433 keeping the isotope effect for N^a at 22% — the latter of which resembles the case for

434 *Pseudomonas fluorescens* (Toyoda et al., 2005).

Four sets of parameter combinations were tested for each denitrification site preference 435 setting: (1) the default initialization previously described, (2) increasing the initial amount of 436 437 N₂O ([N₂O]_i), (3) increasing the isotope effects for N₂O consumption ($^{15}\varepsilon_{cons}$ and $^{18}\varepsilon_{cons}$), and (4) combining (2) and (3). With three sets of isotope effects for N₂O production via denitrification 438 and four parameter combinations, 12 optimizations were performed. The optimized rates and 439 average mean squared error (MSE) for the modeled tracers are listed in Table 1. N_2O 440 consumption is phased-in nonlinearly in this set of experiments, as described in Section 2.4 of 441 Kelly et al., 2021, and the initial rate of consumption and maximum rate of consumption are 442 given for each optimization (Table 1). 443

Table 1: Optimized rates (nM N/day) for N₂O production and consumption within the

445 **ODZ** ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$) at core-ODZ stations. Mean squared error (MSE) is presented as an 446 average of that of the four tracers. Results in boldface are those where the rate constant for 447 consumption converged at the maximum constraint of 2.1 day⁻¹.

		Default	$[N_2O]_i = 60 \text{ nM}$	^{15,18} ε _{cons} x 1.5	$[N_2O]_i = 60 \text{ nM and}$
	Prod. from NO ₂ -	1.19 x 10 ⁻²	3.18 x 10 ⁻¹	2.91 x 10 ⁻²	8.17 x 10 ⁻²
SP = 0%	Prod. from NO ₃ ⁻	1.74 x 10 ⁻⁶	2.06 x 10 ⁻⁷	8.88 x 10 ⁻⁴	3.81 x 10 ⁻²
	Initial Cons.	7.77 x 10 ⁻³	8.88 x 10 ⁻¹	8.99 x 10 ⁻³	4.97 x 10 ⁻²
$^{15}\varepsilon_{NOx \rightarrow N2O}(\alpha) =$	Max. Cons.	6.80 x 10 ⁻¹	1.37 x 10 ¹	7.67 x 10 ⁻¹	$2.72 \text{ x } 10^{0}$
$^{15}\varepsilon_{\text{NOx}\rightarrow\text{N2O}}(\beta) = 22\%$					
	MSE	59.70	47.70	55.84	86.80
	Prod. from NO ₂ ⁻	2.27 x 10 ⁻¹	2.01 x 10 ⁻¹	3.47 x 10 ⁻²	1.23 x 10 ⁻¹
SD = 220/	Prod. from NO ₃ ⁻	4.48 x 10 ⁻¹	9.24 x 10 ⁻¹	5.16 x 10 ⁻²	1.78 x 10 ⁻¹
SF = 22700	Initial Cons.	6.53 x 10 ⁻¹	3.18 x 10 ⁻¹	1.43 x 10 ⁻²	9.51 x 10 ⁻²
15 $_{\rm ENOx \rightarrow N2O}(\alpha) = 0\%$	Max. Cons.	$1.02 \ge 10^1$	$7.96 \ge 10^{\circ}$	$1.09 \ge 10^{\circ}$	$4.18 \ge 10^{0}$
	MSE	44.57	37.69	42.79	77.04
	Prod. from NO ₂ ⁻	3.30 x 10 ⁻⁸	1.90 x 10 ⁻²	3.49 x 10 ⁻⁵	8.48 x 10 ⁻⁴
SD = 220/	Prod. from NO ₃ ⁻	5.78 x 10 ⁻¹	1.50 x 10 ⁻⁷	8.03 x 10 ⁻²	1.17 x 10 ⁻¹
SF = 22700	Initial Cons.	6.53 x 10 ⁻¹	1.41 x 10 ⁻²	1.36 x 10 ⁻²	2.84 x 10 ⁻²
15 $\epsilon_{NOx \rightarrow N2O}(\beta) = 44\%$	Max. Cons.	$1.00 \ge 10^1$	$1.17 \ge 10^{0}$	$1.05 \ge 10^{\circ}$	$1.95 \ge 10^{\circ}$
	MSE	44.90	41.80	62.22	105.04

448

For each experiment, the model was generally able to recreate the breakpoint in N₂O isotopic composition for $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ at core-ODZ stations (Fig. 4). The reported MSE is an average of that of the four tracers ($\delta^{15}N_2O^{\alpha}$, $\delta^{15}N_2O^{\beta}$, SP, and $\delta^{18}O-N_2O$). $\delta^{18}O-N_2O$ had the

- 452 highest MSE for each experiment, likely because the breakpoint in the isotopic data for δ^{18} O-
- 453 N₂O was the most dramatic (Fig. S5), and thus the model performed most poorly in replicating it.
- 454 Additionally, there could be a statistical bias in optimizing for the lowest average MSE when the
- 455 average includes SP, which depends on $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$.





Figure 4: Time-dependent model output from core-ODZ stations. Model output for the 457 default initialization with SP = 0% for N₂O production via denitrification, presented as (a) a time 458 series of [N₂O] and isotopocules and (b) isotopocules against the natural logarithm of [N₂O] for 459 $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ at core-ODZ stations (similar to Fig. S5). The model was optimized at 1000 460 461 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 462 in (a) are output using the maximum and minimum ${}^{15}\varepsilon_{cons}$ and ${}^{18}\varepsilon_{cons}$ from literature. The vertical 463 blue line in each panel indicates the timestep with modeled [N₂O] was closest to the smallest 464 measured [N₂O]. In (b), model output is plotted with a series of lines, while observations are 465 plotted with points of matching color. 466

467

In most experiments, the rate constant for N₂O consumption (k_{cons}) converged within the 468 range of 10⁻²-10⁻¹ day⁻¹; however, three runs (bolded in Table 1) converged at the maximum 469 constraint of 2.1 day⁻¹, implying that additional N₂O consumption might have improved the 470 model fits in those cases. It is also plausible that ${}^{15}\varepsilon_{cons}$ and ${}^{18}\varepsilon_{cons}$ calculated with the Rayleigh 471 model were too low, forcing the model to converge at a high k_{cons} to reach the high observed 472 isotopic values. The ${}^{15}\varepsilon_{cons}$ and ${}^{18}\varepsilon_{cons}$ implemented in the model were calculated from high-N₂O 473 stations to avoid N₂O production lowering the apparent isotope effects (unpublished data; see 474 Kelly et al., 2021). This approach yielded isotope effects of 10.54±0.99‰ and 16.16±2.28‰ for 475 $^{15}\varepsilon_{cons}$ and $^{18}\varepsilon_{cons}$, respectively. However, given that previous work estimated $^{15}\varepsilon_{cons}$ and $^{18}\varepsilon_{cons}$ at 476 20±2‰ and 31±3‰, respectively (Yamagishi et al., 2007), the values calculated here are at the 477 lower end of the spectrum (see Bourbonnais et al., 2017). Increasing ${}^{15}\varepsilon_{cons}$ and ${}^{18}\varepsilon_{cons}$ by a factor 478 of 1.5 (to 15.75‰ and 24‰, respectively) slightly improved MSE in two of the three sets of 479

480 isotope effects tested for N₂O production, and none of the three runs with higher ${}^{15}\varepsilon_{cons}$ and 481 ${}^{18}\varepsilon_{cons}$ forced k_{cons} to converge on the maximum constraint (Table 1).

Initializing the model with a higher $[N_2O]_i$ also reduced error (Table 1). This could be a 482 result of the interpolation method for calculating MSE; increasing the starting amount of N_2O 483 allowed for better interpolation when calculating MSE between model output and observations 484 because three observations had greater [N₂O] than used in the default initialization (Figure 4). 485 Starting with more N₂O also allowed for greater first-order consumption rates. In this way, the 486 higher $[N_2O]_i$ allowed $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$ to reach high values via enhanced N_2O 487 consumption, whereas increasing ${}^{15}\varepsilon_{cons}$ and ${}^{18}\varepsilon_{cons}$ values achieved high $\delta^{15}N_2O^{\alpha}$ and $\delta^{18}O-N_2O$ 488 via higher fractionation at lower extents of consumption. Increasing both $[N_2O]_i$ and 489 fractionation, however, led to the worst fits of the 12 model runs (Table 1). 490

Recently, both tracer experiments and natural abundance isotope ratio measurements 491 have pointed to NO_3^- as the primary substrate for N₂O production in ODZs (Casciotti et al., 492 2018; Frey et al., 2020; Ji et al., 2015, 2018). In this study, production from NO₂⁻ was much 493 494 higher than that from NO₃⁻ in optimizations where production from denitrification had a SP of 0% (Table 1), meaning that production from NO₂⁻ better explained the isotopic composition of 495 N₂O observed in $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ at core-ODZ stations. This finding is corroborated by the 496 pronounced SNM at these stations (Fig. 2c). When a SP for N₂O production was added to the 497 model, however, NO₃⁻ became the primary substrate in several instances (Table 1). With 498 15 $\varepsilon_{NOx \rightarrow N2O}(\beta) = 44\%$, it is likely that production from NO₂⁻ would drive $\delta^{15}N_2O^{\beta}$ much lower 499 than observed values. Therefore, which substrate best fits the N2O data depends on the assumed 500 isotope effects. 501

Another important takeaway is that adding a SP for N₂O production lowered MSE in 502 most model experiments (Table 1). Essentially, setting ${}^{15}\varepsilon_{NOX\to N2O}(\alpha) = 0\%$ allowed $\delta^{15}N_2O^{\alpha}$ to 503 reach higher values and more closely match observations, while setting ${}^{15}\varepsilon_{NOX\to N2O}(\beta) = 44\%$ 504 and switching the substrate to NO₃⁻ allowed $\delta^{15}N_2O^{\beta}$ to reach slightly lower values and more 505 closely match observations. Both had the effect of improving the fit for SP. This result 506 corroborates prior modeling studies (Kelly et al. 2021; Casciotti et al. 2018) which suggest that 507 N₂O production via denitrification with a nonzero SP (Schmidt et al., 2004; Toyoda et al., 2005) 508 could explain the isotopic composition of N₂O in ODZs. 509

Peng and Valentine (2021) recently suggested a potential suboxic niche for fungal 510 denitrification in the ETNP and found that it contributes significantly to N₂O production in the 511 water column. Because fungi produce N₂O with a SP ranging from ~15‰ to ~37‰ (Maeda et 512 al., 2015; Rohe et al., 2014; Sutka et al., 2008), fungal denitrification could partly account for 513 inferred N₂O production via denitrification with a nonzero SP. This picture complicates current 514 interpretations of N₂O isotopocules in the marine environment and the ability of SP to serve as a 515 diagnostic tool for N₂O cycling in the water column, and it highlights the importance of 516 517 modeling $\delta^{15}N_2O^{\alpha}$ and for $\delta^{15}N_2O^{\beta}$ as distinct tracers.

The primary goals of the modeling exercises were to constrain the processes contributing to N₂O cycling in the core of the ODZ and assess whether the regime was in steady state. In each run, the point at which [N₂O] in the model most closely matched the lowest [N₂O] observation from core-ODZ stations (indicated by blue line in Fig. 4) occurred at the part of the time series in

- which $[N_2O]$ was reaching equilibrium; that is, $[N_2O]$ 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 $[N_2O]$ observation (indicated by blue line in Fig. 4), the isotopocules of N_2O were still
- changing dramatically. Thus, while [N₂O] was close to steady state in $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ 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
- 527 values. At best, based on the model output, it can be said that this area of the water column was 528 approaching steady state but not yet in equilibrium — especially from a stable isotope
- perspective. This demonstrates that N_2O 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 N₂O consumption or using a nonzero SP for N₂O production had large impacts on the model output, including the rate of consumption as well as the primary substrate for N₂O production (Table 1). To understand the dynamic cycling of N₂O more fully in ODZs, isotope effects and other environmental conditions need to be constrained.

538 4.2 Contributors to Near-Surface N₂O Accumulation

The near-surface $[N_2O]$ maxima, such as the high $[N_2O]$ at Stns. 8-11, are climatically 539 relevant, as these accumulations may supply N_2O to the mixed layer and subsequently to the 540 atmosphere via air-sea gas exchange. Based on earlier studies of N₂O cycling in and around 541 ODZs, is generally thought that nitrification is the primary pathway in the oxygenated surface 542 waters (Cohen & Gordon, 1979; Dore et al., 1998; Nevison et al., 2003), while denitrification is 543 the primary pathway in the anoxic core of the water column (Babbin et al., 2015; Farías et al., 544 2009), and a combination of nitrification with a high-N₂O yield and incomplete denitrification 545 drives high concentrations in the oxycline (Babbin et al., 2015; Farías et al., 2009). Recent work 546 also suggests the importance of ammonia-oxidizing archaea to N2O production in the ETNP 547 (Trimmer et al., 2016). However, high concentrations of N₂O are not uniform across the ETNP, 548 as seen in variability across this and other studies (Babbin et al., 2015; Kelly et al., 2021; 549 Yamagishi et al., 2007). 550

In a global model, Yang et al. (2020) correlated the N₂O seasonality of the Eastern 551 Tropical Pacific with Chl-a and apparent oxygen utilization. In our study, the high-N₂O stations 552 553 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-N₂O stations 554 contained a deep Chl-a maximum. In order to quantify the relationship between [N₂O], [O₂], and 555 Chl-a, near-surface averages were calculated for each property at each station by linearly 556 interpolating values to every 0.05 kg/m³ density interval, taking a trapezoidal sum for $\sigma_{\theta} < 26$ 557 kg/m^3 , and then dividing by the density range the integral was taken over (26 kg/m³ minus 558 559 surface density at each station). Regression analysis revealed strong relationships between

560 average [N₂O] and average [O₂] ($R^2 = 0.90$, p < 0.001) and average [N₂O] and average Chl-*a* ($R^2 = 0.76$, p < 0.001) along the transect (Fig. 5), affirming these two predictors on a local scale.

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Figure 5: Relationships between [N₂O], Chl-a, and [O₂]. Regressions between (a) the average [N₂O] and average Chl-*a* and (b) the average [N₂O] and average [O₂] for $\sigma_{\theta} < 26$ kg/m³ at each station in the transect. Averages were calculated by linearly interpolating values to every 0.05 kg/m³ density interval, taking a trapezoidal sum for $\sigma_{\theta} < 26$ kg/m³, and then dividing by the density range the integral was taken over.

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There are likely both biogeochemical and physical mechanisms contributing to the N₂O 570 accumulation in the near-surface maxima. The shoaling of the oxycline brings the suboxic-571 572 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 573 increased N₂O production. The enhancement of denitrification by particulate organic matter has 574 been observed in ODZs before (Babbin et al., 2014; Chang et al., 2014; Ward et al., 2008), and, 575 in incubations, particulate matter additions have been shown to increase N₂O production via 576 577 denitrification up to 5-fold (Frey et al., 2020). Similarly, nitrification fueled by enhanced organic matter regeneration could contribute to enhanced N₂O production under these conditions. The 578 high Chl-a estimates at high-N₂O stations (Fig. S2b) potentially indicate higher levels of 579 particulate organic matter, facilitating higher levels of N₂O production by either mechanism. It is 580 likely that organic matter and a shallow oxycline fuel the N₂O accumulation at these stations, in 581 conjunction with a relatively strong pycnocline (Fig. S2a) preventing the N₂O from escaping to 582 the surface. The isotopic measurements conducted in this study can be used to constrain the 583 sources of N₂O in this near-surface feature. 584

 N_2O isotopic ratios in samples with $\sigma_{\theta} < 25.8 \text{ kg/m}^3$ were plotted against the inverse of N₂O concentration, 1/[N₂O], to compare near-surface sources at high-N₂O stations to those in the

rest of the transect. Under the framework of a background pool of N_2O to which N_2O is added,

the y-intercepts of regressions performed on such "Keeling plot" analyses (C. D. Keeling, 1961)

yield the isotopic compositions of the N₂O source (Casciotti et al., 2018; Fujii et al., 2013; Ji et al., 2019; Yamagishi et al., 2007). The y-intercepts of δ^{18} O-N₂O, SP, δ^{15} N₂O^{α}, δ^{15} N₂O^{bulk}, and

 $\delta^{15}N_2O^{\beta}$ on 1/[N₂O] for high-N₂O 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‰,

593 6.4‰, and 1.4‰, respectively (Fig. S8). SP was the only tracer that was not significantly

594 different (outside standard error) between the two sets of stations, while intercepts for $\delta^{15}N_2O^{\alpha}$,

595 $\delta^{15}N_2O^{\beta}$, and $\delta^{15}N_2O^{bulk}$ were the most different.

Although SP is thought to reflect the N₂O production processes independently of the 596 isotopic composition of substrates (Schmidt et al., 2004; Sutka et al., 2004; Yoshida & Toyoda, 597 2000), these Keeling analyses show the importance of looking at $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$ along 598 with SP. While SP as a tracer can distinguish between oxidative (nitrification) and reductive 599 (denitrification) N₂O production, the individual variations in $\delta^{15}N_2O^{\alpha}$, $\delta^{15}N_2O^{\beta}$, and $\delta^{15}N_2O^{bulk}$ 600 help further distinguish between substrate pools (NO_{3⁻} vs. NO_{2⁻}) and different reductive 601 processes (denitrification vs. nitrifier-denitrification). With respect to the mix of production 602 processes, the lower $\delta^{15}N_2O$ intercepts at high-N₂O stations could indicate a stronger 603 contribution from either denitrification or nitrifier-denitrification, as the low $\delta^{15}N_2O^{bulk}$ signals 604 are commonly observed during nitrifier-denitrification (Casciotti et al., 2018; Frame & Casciotti, 605 2010; Sutka et al., 2004; Webster & Hopkins, 1996; Yoshida, 1988). 606

The intercepts for high-N₂O stations were remarkably like those determined in 607 Yamagishi et al. (2007), which concluded that nitrification contributed more to N_2O production 608 than denitrification in the oxycline of the ETNP (Yamagishi et al., 2007). More recent work, 609 however, has attributed denitrification as the dominant source of N₂O in ODZs, especially in the 610 oxycline (Frey et al., 2020; Ji et al., 2015, 2018; Kelly et al., 2020). To better constrain the mix 611 of processes that may contribute to the flux of N₂O to the atmosphere, the time-dependent model 612 and optimization procedure described above for the core-ODZ depths were applied to the near-613 614 surface.

For these model experiments, N₂O production and consumption via denitrification were 615 parameterized as they were in the ODZ core. To simulate an area shallower in the water column, 616 nitrification was added as a potential source of N₂O. The isotope systematics for N₂O production 617 from ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were added to 618 the model using values from the literature (Table S2: Frame & Casciotti, 2010: Santoro et al., 619 2011; Sutka et al., 2006). Rather than starting with a pool of N₂O and simulating the decrease in 620 [N₂O] as in the runs for the anoxic core, the opposite was done for this set of experiments. The 621 smallest [N₂O] measurement of the transect and its associated isotopic value within the model 622 domain $\sigma_{\theta} = 23.5 \cdot 25.8 \text{ kg/m}^3$ were used to initialize the model, and the model was optimized to 623 624 match N_2O isotopic values as $[N_2O]$ increased to the maximal value of the transect within the model domain. This density layer was chosen because it contained the highest $[N_2O]$ values at 625 each station, without showing dominant consumption signals. Isotopic values for NO₂⁻ and NO₃⁻ 626 were taken from the density layer at the high-N₂O stations. Because [NO₂⁻] was often too low to 627 measure δ^{15} N and δ^{18} O, two sets of initializations were tested — one using the δ^{15} N and δ^{18} O for 628 NO₂⁻ from the PNM and one using the δ^{15} N and δ^{18} O for NO₂⁻ from the SNM. Neither the PNM 629 630 nor SNM were exactly in the density layer of interest, but these values were used as two potential

- 631 endmembers to evaluate the difference between. In addition, the isotope effects for N₂O
- 632 consumption were varied as described for the ODZ formulation. Unlike the previous
- 633 experiments, k_{cons} was held constant and did not evolve nonlinearly. In addition, the isotope
- 634 effects for N₂O consumption were varied as described for the ODZ formulation. Unlike the
- k_{cons} previous experiments, k_{cons} was held constant and did not evolve nonlinearly. While k_{cons} was
- $_{22}$ constant, the rates (Table 2) still evolved because [N₂O] 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.

639Table 2: Optimized rates (nM N/day) for N2O production and consumption above the ODZ

- $(\sigma_{\theta} = 23.5 25.8 \text{ kg/m}^3)$, using data from all stations. Mean squared error (MSE) is presented as
- 641 an average of that of the four tracers.

		NO2 ⁻ PNM	NO2 ⁻ SNM	$^{15,18}\varepsilon_{cons} \ge 1.5$	$^{15,18}\varepsilon_{cons} \ge 1.5$
				and NO _{2⁻PNM}	and NO _{2⁻SNM}
	Prod. from NO ₂ ⁻	0	0	0	0
	Prod. from NO ₃ -	8.33 x 10 ⁻¹	8.33 x 10 ⁻¹	6.20 x 10 ⁻¹	6.20 x 10 ⁻¹
SP = 0%	Initial Cons.	2.01 x 10 ⁻¹	2.01 x 10 ⁻¹	1.17 x 10 ⁻¹	1.17 x 10 ⁻¹
	Max. Cons.	$2.28 \ge 10^{\circ}$	2.28×10^{0}	$1.32 \ge 10^{0}$	$1.32 \ge 10^{\circ}$
$^{15}\varepsilon_{NOx \rightarrow N2O}(\alpha) =$	Prod. from NH ₄ ⁺ , AOA	5.10 x 10 ⁻¹	5.10 x 10 ⁻¹	3.65 x 10 ⁻¹	3.65 x 10 ⁻¹
$^{15}\varepsilon_{NOx \rightarrow N2O}(\beta) = 22\%$	Prod. from NH4 ⁺ , AOB	0	0	0	0
	MSE	23.74	23.74	22.88	22.88
	Prod. from NO ₂ ⁻	1.93 x 10 ⁻⁶	3.34 x 10 ⁻⁹	1.92 x 10 ⁻⁷	4.31 x 10 ⁻³
	Prod. from NO ₃ ⁻	$5.63 \ge 10^1$	5.39 x 10 ¹	5.63 x 10 ¹	$5.35 \ge 10^1$
SD = 220/	Initial Cons.	$1.25 \ge 10^{\circ}$	$1.28 \ge 10^{\circ}$	$1.25 \ge 10^{\circ}$	1.97 x 10 ⁰
SP = 22700	Max. Cons.	$1.13 \ge 10^2$	$1.13 \ge 10^2$	$1.13 \ge 10^2$	1.13 x 10 ²
15_{22} (a) = 0%	Prod. from NH ₄ ⁺ , AOA	1.74 x 10 ⁻⁹	1.75 x 10 ⁻⁴	1.52 x 10 ⁻³	7.14 x 10 ⁻²
$10 \epsilon_{\text{NOx} \rightarrow \text{N2O}} (\alpha) = 0.000$	Prod. from NH4 ⁺ , AOB	1.64 x 10 ⁻¹²	$2.46 \ge 10^{0}$	1.88 x 10 ⁻⁷	$3.10 \ge 10^{\circ}$
	MSE	42.32	40.69	42.45	40.49
	Prod. from NO ₂ ⁻	8.48 x 10 ⁻⁹	8.48 x 10 ⁻⁹	0	6.24 x 10 ⁻⁴
	Prod. from NO ₃ ⁻	$8.78 \ge 10^{\circ}$	8.78 x 10 ⁰	6.20 x 10 ⁻¹	1.44 x 10 ⁻¹
SD = 220/	Initial Cons.	$1.82 \ge 10^{\circ}$	$1.82 \ge 10^{\circ}$	1.17 x 10 ⁻¹	3.69 x 10 ⁰
SP = 22%00	Max. Cons.	$1.13 \ge 10^2$	$1.13 \ge 10^2$	$1.32 \ge 10^{\circ}$	$4.53 \ge 10^{1}$
15_{010} (B) $-4.40/$	Prod. from NH4 ⁺ , AOA	3.81×10^{1}	$3.81 \ge 10^1$	3.65 x 10 ⁻¹	$1.55 \ge 10^{1}$
$\epsilon_{NOx \rightarrow N2O}(p) = 44\%$	Prod. from NH4 ⁺ , AOB	9.73 x 10 ⁰	$9.73 \ge 10^{\circ}$	0	6.99 x 10 ⁰
	MSE	71.94	71.94	22.88	57.44

642

643 The model was generally able to recreate the Keeling plot for $\sigma_{\theta} = 23.5-25.8 \text{ kg/m}^3$,

644 including the sharp increase in isotopic values at high-N₂O points (Fig. 6). Unlike in the ODZ,

runs with the set of isotope effects for N₂O production via denitrification with SP = 0%

646 performed much better than those with SP = 22% (Table 2). In several instances, the

optimization converged at the same rates and mechanisms of N₂O cycling, regardless of whether

648 NO₂⁻ was initialized with δ^{15} N and δ^{18} O from the PNM or SNM (Table 2). This is largely

because the rates of N₂O production from NO_2^- were negligible in the near-surface maximum,

650 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 653 default initialization with SP = 0%, presented as (a) a time series of $[N_2O]$ and isotopocules and 654 (b) a recreated Keeling plot for $\sigma_{\theta} = 23.5 \cdot 25.8 \text{ kg/m}^3$. The maximum and minimum bounds for 655 the time series in (a) are output using the maximum and minimum $^{15}\varepsilon_{cons}$ and $^{18}\varepsilon_{cons}$ from 656 literature. In (b), model output is plotted with a set of lines, while observations are plotted with 657 color-matched points. While the previous experiment used only data points from core-ODZ 658 stations, this set of runs used data points within the density layer from all stations. Since this set 659 of runs began with a small amount of N₂O, which gradually increased, time progresses on the 660 661 Keeling plot from right to left.

662

The model runs with the best fit each included N₂O production via denitrification from 663 NO₃⁻, N₂O production via nitrification by AOA, and N₂O consumption via denitrification; rates 664 for denitrification from NO₂⁻ and nitrification via AOB were optimized at or near zero in several 665 of the experiments (Table 2). While the experiments in the anoxic core primarily used NO₂⁻ as 666 the substrate for denitrification (Table 1), these runs at the base of the oxycline primarily used 667 NO_3^{-} , which is in line with tracer experiments and natural abundance isotope ratio measurements 668 (Casciotti et al., 2018; Frev et al., 2020; Ji et al., 2015, 2018). Furthermore, this set of 669 experiments provides support for the idea that AOA and not AOB are largely responsible for the 670 ammonia-derived source of N₂O near the surface, as set forth by Santoro et al. (2011) and 671 672 Trimmer et al. (2016).

N₂O 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 N₂O 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, N₂O consumption in this region allowed
- the reconstructed Keeling plot to recreate the sharp increase in isotopic values at high-N₂O points
- 679 (Fig. 6b). Rates of the two most significant sources of N_2O production from NO_3^- by
- denitrifiers and production from NH_4^+ by AOA were the same order of magnitude in the bestfit model runs, with denitrification producing N₂O at a slightly higher rate (Table 2). These
- 681 fit model runs, with denitrification producing N_2O at a slightly higher rate (Table 2). Thes 682 results thus reinforce the importance of incomplete denitrification as a driver of N_2O
- 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 685 considerable variability when isotope effects (SP for production via denitrification or $^{15}\varepsilon_{cons}$ and 686 $^{18}\varepsilon_{cons}$) were varied. In the anoxic core, N₂O production via denitrification with SP = 22‰ was 687 able to better match isotopic observations (Table 1). Here, however, N₂O production via 688 denitrification with SP = 22% worsened the fit of the optimization (Table 2), highlighting the 689 need to constrain these isotope effects for denitrification throughout the water column. These 690 model results suggest a variable SP for microbial denitrification under different environmental 691 conditions or microbial communities, or variable roles of fungal denitrification throughout the 692 693 water column.

694 **4.3 Eddies and the Variability of N₂O Cycling in the ETNP**

The unique features of the high-N₂O stations relative to the rest of the transect are made 695 more interesting by the presence of an anticyclonic eddy at these stations (Fig. S4). Previous 696 surveys linking mesoscale eddies to the N₂O cycle have focused on the ODZ of the Eastern 697 Tropical Southern Pacific (Arévalo-Martínez et al., 2016; Bourbonnais et al., 2015; Cornejo 698 D'Ottone et al., 2016; Frey et al., 2020). There, young eddies are associated with high [N₂O] and 699 high N₂O production rates (Frey et al., 2020), while eddy aging generally leads to decreasing 700 [N₂O] throughout the water column due to the reduced supply of organic matter to fuel 701 denitrification processes (Arévalo-Martínez et al., 2016). The highest N₂O production rates for 702 denitrification ever measured in the ETSP were in an anticyclonic mode water eddy (Frey et al., 703 2020). A recent study of an anticyclonic mode water eddy in the eastern tropical North Atlantic 704 — a region where $[N_2O]$ is typically low relative to ODZs — found N₂O concentrations up to 705 115 nM (Grundle et al., 2017). These studies show that environments created by mesoscale 706 707 eddies are conducive to high N₂O production and enhanced denitrification in the water column.

708 This study represents the first in the ETNP to link N₂O isotopocule measurements to a mesoscale eddy. While relatively high sea surface height anomalies (Fig. S4) and depressed 709 isopycnals in deeper water (Fig. S2a) indicate that the eddy was anticyclonic, several other 710 hydrographic features of the high-N₂O stations are not typically associated with downwelling 711 eddies. For example, the oxycline was shallower relative to the other stations (Section 3.1), and 712 the water column was more stratified (Supp Fig. 2b). Typically, anticyclonic eddies decrease 713 714 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, 715 given that section profiles of similar transects through the ETNP show that the oxycline of 716 waters around 14-16°N historically occurs on lower-density isopycnals relative to the northern 717

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 720 of the transect (Fig. S2a; Fig. 5). Enhanced Chl-a has been previously observed in anticyclonic 721 722 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 723 the density fronts on the eddy periphery, bringing up nutrients to the surface (Callbeck et al., 724 725 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 726 associated with the density front and subsequent mixing within the eddy could thus explain the 727 enhanced productivity at high-N₂O stations. 728

729 Mesoscale eddies and the submesoscale processes within add new complexity to the spatiotemporal variability of the ETNP N₂O cycle. Based on biogeochemical and isotopic 730 measurements, this transect alone contained three different regimes of N_2O cycling (Section 2). 731 732 The influence of eddies on this variability is unknown, but in this study the highest N₂O concentrations were associated with the site of an anticyclonic eddy and its shallow oxycline and 733 high primary productivity. Further study on the connection between different types of eddies and 734 N₂O cycling in the ETNP will be of great importance to modeling the outgassing of N₂O in this 735 region. 736

737 **5 Conclusion**

Three distinct regimes of N₂O cycling were identified along this north-south transect 738 within the ETNP. The patterns of isotopocule distributions at low [N₂O] in the core of the ODZ 739 740 suggested that N₂O was simultaneously being produced and consumed under anoxia. A time-741 dependent model of N₂O isotopocules confirmed that production via denitrification was a significant process and that the continuing evolution of isotopologues past the plateau of $[N_2O]$ is 742 consistent with a longer time scale for isotopic steady state. Denitrification from NO3⁻ and/or 743 NO₂⁻ with a nonzero SP allowed the model to fit the data well, providing evidence that this 744 745 process — either in the form of fungal denitrification or bacterial denitrification with a variable 746 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. 747

Implementation of the time-dependent model in the near-surface $[N_2O]$ maximum led to 748 749 the conclusion that AOA, and not AOB, were the major nitrifying source of N₂O; incomplete denitrification of NO₃⁻ also remained a significant contributor to the high [N₂O]. This 750 accumulation of N₂O coincided with high levels of primary productivity and a shallower 751 oxycline relative to the rest of the transect. A shallower niche for high-yield N₂O processes and 752 greater levels of organic matter could explain the accumulation. Finally, the presence of a 753 mesoscale eddy at the site of the N_2O accumulation adds to the heterogeneity of the cycle in the 754 ETNP. By modeling the rates of nitrification and denitriciation processes and connecting them to 755 hydrographic parameters, this study adds new insight to the cycling of this important greenhouse 756 gas. 757

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767 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
- 775 Copernicus Marine Environmental Monitoring Service (see supplement), and the World Ocean
- Atlas 2018 product was downloaded as an Ocean Data View collection
- 777 (https://odv.awi.de/data/ocean/world-ocean-atlas-2018/).
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Global Biogeochemical Cycles

Supporting Information for

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

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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 N₂O 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 N₂O 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 N₂O isotopic ratios against the natural logarithm of [N₂O] between σ_{θ} 26-27 kg/m³ for all stations, (6) regressions for calculating apparent isotope effects for N₂O consumption at high N₂O stations, (7) an illustration of the rate of change of N₂O concentration and isotopic composition in the ODZ N₂O model, and (8) near-surface N₂O 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 N₂O above the ODZ. Isotopocules of N₂O were tracked through time as five variables (¹⁴N, ¹⁵N^{α}, ¹⁵N^{β}, ¹⁶O, and ¹⁸O) in N₂O modified by five processes: N₂O production from NO₃⁻ via denitrification, N₂O production from NO₃⁻ via

denitrification, N₂O production from NH_4^+ via nitrification by ammonia-oxidizing archaea (AOA), and N₂O production from NH_4^+ via nitrification by ammonia-oxidizing bacteria (AOB). ¹⁴N and ¹⁶O were modeled with the following equations, with new terms shown in bold:

$$[{}^{14}N_2O]_{n+1} = [{}^{14}N_2O]_n + k_{NO3 \to N20} [{}^{14}NO_3]_n + k_{NO2 \to N20} [{}^{14}NO_2]_n - k_{cons} [{}^{14}N_2O]_n + k_{NH4 \to N20,AOA} [{}^{14}NH_4]_n + k_{NH4 \to N20,AOB} [{}^{14}NH_4]_n$$
(S1)

$$[N_{2}^{16}O]_{n+1} = [N_{2}^{16}O]_{n} + \frac{1}{2}k_{NO3 \to N20}\frac{1}{3}[N^{16}O_{3}]_{n} + \frac{1}{2}k_{NO2 \to N20}\frac{1}{2}[N^{16}O_{2}]_{n} - k_{cons}[N_{2}^{16}O]_{n} + \frac{1}{2}k_{NH4 \to N20,AOA}[^{14}NH_{4}]_{n} + \frac{1}{2}k_{NH4 \to N20,AOB}[^{14}NH_{4}]_{n}$$
(S2)

where $[{}^{14}N_2O]_n$ is the concentration of ${}^{14}N_-{}^{14}N_-{}^{16}O$ in micromoles N per liter (µM) at the "nth" timestep and $[N_2{}^{16}O]_n$ is the concentration of ${}^{16}O$ -containing N₂O (predominantly ${}^{14}N_-{}^{16}N_-{}^{16}O$). The ${}^{12}_{2}$ factor in front of terms producing $[N_2{}^{16}O]$ represents the 1:2 ratio of oxygen to nitrogen atoms in N₂O (e.g., for every two nitrogen atoms added to the N₂O pool, one oxygen atom is added). In line with this, the initial $[N_2{}^{16}O]$ was calculated by multiplying the initial $[{}^{14}N_2O]$ by ${}^{12}_{2}$. Note also that ${}^{13}_{3}[N^{16}O_3]_n$ (eqn. S2) is approximately equal to $[{}^{14}NO_3]_n$ (eqn. S1) and ${}^{12}_{2}[N^{16}O_2]_n$ (eqn. S2) is equivalent to $[{}^{14}NO_2]_n$ (eqn. S1). The substrate pools were held constant in these experiments, so $[{}^{14}NO_3]$ was always equal to the initial concentration of NO₃⁻ and $[N^{16}O_3]$ was 3 times that (3:1 ratio of oxygen to nitrogen in NO₃⁻). Similarly, $[{}^{14}NO_2]$ was always equal to the initial concentration of NO₂⁻ and $[N^{16}O_2]$ was 2 times that. The factors of ${}^{12}_{2}$ for NO₂⁻ and ${}^{13}_{3}$ for NO₃⁻ were used to account for the ratio of oxygen to nitrogen in each species of NO_x. k values represent the first-order reaction rate constants for each reaction.

In nitrification, since the oxygen atom in N₂O is derived from dissolved O₂, the model assumed that dissolved oxygen is in excess and reacts in a stoichiometric ratio with NH₄⁺. Thus, [¹⁴NH₄] 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^{light}/k^{heavy}, or $\epsilon/1000 + 1$. ¹⁵N^{α} and ¹⁵N^{β} were modeled separately, as they have different isotope effects:

$$[{}^{15}N_{2}O^{\alpha}]_{n+1} = [{}^{15}N_{2}O^{\alpha}]_{n} + \frac{k_{N03 \to N20}}{\alpha^{15,\alpha}_{N0x \to N20}} [{}^{15}NO_{3}]_{n} + \frac{k_{N02 \to N20}}{\alpha^{15,\alpha}_{N0x \to N20}} [{}^{15}NO_{2}]_{n} - \frac{k_{cons}}{\alpha^{15,\alpha}_{cons}} [{}^{15}N_{2}O^{\alpha}]_{n} + \frac{k_{NH4 \to N20,A0A}}{\alpha^{15,\alpha}_{NH4 \to N20,A0A}} [{}^{15}NH_{4}]_{n} + \frac{k_{NH4 \to N20,A0B}}{\alpha^{15,\alpha}_{NH4 \to N20,A0B}} [{}^{15}NH_{4}]_{n}$$

$$(S3)$$

.

$$\begin{split} [{}^{15}N_{2}O^{\beta}]_{n+1} &= [{}^{15}N_{2}O^{\beta}]_{n} + \frac{k_{N03 \to N20}}{\alpha^{15,\beta}_{N0x \to N20}} [{}^{15}NO_{3}]_{n} + \frac{k_{N02 \to N20}}{\alpha^{15,\beta}_{N0x \to N20}} [{}^{15}NO_{2}]_{n} - \\ &- \frac{k_{cons}}{\alpha^{15,\beta}_{cons}} [{}^{15}N_{2}O^{\beta}]_{n} + \frac{k_{NH4 \to N20,A0A}}{\alpha^{15,\beta}_{NH4 \to N20,A0A}} [{}^{15}NH_{4}]_{n} + \frac{k_{NH4 \to N20,A0B}}{\alpha^{15,\beta}_{NH4 \to N20,A0B}} [{}^{15}NH_{4}]_{n} \end{split}$$

$$(S4)$$

where $[{}^{15}N_2O^{\alpha}]$ and $[{}^{15}N_2O^{\beta}]$ represent concentrations of ${}^{14}N_{-}{}^{15}N_{-}{}^{16}O$ and ${}^{15}N_{-}{}^{14}N_{-}{}^{16}O$, respectively. Initial values were calculated using the initialized $\delta^{15}N_2O^{\alpha}$ and $\delta^{15}N_2O^{\beta}$ values. In the same way, ${}^{15}N$ concentrations of substrates were also calculated from their respective initialized isotopic ratios. The equation for ${}^{18}O$ includes branching isotope effects (Casciotti et al., 2007) in addition to kinetic isotope effects:

$$\begin{split} [N_{2}^{18}O]_{n+1} &= [N_{2}^{18}O]_{n} + \frac{1}{2} \frac{k_{NO3 \to N20} \alpha^{18}_{NO3 \to NO2, branching} \alpha^{18}_{NO2 \to N20, branching}}{\alpha^{18}_{NO2 \to N20, kinetic}} \frac{1}{3} [N^{18}O_{3}]_{n} \\ &+ \frac{1}{2} \frac{k_{NO2 \to N20} \alpha^{18}_{NO2 \to N20, branching}}{\alpha^{18}_{NO2 \to N20, branching}} \frac{1}{2} [N^{18}O_{2}]_{n} - \frac{k_{cons}}{\alpha^{18}_{cons}} [N_{2}^{18}O]_{n} \\ &+ \frac{1}{2} \frac{k_{NH4 \to N20, AOA}}{\alpha^{18}_{NH4 \to N20, AOA}} [^{18}O_{NH4 \to N20}]_{n} + \frac{1}{2} \frac{k_{NH4 \to N20, AOB}}{\alpha^{18}_{NH4 \to N20, AOB}} [^{18}O_{NH4 \to N20}]_{n} \end{split}$$

$$(S5)$$

where $[N_2^{18}O]_n$ is the concentration of ¹⁸O in ¹⁴N-¹⁴N-¹⁸O, and initial ¹⁸O concentrations of substrates were calculated with initialized δ^{18} O values. The ¹⁸O 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 ¹⁶O, 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, (δ (‰) = 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_2O 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.

Variable	Value	Source
$^{15}\varepsilon_{NOx \to N2O}(\alpha)$	22‰	Toyoda et al., 2005
¹⁵ $\varepsilon_{NOx \to N2O}$ (β)	22‰	Toyoda et al., 2005
$^{15}\varepsilon_{cons}(\alpha)$	10.5‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
$^{15}\varepsilon_{cons}(\beta)$	0‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
¹⁸ €NO3→NO2,	24‰	Casciotti and McIlvin, 2007
branching		
$^{18}\varepsilon_{NO2\rightarrow N2O}$,	12‰	Casciotti and McIlvin, 2007
branching		
¹⁸ €NO2→N2O,	-2‰	Martin and Casciotti, 2016
kinetic		
¹⁸ ε _{cons} , kinetic	16‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
$[N_2O]_i$	39.05 nM	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, background stations)
$\delta^{15} N_2 O^{lpha}{}_i$	19.73‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, background stations)
$\delta^{15} N_2 O^{\beta}{}_i$	4.29‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, background stations)
$\delta^{I8} O$ - $N_2 O_i$	59.48‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, background stations)
$[NO_3]$	25.50 μM	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$\delta^{15}NO_3^-$	18.09‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$\delta^{18}O$ -NO $_3$ -	16.55‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$[NO_2^-]$	1.023 μM	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$\delta^{15}NO_2^-$	-20.42‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$\delta^{18}O$ -NO ₂ -	15.52‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, core-ODZ stations)
$k_{NO3 \rightarrow N2O}$	5.25 x 10 ⁻⁵ day ⁻¹	Kelly et al., 2021 (used as starting point of optimization)
$k_{NO2 \rightarrow N2O}$	3.962 x 10 ⁻⁶ day ⁻¹	Kelly et al., 2021 (used as starting point of optimization)
k _{cons}	2.1 day ⁻¹	Babbin et al., 2015; Kelly et al., 2021 (used as starting point of optimization)

Variable	Value	Source
$^{15}\varepsilon_{NOx \to N2O}(\alpha)$	22‰	Toyoda et al., 2005
$^{15}\varepsilon_{NOx \to N2O}(\beta)$	22‰	Toyoda et al., 2005
$^{15}\varepsilon_{cons}(\alpha)$	10.5‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
$^{15}\varepsilon_{cons}(\beta)$	0‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
$^{18}\varepsilon_{NO3\rightarrow NO2}$,	24‰	Casciotti and McIlvin, 2007
branching		
$^{18}\varepsilon_{NO2\rightarrow N2O}$	12‰	Casciotti and McIlvin, 2007
branching		
$^{18}\varepsilon_{NO2\rightarrow N2O}$,	-2‰	Martin and Casciotti, 2016
kinetic		
¹⁸ ε _{cons} , kinetic	16‰	This study ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$, high-N ₂ O stations)
$^{15}\varepsilon_{NH4\rightarrow N2O}(\alpha),$	-21.3‰	Santoro et al., 2011(adjusted based on $\delta^{15}NH_4^+$ value)
AOA		
$^{15}\varepsilon_{NH4\rightarrow N2O}(\beta),$	9‰	Santoro et al., 2011 (adjusted based on $\delta^{15}NH_4^+$ value)
AOA		
¹⁸ Е _{NH4→N2O} ,	-10.5‰	Santoro et al., 2011 (adjusted based on $\delta^{18}O_{O2}$ value)
AOA		
$^{15}\varepsilon_{NH4\rightarrow N2O}(\alpha),$	62.5‰	Sutka et al., 2006
AOB		
$^{15}\varepsilon_{NH4\rightarrow N2O}(\beta),$	31.5‰	Sutka et al., 2006
AOB		
¹⁸ €NH4→N2O ,	3‰	Frame and Casciotti, 2010
AOB		
$[N_2O]_i$	11.14 nM	This study (minimum [N ₂ O] data point in $\sigma_{\theta} = 23.5-25.8 \text{ kg/m}^3$)
$\delta^{15} N_2 O^{lpha}{}_i$	15.77‰	This study (minimum [N ₂ O] data point in $\sigma_{\theta} = 23.5-25.8 \text{ kg/m}^3$)
$\delta^{15} N_2 O^{\beta}{}_i$	-1.42‰	This study (minimum [N ₂ O] data point in $\sigma_{\theta} = 23.5-25.8 \text{ kg/m}^3$)
$\delta^{I8} O$ - $N_2 O_i$	45.45‰	This study (minimum [N ₂ O] data point in $\sigma_{\theta} = 23.5-25.8 \text{ kg/m}^3$)
[NO3 ⁻]	22.27 μM	This study ($\sigma_{\theta} = 23.5 - 25.8 \text{ kg/m}^3$, high-N ₂ O stations)
$\delta^{15}NO_3^-$	9.91‰	This study ($\sigma_{\theta} = 23.5 - 25.8 \text{ kg/m}^3$, high-N ₂ O stations)
$\delta^{18}O$ -NO $_3$ -	8.57‰	This study ($\sigma_{\theta} = 23.5 - 25.8 \text{ kg/m}^3$, high-N ₂ O stations)
$[NO_2]$	0.286 μM	This study ($\sigma_{\theta} = 23.5 - 25.8 \text{ kg/m}^3$, high-N ₂ O stations)
$\delta^{15} NO_{2^{-}PNM}$	1.58‰	This study (PNM of Stn. 8)
$\delta^{l8}O extsf{-}NO_2 extsf{-}_{PNM}$	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 μM	Unpublished data from transect ($\sigma_{\theta} = 23.5 - 25.8 \text{ kg/m}^3$, high-N ₂ O stations)
$\delta^{15}NH_4{}^+$	7‰	arbitrary value
$\delta^{18}O_{ m NH4 ightarrow m N2O}$	23.5‰	average δ^{18} O of dissolved oxygen in seawater, which is incorporated into
		N ₂ O during nitrification.
$k_{NO3 \rightarrow N2O}$	3.9 x 10 ⁻⁶ day ⁻¹	starting point that led to best optimization in sensitivity tests
$k_{NO2 \rightarrow N2O}$	1.1 x 10 ⁻³ day ⁻¹	starting point that led to best optimization in sensitivity tests
k _{cons}	2.7 x 10 ⁻¹ day ⁻	starting point that led to best optimization in sensitivity tests
$k_{NH4 \rightarrow N2O}, AOA$	1.4 x 10 ⁻¹ day ⁻	starting point that led to best optimization in sensitivity tests
$k_{NH4 \rightarrow N2O}$, AOB	4.7 x 10 ⁻⁵ day ⁻	starting point that led to best optimization in sensitivity tests

Table S2. Initialization conditions for the time-step model of N_2O isotopocules above the ODZ (denitrification and nitrification processes), discussed in Section 2.3 and Section 4.2. These are input into equations S1-S5.

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 µmol/kg. Average σ_{θ} minimum represents the mean of each station's surface density in a particular regime.

Regime	Stations	General Summary	ODZ Upper Boundary	Average σ _θ Minimum	Average ODZ [N2O] Minimum	Average Above-ODZ [N2O] Maximum
Back- ground	1-3	Closest to Baja Peninsula; no dramatic [N ₂ O], [NO ₂ ⁻], $\delta^{15}N_2O^{\alpha}$, $\delta^{18}O$ -N ₂ O, and SP profiles relative to other stations.	124.7 m (25.59 kg/m ³)	23.01 kg/m ³	23.34 nM	44.55 nM
Core ODZ	4-7&12	Most pronounced [N ₂ O] minima and secondary nitrite maxima in ODZ; high $\delta^{15}N_2O^{\alpha}$, $\delta^{18}O-N_2O$, and SP in ODZ; low $\delta^{15}N_2O^{\beta}$ in ODZ; eddy at Stn. 7	98 m (25.24 kg/m ³)	21.88 kg/m ³	3.54 nM	69.26 nM
High N ₂ O	8-11	Some ODZ properties similar to core-ODZ stations; highest near- surface [N ₂ O] and Chl- a of the transect; strongest primary nitrite maxima; shallowest oxycline and nitracline relative to other stations; mesoscale eddy	72 m (24.59 kg/m ³)	20.88 kg/m ³	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-N₂O 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 (σ_{θ}) 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-N₂O stations. (b) Section profile of Chl-*a* (calculated from CTD fluorometer) plotted against σ_{θ} , showing the highest surface Chl-*a* at high-N₂O stations.



Figure S3. NO₂⁻ (a, b) and NO₃⁻ (c, d) isotopologues plotted against σ_{θ} . If [NO₂⁻] is too low (< 0.5 umol/L), isotopic measurements cannot be made, which is why the points are not connected in the profiles for (a) δ^{15} NO₂⁻ and (b) δ^{18} O-NO₂⁻. 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 (<u>http://marine.copernicus.eu/</u>) 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 N₂O isotopic ratios against the natural logarithm of [N₂O] (where [N₂O] is given in nM) within $\sigma_{\theta} = 26-27 \text{ kg/m}^3$ across all stations. Using a segmented regression model (Muggeo, 2003, 2008), a breakpoint was detected for δ^{18} O-N₂O, δ^{15} N₂O^{α}, and SP at ln([N₂O]) = 1.56\pm0.11, 1.56±0.07, and 1.50±0.16, respectively. What is more, most of the points after the breakpoint were from core-ODZ stations. These could possibly represent N₂O production co-occurring with consumption. Adjusted R² 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 N₂O is consumed ([N₂O] decreases).



Figure S6. Regressions for calculating the apparent isotope effects (ϵ) for N₂O consumption for (a) $\delta^{15}N_2O^{\alpha}$, (b) $\delta^{18}O$ -N₂O, and (c) $\delta^{15}N_2O^{\beta}$. Data from $\sigma_{\theta} = 26-27$ kg/m³ at high-N₂O 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-N₂O were chosen to calculate the isotope effects for N₂O 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 N₂O remaining, and was hence calculated as [N₂O]_{observed} divided by the maximum N₂O 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 $\delta^{15}N_2O^{\beta}$ because the regression slope was insignificant.



Figure S7. An example of the rate of change of N₂O concentration and isotopic composition in the ODZ model ($\sigma_{\theta} = 26-27 \text{ kg/m}^3$) 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 [N₂O] was closest to the lowest measured [N₂O] 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, [N₂O] is approaching equilibrium (low rates of change) while the isotopocule measurements continue to evolve at high rates.



Figure S8. Keeling plot for N₂O samples above the ODZ ($\sigma_{\theta} < 25.8 \text{ kg/m}^3$) at (a) background and core-ODZ stations and (b) high-N₂O stations. Each regression was statistically significant. A box representing the atmospheric source of N₂O was also plotted, using values from Mohn et al., 2014.

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