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Marine N₂O cycling from high spatial resolution concentration, stable isotopic and isotopomer measurements along a meridional transect in the eastern Pacific Ocean

Annie Bourbonnais[1]*, Bonnie X. Chang[2], Rolf E. Sonnerup[2], Scott C. Doney[3] and Mark A. Altabet[4]

[1]School of the Earth, Ocean and Environment, University of South Carolina, Columbia, SC, United States, [2]Cooperative Institute for Climate, Ocean, and Ecosystem Studies, University of Washington, Seattle, WA, United States, [3]Department of Environmental Sciences, University of Virginia, Charlottesville, VA, United States, [4]School for Marine Science and Technology, University of Massachusetts, Dartmouth, MA, United States

Nitrous oxide (N₂O) is a potent greenhouse gas and ozone depleting substance, with the ocean accounting for about one third of global emissions. In marine environments, a significant amount of N₂O is produced by biological processes in Oxygen Deficient Zones (ODZs). While recent technological advances are making surface N₂O concentration more available, high temporal and spatial resolution water-column N₂O concentration data are relatively scarce, limiting global N₂O ocean models' predictive capability. We present a N₂O concentration, stable isotopic composition and isotopomer dataset of unprecedently large spatial coverage and depth resolution in the broader Pacific, crossing both the eastern tropical South and North Pacific Ocean ODZs collected as part of the GO-SHIP P18 repeat hydrography program in 2016/2017. We complement these data with dissolved gases (nitrogen, oxygen, argon) and nitrate isotope data to investigate the pathways controlling N₂O production in relation to apparent oxygen utilization and fixed nitrogen loss. N₂O yield significantly increased under low oxygen conditions near the ODZs. Keeling plot analysis revealed different N₂O sources above the ODZs under different oxygen regimes. Our stable isotopic data and relationships between the N₂O added by microbial processes (ΔN₂O) and dissolved inorganic nitrogen (DIN) deficit confirm increased N₂O production by denitrification under low oxygen conditions near the oxycline where the largest N₂O accumulations were observed. The slope for δ^{18}O-N₂O versus site preference (SP, the difference between the central (α) and outer (β) N atoms in the linear N₂O molecule) in the eastern tropical North Pacific ODZ was lower than expected for pure N₂O reduction, likely because of the observed decrease in $\delta^{15}N^\beta$. This trend is consistent with prior ODZ studies and attributed to concurrent production of N₂O from nitrite with a low δ^{15}N or denitrification with a SP >0‰. We estimated

apparent isotope effects for N_2O consumption in the ETNP ODZ of 3.6‰ for $^{15}N^{bulk}$, 9.4‰ for $^{15}N^{\alpha}$, -2.3‰ for $^{15}N^{\beta}$, 12.0‰ for ^{18}O, and 11.7‰ for SP. These values were generally within ranges previously reported for previous laboratory and field experiments.

1 Introduction

Nitrous oxide (N_2O) is mainly produced by microbial processes and contributes to climate change as a tropospheric greenhouse gas approximately 275 times more potent than CO_2 on a per molecule basis (Forster et al., 2021). N_2O produced at the Earth's surface has a long atmospheric residence time of more than 100 years and is the main source of ozone-depleting nitric-oxide radicals in the stratosphere (Nevison and Holland, 1997; Ravishankara et al., 2009). Biogeochemical models estimate that the ocean accounts for about one third of global N_2O emissions (Freing et al., 2012; Ciais et al., 2013; Tian et al., 2020; Forster et al., 2021) with a global oceanic flux estimate of 4.2 ± 1.0 Tg N y^{-1} (Yang et al., 2020). Coastal upwelling systems associated with Oxygen Deficient Zones (ODZs) account for up to 35% of the oceanic N_2O total emissions (Bakker et al., 2014; Arévalo-Martínez et al., 2015; Yang et al., 2020), yet temporal variability from these productive coastal waters is still not well quantified.

N_2O is produced in oxic ocean waters as a by-product of nitrification through the oxidation of hydroxylamine (NH_2OH), an intermediate during ammonia (NH_3) oxidation to nitrite (NO_2^-) by both archaeal and bacterial nitrifiers (Caranto and Lancaster, 2017) (Figure 1). Under low oxygen (O_2)-conditions, ammonia oxidizing bacteria (AOB) produce N_2O by nitrifier-denitrification, the sequential NO_2^- reduction to N_2O during respiration (Goreau et al., 1980; Wrage et al., 2001; Frame and Casciotti, 2010). N_2O is also produced by ammonia oxidizing archaea (AOA) following a hybrid pathway where one atom in the N_2O molecule is derived from NH_3 (e.g., NH_2OH) and the other from NO_2^- (Trimmer et al., 2016). N_2O production by nitrification leads to strong positive correlations between Apparent Oxygen Utilization (AOU) and ΔN_2O (i.e., the difference between N_2O measured and at atmospheric equilibrium) and nitrate (NO_3^-) concentrations (Yoshinari and Knowles, 1976; Cohen and Gordon, 1979; Nevison et al., 2003). N_2O production yield per NO_2^- generated during nitrification by either AOA or AOB is generally low, varying from 0 to 2% of NO_3^- production (Yoshida et al., 1989; Frame and Casciotti, 2010; Santoro et al., 2010; Santoro et al., 2011; Löscher et al., 2012; Ryabenko et al., 2012) but is generally higher for AOB (Hink et al., 2017a; Hink et al., 2017b) and enhanced under low-O_2 conditions according to both culture and field observations (up to 10% at low O_2; Goreau et al., 1980; Ji et al., 2015).

Under anoxic conditions, denitrification, the sequential reduction of NO_3^-, NO_2^-, and NO to N_2O, both produces and consumes N_2O. Enhanced N_2O production occurs under low-O_2 conditions (generally below 10 μmol kg^{-1}; Frey et al., 2020) due to different O_2 sensitivities for the different steps involved in denitrification, with nitrous oxide reductase being inhibited by even nanomolar O_2 concentrations (Dalsgaard et al., 2014). N_2O produced by incomplete denitrification significantly accumulates in low-O_2 conditions near the upper oxycline in the eastern tropical North Pacific (ETNP) and eastern tropical South Pacific (ETSP) ODZs (e.g., Ji et al., 2015; Kock et al., 2016; Bourbonnais et al., 2017; Casciotti et al., 2018; Frey et al., 2020; Kelly et al., 2021; Monreal et al., 2022). High N_2O accumulations of up to 1 μmol L^{-1} were observed in surface waters off the Peru coast, where anoxic waters from the ETSP ODZ are continuously upwelled (Arévalo-Martínez et al., 2015). N_2O accumulation is caused by a decoupling between N_2O production and its reduction to N_2 by denitrification, the latter process being less O_2 tolerant (Dalsgaard et al., 2014). N_2O is generally completely reduced to non-bioavailable N_2 in anoxic waters. ODZs are thus generally net N_2O sinks, with concentrations near or below atmospheric equilibrium concentrations (Bange et al., 2001; Yamagishi et al., 2007; Kock et al., 2016).

Natural stable isotopes are broadly used as tracers of N-cycle processes in the ocean which integrate over space and time (e.g., Sigman et al., 2005; Altabet, 2006; Bourbonnais et al., 2009;

FIGURE 1

N_2O production and consumption processes. N_2O is produced by hydroxylamine oxidation (1, green) or nitrifier-denitrification (2, purple) during ammonia oxidation and denitrification (3, light blue) by bacteria and archaea. Modified from Bourbonnais et al. (2021).
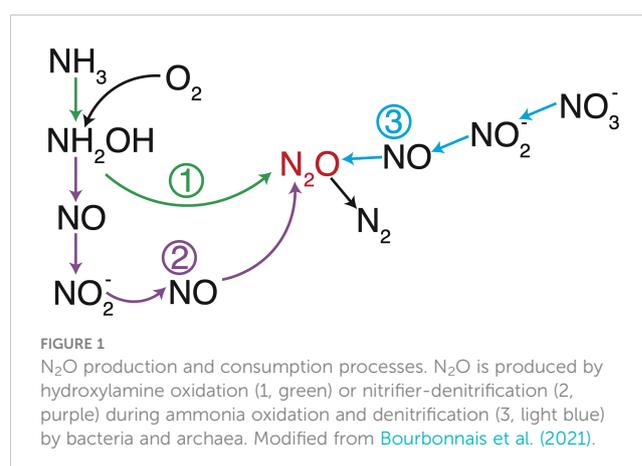
Bourbonnais et al., 2015). The N_2O molecule contains both bulk ($\delta^{15}N$ and $\delta^{18}O$) and, given its asymmetric arrangement of atoms, ^{15}N site specific signatures that are valuable for identifying production and consumption processes. Bulk $\delta^{15}N$ and $\delta^{18}O$ are expressed as:

$$\delta^{15}N \text{ or } \delta^{18}O = ((R_{sample}/R_{reference} - 1) \times 1000 \qquad \text{(eq. 1)}$$

Units are in parts per thousand or per mil (‰) and R is the ratio of $^{15}N/^{14}N$ or $^{18}O/^{16}O$. Reference materials are atmospheric N_2 for N (scale AIR-N_2) and mean ocean water for O (scale Vienna Standard Mean Ocean Water, V-SMOW). The bulk isotopic composition ($\delta^{15}N$ and $\delta^{18}O$) of N_2O depends in part on the isotopic composition of its substrates. For instance, for hydroxylamine oxidation, bulk N_2O $\delta^{15}N$ and $\delta^{18}O$ depend on the $\delta^{15}N$ of the source NH_3 and $\delta^{18}O$ of dissolved O_2. Conversely for nitrifier-denitrification and denitrification, N_2O $\delta^{15}N$ and $\delta^{18}O$ is dependent on the $\delta^{15}N$ and $\delta^{18}O$ of source NO_3^- and/or NO_2^- (Ostrom and Ostrom, 2012). Additionally, significant O exchange usually occurs between NO_2^- and H_2O during N_2O production by nitrifier-denitrification or denitrification (Buchwald and Casciotti, 2010; Kool et al., 2011), which acts to decouple the $\delta^{18}O$ values of source and product.

Isotopic fractionation during nitrification and denitrification is the other major influence on the $\delta^{15}N$ and $\delta^{18}O$ of N_2O. Kinetic isotope fractionation occurs as the molecules containing the lighter isotopes (e.g., ^{14}N, ^{16}O) react more quickly leaving the residual substrate enriched in heavier isotopes (e.g., ^{15}N and ^{18}O). The isotope effect (ε) is defined by:

$$\varepsilon(‰) = ((k_2/k_1) - 1) \times 1000 \qquad \text{(eq. 2)}$$

where k_1 and k_2 are the specific reaction rates for the lighter and heavier isotope, respectively. N and O isotope effects ($^{15}\varepsilon$, $^{18}\varepsilon$) during N_2O production and consumption vary substantially in laboratory culture as well as in the environment (Bourbonnais et al., 2017 and references therein).

In contrast to bulk isotope values, N_2O Site Preference (SP) is independent of initial isotopic composition of the substrate (Toyoda et al., 2002; Schmidt et al., 2004; Sutka et al., 2006). Thus, SP is generally process-dependent and can be used as a tracer to identify the source of N_2O. SP is calculated from the difference in $\delta^{15}N$ between the central (α) and outer (β) N atoms in the linear, asymmetrical N_2O molecule (NNO):

$$SP = \delta^{15}N^\alpha - \delta^{15}N^\beta \qquad \text{(eq. 3)}$$

Non-zero SP arises from the differential biochemical bond making and breaking experienced by each of the two N atoms as a consequence of their different molecular positions. Low SP isotopic signatures (-11 to 0‰) are associated with N_2O production via NO_2^- reduction by nitrifier-denitrification or denitrification. Much higher SP values are indicative of abiotic N_2O formation (Heil et al., 2014) or production by hydroxylamine oxidation (30-36‰) (Sutka et al., 2006; Frame and Casciotti, 2010). N_2O isotopic signatures have consequently been classified into distinct compositional fields used to evaluate the relative contribution from different processes (e.g., Wankel et al., 2017

and reference therein). However, SP does increase as a result of isotope fractionation during consumption by denitrification as discussed above (Yamagishi et al., 2005; Ostrom et al., 2007; Yamagishi et al., 2007).

The main objectives of this study are to better understand nitrous oxide (N_2O) distribution and production mechanisms along the meridional P18 transect in the eastern Pacific sampled as part of the U.S. GO-SHIP program (Figure 2). We sampled at an unprecedently high spatial resolution to investigate how N_2O dynamics respond to changes in O_2 concentrations and dissolved inorganic nitrogen (DIN) loss. We then evaluated pathways responsible for N_2O production in sub-oxic waters ($O_2 > 5$ μmol kg^{-1}) overlying the ETNP and ETSP ODZs as well as deep waters using stable isotopes and isotopomers and Keeling plot analysis. We also investigated N_2O cycling within the ETNP ODZ and estimated apparent isotope effects for N_2O consumption.

2 Methods

The U.S. GO-SHIP Program conducted a hydrographic survey along the P18 section in 2016/2017 (Figure 2). Core physical and biogeochemical data from the cruise (e.g., temperature and salinity, O_2 and nutrient concentrations, transient tracers, radiocarbon) are publicly available at https://cchdo.ucsd.edu/cruise/33RO20161119. Samples for N_2/Ar were collected every ~2 degrees latitude during leg 1 at 25 stations in total. N_2O stable isotope and isotopomer samples were collected at 44 stations, every ~2 degrees latitude during leg 1 and every 3 to 4 degrees during leg 2, except between 3°
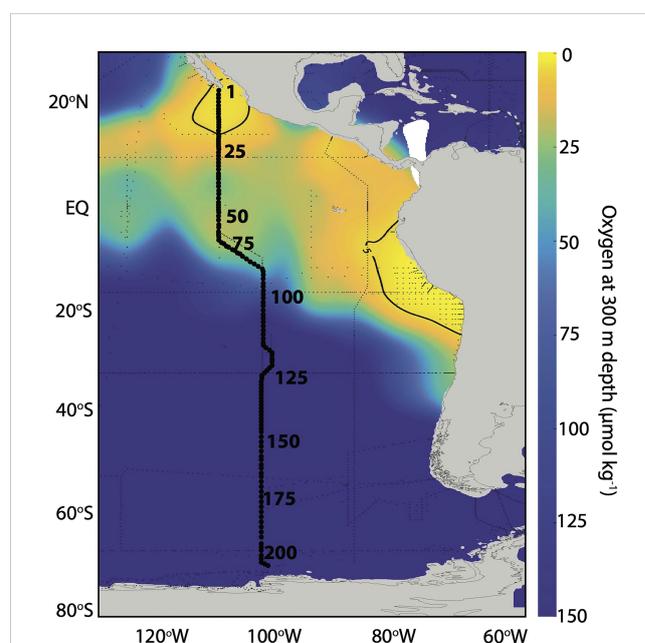
FIGURE 2
Stations sampled as part of the U.S. GO-SHIP Program along the P18 section in 2016/2017. Representative station numbers (every 25 stations) are shown along the transect. Colors indicate O_2 concentration at 300 m depth from the Global Ocean Data Analysis Project (GLODAPv2.2022). The 5 μmol kg^{-1} O_2 contour is shown.

N and 3°S where the resolution was increased to ~1 degree. Samples for $\delta^{15}N$ of NO_3^- analysis were collected during leg 1 at every degree of latitude, but only analyzed at station 32 in the ETNP and stations 53, 55, 62, 68, 74, 78, 90, 92, 96, 100, 104, 108 and 112 near the equator and the ETNP. We usually collected water column profiles from the surface to 2000 m depth, with deeper profiles at 3 stations.

2.1 N₂/Ar

N₂/Ar and $\delta^{15}N_2$ samples were collected and preserved as in Charoenpong et al. (2014) and analyzed at the University of Massachusetts Dartmouth (UMass Dartmouth). In total, approximately 600 samples were collected in 60 mL serum bottles. Water samples were pumped, at 5 to 10 ml min⁻¹, through a continuous sparger which transfers dissolved gases quantitatively to a continuous flow of helium (He) carrier gas. Analysis time was about 10 minutes. Carrier gas was passed through water, carbon dioxide (CO₂), and software selectable hot-copper O₂ traps before admittance *via* an open split to an isotope-ratio mass spectrometer (IRMS). The GV IsoPrime IRMS was fitted with collectors for simultaneous measurement of N₂ (masses 28 and 29), O₂ (masses 32, 33, and 34), and Ar (mass 40). Gas and isotopic ratios were measured against artificial compressed gas mixtures of N₂, O₂, and Ar close to expected dissolved gas ratios. Reproducibility of N₂/Ar measurements were better than 0.5‰. Daily calibration against an atmosphere reference (water equilibrated with air) was carried out at precisely controlled temperatures of 10.0 and 20.0°C. Excess (biogenic) N₂ was calculated against equilibrium values expected from *in situ* temperature and salinity as in Charoenpong et al. (2014). Subtraction of the background N₂ excess was done as in Bourbonnais et al. (2015) using N₂/Ar values in source waters near the equator.

2.2 N₂O concentrations, stable isotopes and isotopomers

Samples for dissolved N₂O were collected in a similar fashion as for dissolved O₂/N₂/Ar samples. Tygon tubing was attached to the Niskin bottle and a 165 mL serum glass bottle was filled and overflowed with seawater at least 2 times before capping with a butyl stopper and crimp sealed with aluminum. This procedure was executed underwater in a plastic container to avoid air bubbles. After collection, 0.2 mL of a saturated HgCl₂ solution was injected to prevent biological activity. In total, approximately 800 samples were collected.

Samples were analyzed at UMass Dartmouth using a GV IsoPrime Continuous Flow, MultiCollector, IRMS (CF-MC-IRMS) coupled to an automated gas extraction as described in Bourbonnais et al. (2017). The collector configuration included masses 30, 31 for the NO⁺ fragment of N₂O (used for the determination of $\delta^{15}N^\alpha$) and 44, 45, and 46 (used for the determination of $\delta^{15}N^{bulk}$ and $\delta^{18}O$). Briefly, dissolved N₂O was pumped through a gas extractor, completely extracted through bubbling with a continuous flow of He and concentrated and purified in a purge-trap system. CO₂ and H₂O vapor were removed using chemical and cryogenic traps. N₂O was cryo-focused with two liquid N₂ traps and passed through a capillary gas chromatography (GC) column prior to IRMS analysis. Results were reproducible even at low N₂O concentration (down to ~5 nmol L⁻¹). N₂O concentrations in our samples were calculated from relative peak heights between the samples and a seawater standard of known N₂O concentration equilibrated with seawater at 5°C (12.5 nmol L⁻¹ at salinity 34 as calculated using the Weiss and Price (1980) equation). Equilibrium N₂O concentrations at depth were calculated using historical reconstructions of atmospheric N₂O and water mass age distributions estimated from chlorofluorocarbon and sulfur hexafluoride tracers as described in section 2.4.

The N₂O concentrations measured with our IRMS agreed well with those measured independently using gas chromatography and an electron capture detector (ECD) at sea during the same research expedition (data available at https://cchdo.ucsd.edu/cruise/33RO20161119).

The reproducibility of $\delta^{15}N^{bulk}$, the average of $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$, $\delta^{18}O$ and SP as well as any instrumental drift were determined from measurements of the 5°C seawater standard distributed throughout an analytical run. We calibrated measurements and corrected for scrambling between the α and β positions (Westley et al., 2007) using four N₂O standards covering a large range of SP (as well as $\delta^{15}N^{bulk}$ and $\delta^{18}O$) composition (see Mohn et al., 2014). These standards were analyzed in duplicate for each run to quantify the scrambling effect and potential offsets, and we iteratively solved for the different calibration parameters as described in Frame and Casciotti (2010) and Mohn et al. (2014). Correction for isobaric interference from ¹⁷O was included in these procedures. Standard deviations for triplicate measurements of our N₂O standards were typically below 0.1‰ for $\delta^{15}N^{bulk}$ N₂O, 0.1‰ for $\delta^{18}O$-N₂O and 1.0‰ for SP, which were comparable to values reported by Mohn et al. (2014).

2.3 Nitrate isotopes

The $\delta^{15}N$ of NO_3^- was measured using the Ti (III) reduction method for nitrate conversion to N₂O (Altabet et al., 2019). The product N₂O was purified and analyzed at UMass Dartmouth using a GV Isoprime continuous flow IRMS equipped with a custom purge trap extraction system and a PAL autosampler. Target sample size was 10 nmoles. Samples were standardized using a three-point correction with the international standards IAEA-N3 ($\delta^{15}N = 4.7$‰ *vs* AIR), USGS-34 ($\delta^{15}N = -1.8$‰ *vs* AIR) and USGS-35 (2.7‰ *vs* AIR). The isotope ratios are expressed in δ (‰) relative to AIR for N. The average standard deviation for duplicate samples was better than ± 0.3‰ for $\delta^{15}N$.

2.4 Calculation of N₂O at equilibrium using transit time distributions

In the same water parcel as N₂O, three transient tracers [chlorofluorocarbon (CFC)-11, CFC-12, and sulfur hexafluoride (SF₆)] were measured, each of which are affected by mixing

differently due to their differing atmospheric histories. This concurrence provides a means of estimating, from multiple tracer ages, the impact of mixing on the ages. To estimate mean ventilation timescales, age distributions, otherwise known as transit-time distributions (TTDs), were approximated using the inverse Gaussian form:

$$G(t) = \sqrt{\frac{\Gamma^3}{4\pi\Delta^2 t^3}} \exp\left[-\frac{\Gamma(t-\Gamma)^2}{4\Delta^2 t}\right] \qquad \text{(eq. 4)}$$

where Γ is the mean age and Δ is the width, or standard deviation, of the distribution (Waugh et al., 2003).

Each water sample's measured CFC-11, CFC-12, and SF_6 were matched to a TTD lookup table (Stanley et al., 2012; Sonnerup et al., 2015) that allowed for Δ/Γ to range, in 0.1 increments, from 0.3 up to 1.8, the range that can be constrained by those three tracers (Stöven et al., 2015), and included each TTD's atmospheric N_2O, below. The matching criterion was within $\pm$ 3 σ of the combined tracer measurement and tracer saturation level uncertainties. The saturation levels dominate the uncertainty. Saturation levels of CFC-11, CFC-12, and SF_6 were estimated from the outcropping region of the respective isopycnals.

2.4.1 N_2O atmospheric history

The N_2O atmospheric history post-1800 was estimated using the polynomial fit presented in Freing et al. (2009), updated to the present using annual global mean N_2O atmospheric mixing ratios from the NOAA Earth System Research Laboratory (ESRL) Global Monitoring Division (https://www.esrl.noaa.gov/gmd/hats/combined/N2O.html). Pre-1800, a constant N_2O mixing ratio of 274.81 ppb was used.

2.4.2 Using TTDs to constrain biogenic N_2O

The total N_2O in a water parcel (N_2O_{obs}) is the sum of N_2O from two different sources: 1) equilibrated from the atmosphere at the time it was last at the surface (N_2O_{bkg}) and 2) added from microbial processes (ΔN_2O or N_2O_{prod}):

$$\Delta N_2O = [N_2O]_{prod} = [N_2O]_{obs} - [N_2O]_{bkg} \qquad \text{(eq. 5)}$$

$$[N_2O]_{bkg} = X_{N2O} \times P \times F_{T,S} \qquad \text{(eq. 6)}$$

where X_{N2O} is the atmospheric mixing ratio of N_2O, P is the atmospheric pressure, and $F_{T,S}$ is the temperature and salinity dependent solubility of N_2O in seawater (Weiss and Price, 1980). The N_2O mixing ratio of the TTDs for each water parcel were calculated using the atmospheric history of N_2O. The TTDs' N_2O mixing ratio were used to calculate the N_2O concentration at atmospheric equilibrium (N_2O_{bkg}) for each water sample. For values where the CFC-11 was below detection (< 0.001 pmol kg^{-1}), an atmospheric N_2O mixing ratio of 274.81 ppb was used.

2.5 Keeling plot analysis

In order to determine the sources of high ΔN_2O near the oxycline in the ETNP and ETSP ODZs, the isotopic and

isotopomer compositions of the N_2O produced within given water masses and at different O_2 concentrations were estimated using Keeling plot analysis (Keeling, 1961; Pataki et al., 2003; Yamagishi et al., 2007; Fujii et al., 2013; Casciotti et al., 2018). The isotopic composition of source N_2O was determined using equation 5 and:

$$\delta_{obs} \times [N_2O]_{obs} = \delta_{bkg} \times [N_2O]_{bkg} + \delta_{prod} \times [N_2O]_{prod} \qquad \text{(eq. 7)}$$

where the obs, prod and bkg subscripts refer to N_2O measured, newly produced and at equilibrium, respectively.

Rearranging equations 5 and 7 gives:

$$\delta_{obs} = 1/[N_2O]_{obs} \times (\delta_{bkg} - \delta_{prod} \times [N_2O]_{bkg} + \delta_{prod}) \qquad \text{(eq. 8)}$$

The intercepts of the linear regression between the inverse of measured N_2O concentration and the $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$ and SP thus represents the isotopic composition of produced N_2O.

2.6 Isotope effects during N_2O consumption

We estimated isotope effects associated with N_2O consumption in the ETNP ODZ using a closed-system Rayleigh model (Mariotti et al., 1981). Only data in the ETNP ODZ at $[O_2]$ < 5 µmol kg^{-1} where N_2O consumption occurs were selected for this analysis.

$$\delta N_2O = \delta N_2O_{initial} - \varepsilon \ln([N_2O]_{obs}/[N_2O]_{initial}) \qquad \text{(eq. 9)}$$

where $\delta^{15}N_2O$ is the $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$ or SP for samples at O_2 < 5 µmol kg^{-1} (mostly in the ETNP ODZ), and the subscript refers to N_2O measured and initial concentrations and isotopic values before the onset of N_2O consumption. The isotope effects were estimated as the slopes of the linear regressions between δN_2O versus $-ln[N_2O]$, $-ln[N_2O]$ being an approximation of the $[N_2O]_{obs}/[N_2O]_{initial}$ term.

3 Results

3.1 Water mass characterization

Water masses were characterized according to Emery (2001). The main surface water (0 – 500 m depth) masses along the P18 transects, listed from north to south with potential temperature and absolute salinity ranges in brackets, were Eastern North Pacific Central Water (ENPCW; 12.0-20°C; 34.2-35.0), Eastern North Pacific Transition Water (ENPTW; 11.0-20.0°C, 33.3-34.3), Pacific Equatorial Water (PEW; 7.0-23.0°C, 34.5-36.0), Eastern South Pacific Central Water (ESPCW; 8.0-24°C; 34.4-36.4), Eastern South Pacific Transition Water (ESPTW; 14.0-20.0°C; 34.6-35.2), and Antarctic Surface Water (AASW; -1.0-1.0°C, 34.0-34.6). Intermediate water masses (500 – 1500 m depth) include Eastern South Pacific Intermediate Water (ESPIW; 10.0–12.0°C, 34.0-34.3) and Antarctic Intermediate Water (2-10°C, 33.8-34.5). Circumpolar Deep Water (CDW; 0.1-2.0°C, 34.62-34.73) was observed below 1500 m depth (Figure 3).

FIGURE 3
Potential temperature-absolute salinity plot showing the main water masses along the P18 transect from discrete water samples and ΔN₂O values as the color of the symbols (color bar). Smaller black dots indicate samples for which ΔN₂O was not measured. The different water masses abbreviations are for deep waters: Circumpolar Deep Water (CDW), intermediate waters: Eastern South Pacific Intermediate Water (ESPIW), and Antarctic Intermediate Water (AAIW), and surface waters: Pacific Equatorial Water (PEW), Eastern South Pacific Transition Water (ESPTW), Eastern South Pacific Central Water (ESPCW), Eastern North Pacific Central Water (ENPCW), Eastern North Pacific Transition Water (ENPTW), and Antarctic Surface Water (AASW). Lines of equal potential density (sigma-theta (σ_θ)) are shown (dashed grey lines).

3.2 Distribution of O_2, nutrient concentrations, NO_3^- isotopes, DIN deficit and biogenic N

The P18 transects crossed the ETNP ODZ and the fringe of the ETSP ODZ. O_2 concentrations decreased to < 5 µmol kg^{-1} between 120 m and 845 m depth in the northernmost part of the transect in the ETNP ODZ. In the ETSP, O_2 concentrations generally remained > 5 µmol kg^{-1}, except at few stations/depths between 4.5°S and 9.7°S and 195 and 495 m depth. The oxycline depth varied between 70 m at 10.5°N to 285 m at 16°S (Figure 4A).

Nitrite concentrations were generally low (<2.7 µmol kg^{-1}), with highest concentrations between 13°N and 16°N and 110 m to 285 m depth in the ETNP ODZ. Nitrite also accumulated at the fringe of the ETSP ODZ, with maximum concentrations between 7.5°S and 16.5°S near 100 m depth. Modest nitrite accumulations (<0.5 µmol kg^{-1}) were also observed in the upper 200 m from 34°S to 60°S (Figure 4B). Nitrate concentrations were generally depleted in surface waters, with the deepest maximum nitracline found at 350 m depth in the oligotrophic subtropical South Pacific from 21°S to 31.5°S (Figure 4C). A high $\delta^{15}N$ of NO_3^- of up to ~15‰ was observed in surface waters (Figure 4D), as expected following fractionation during NO_3^- assimilation by phytoplankton (Altabet, 2001; Granger et al., 2004). Nitrate concentrations were

FIGURE 4
Section plots showing **(A)** dissolved O_2 concentration, **(B)** NO_2^- concentration, **(C)** NO_3^- concentration, **(D)** $\delta^{15}N$-NO_3^-, **(E)** DIN deficit calculated from nutrient concentrations, and **(F)** biogenic N (calculated from N_2/Ar measurements) along the P18 transect in 2016/2017.
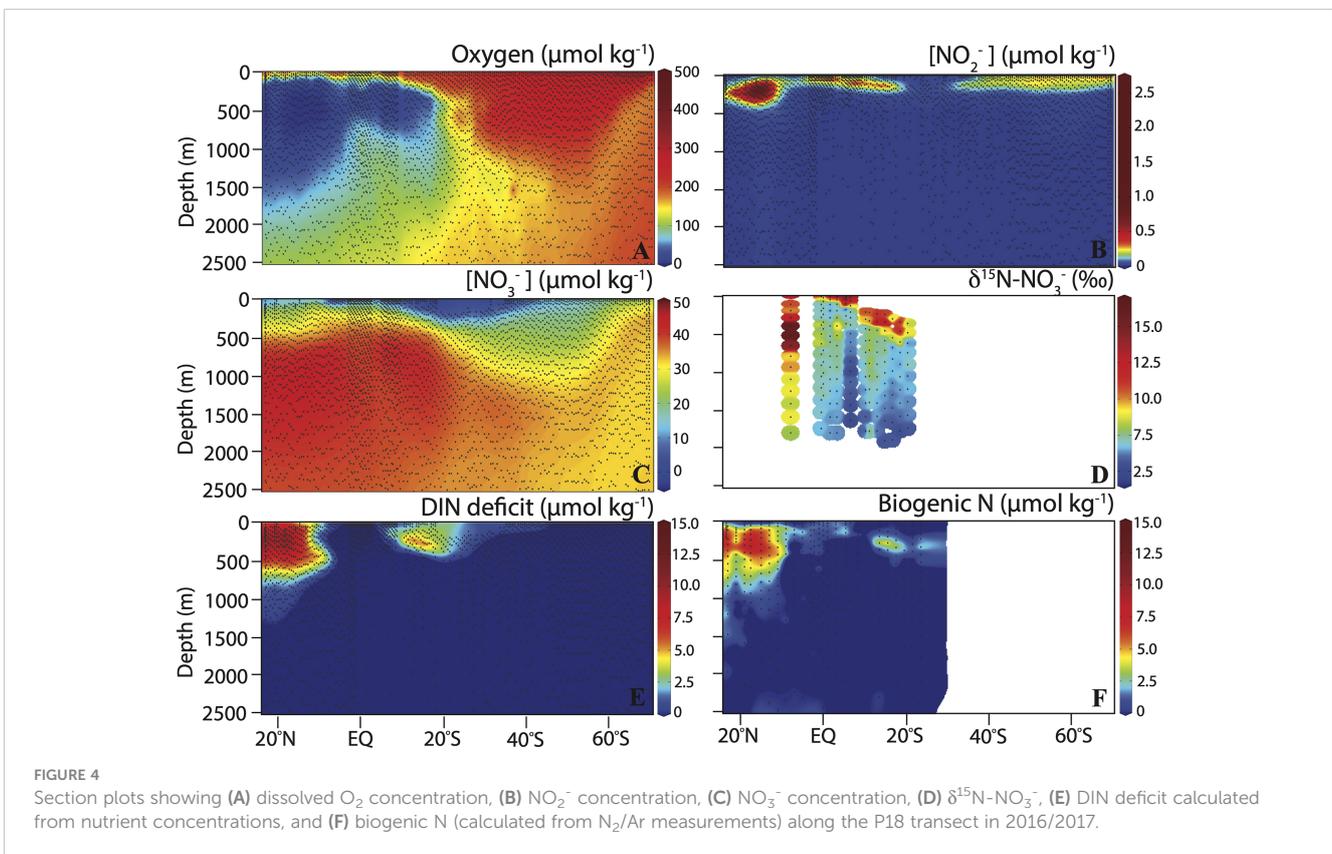
moderate in the upper 500 m of the ETNP ODZ (minimum $[NO_3^-]$ of 21 μmol kg^{-1}), corresponding to ENPCW. The $\delta^{15}N$ of NO_3^- was also elevated in the ODZ (up to 16.7‰ at 8.5°N and 425 m depth), consistent with fractionation during denitrification (Cline and Kaplan, 1975; Granger et al., 2008). Nitrate accumulation of up to 45 μmol kg^{-1} were observed between 625 and 1750 m depth and was more pronounced in the ETNP. Nitrate concentrations remained relatively constant below 2000 m depth, with higher concentrations (~38 μmol kg^{-1}) in the northern portion of the transect compared to the South (~33 μmol kg^{-1}) (Figures 4C, D).

The DIN deficit (Howell et al., 1997) was calculated using the following equations:

$$DIN\ deficit\ =\ DIN_{exp} - DIN_{obs} \qquad (eq.\ 10)$$

$$DIN_{exp} = m \times [PO_4^{3-}]_{obs} + b \qquad (eq.\ 11)$$

where DIN_{exp} is the concentrations of dissolved inorganic nitrogen expected assuming Redfield stoichiometry (typically 16N:1P), DIN_{obs} is the concentration of nitrate plus nitrite measured and m and b are the slope and intercept, respectively, of the relationship between DIN and phosphate (PO_4^{3-}) concentrations for source waters outside of the ETNP and ETSP ODZs. DIN_{exp} was calculated according to Chang et al. (2010; Chang et al., 2012). DIN deficit was particularly elevated (up to 13.75 μmol kg^{-1}) between 50 and 580 m depth in the ETNP ODZ. DIN deficit was also elevated at the fringes of the ETSP ODZ, with values of up to 12.4 μmol kg^{-1} at 9.7°S and 205 m depth. Biogenic N

derived from N_2/Ar data (as μmol N kg^{-1}) were generally comparable to DIN deficit, although generally a bit lower. Discrepancy between these two measurements could be caused by collection (N_2/Ar was not a prioritized gas sample during the P18 cruise) and/or storage effects for the N_2/Ar samples (Figures 4E, F).

3.3 ΔN_2O, N_2O stable isotopes and isotopomers

The largest ΔN_2O accumulation of 75.7 nmol kg^{-1} at $[O_2] < 5$ μmol kg^{-1} was observed at a depth of 95 m (σ_θ = 25.65), near the oxycline in the ENPCW water mass in the ETNP ODZ (14.5°N) (Figure 5A). High ΔN_2O of up to 73 nmol kg^{-1} were also observed under low-O_2 conditions (15 μmol kg^{-1}) at the fringe of the ETSP ODZ (7-9°S) at 180-195 m depth (σ_θ = 26.4), which corresponds to the PEW water mass (Figures 3, 5). Overall, elevated ΔN_2O concentrations (>30 nmol kg^{-1} were observed from the northern portion of the P18 transect to about 20°S, clearly associated with low-O_2 waters from the ETNP and ETSP ODZs. In contrast, slightly negative ΔN_2O (undersaturation) (~-1 nmol kg^{-1}) values were observed in surface waters at the southern part of the transect associated with the subduction of AASW forming AAIW (Emery, 2001). Rapid cooling and sinking of surface water, with insufficient time for re-equilibration with the atmosphere, is likely causing the observed N_2O undersaturation in near surface waters in this region. A slight increase in ΔN_2O (up to ~20 nmol kg^{-1}) was observed from
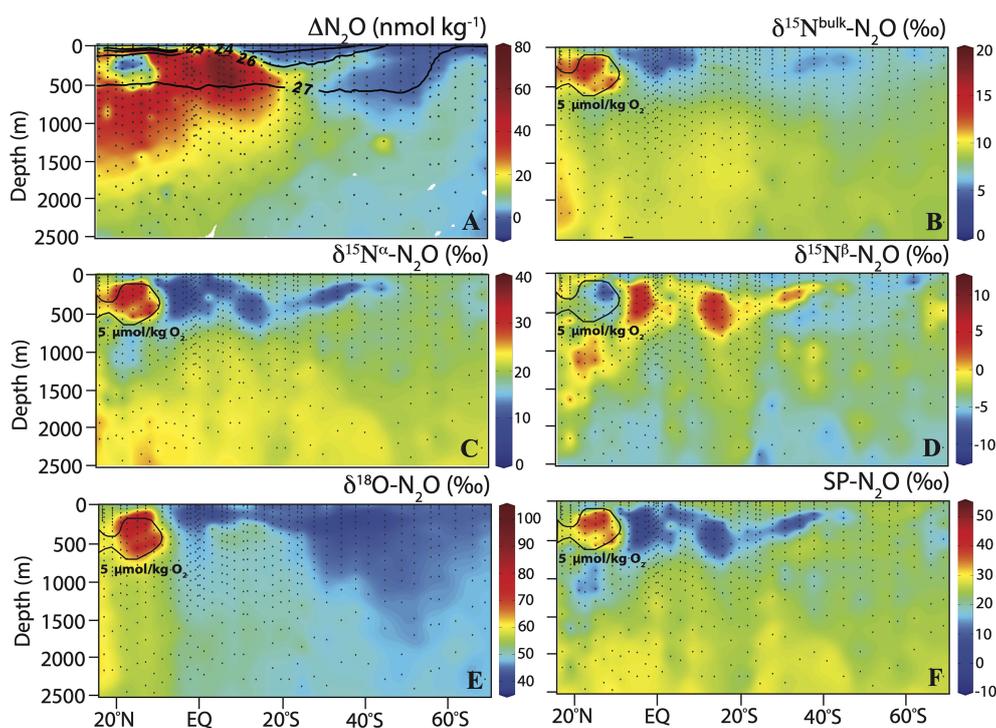
FIGURE 5
Section plots of **(A)** ΔN_2O with sigma-theta (σ_θ; kg m^{-3}) in overlay, **(B)** $\delta^{15}N^{bulk}$-N_2O, **(C)** $\delta^{15}N^{\alpha}$-N_2O, **(D)** $\delta^{15}N^{\beta}$-N_2O, **(E)** $\delta^{18}O$-N_2O, and **(F)** SP-N_2O along the P18 transect. The 5 μmol kg^{-1} O_2 contour in the ETNP is shown in **(B−F)**.

60°S to 20°S in AAIW waters. Negative ΔN_2O (minimum of ~-5 nmol kg^{-1}) were observed within the anoxic zone ($O_2 < 5$ µmol kg^{-1}) of the ETNP (Figure 5A).

The $\delta^{15}N^{bulk}$-N_2O was lowest in the first 500 m depth near the equator, with values less than 5‰, indicating production (Figure 5B). The highest $\delta^{15}N^{bulk}$-N_2O values (up to 18‰) were observed within the ETNP ODZ, consistent with the observed low ΔN_2O and indicating net consumption (Ostrom et al., 2007). $\delta^{15}N^{bulk}$-N_2O remained approximately 8 to 10‰ in waters deeper than 1000 m depth. The $\delta^{15}N^{\alpha}$-N_2O nearly followed the same distribution pattern as $\delta^{15}N^{bulk}$-N_2O, with overall higher values (up to 42.5‰) in the ETNP ODZ (Figure 5C). The $\delta^{15}N^{\beta}$-N_2O contrasted with the $\delta^{15}N^{bulk}$-N_2O and $\delta^{15}N^{\alpha}$-N_2O distributions, with highest values (up to 9.4‰) within the first 500 m flanking the equator and lowest values (minimum of -11.3‰) in the ETNP ODZ (Figure 5D). The $\delta^{18}O$-N_2O decreased along the north-south gradient of the P18 transect, with highest values (up 100‰) in the ETNP ODZ (Figure 5E). $\delta^{18}O$-N_2O remained fairly constant below 2000 m depth and increased from ~48 to 56‰ in CDW from south to north along the P18 transect. SP distribution was similar to that of $\delta^{15}N^{\alpha}$-N_2O, with lowest values (minimum of -6.1‰) in low O_2 waters (>5 µmol kg^{-1}) in the upper 500 m near the equator and highest values (up to 49‰) in the ETNP ODZ (Figure 5F). In AASW and AAIW at the southern portion of the transect near Antarctica, $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$ and SP were close to

atmospheric N_2O values (6.2‰ for $\delta^{15}N^{bulk}$, 15.8‰ for $\delta^{15}N^{\alpha}$, -3.4‰ for $\delta^{15}N^{\beta}$, 44.3‰ for $\delta^{18}O$ and 19.2‰ for SP; Kelly et al., 2021). The isotopic signatures of N_2O, together with the observed slight N_2O undersaturation, indicate no N_2O production in this region.

3.4 Relationships between ΔN_2O, AOU and N deficit

The ratio between ΔN_2O/AOU, indicative of cumulative N_2O production yield, increased at low O_2 concentration to 0.3 (Figure 6A). Other high ΔN_2O/AOU ratios observed at higher O_2 concentrations were clearly influenced by the contrasting effect of photosynthesis on AOU, as indicated by higher pH at these shallower isopycnal ranges (Supplementary materials, Figure S1). The lowest SPs were observed at highest ΔN_2O and ΔN_2O/AOU ratios (Figure 6B). Positive relationships between ΔN_2O and AOU were observed in surface waters $\sigma_\theta < 26$ kg m^{-3} in the ETNP and ETSP (Supplementary materials, Figure S2). No significant relationship between ΔN_2O and AOU was observed at $26 < \sigma_\theta < 27$ kg m^{-3} where highest ΔN_2O values were generally observed.

Significant positive relationships between ΔN_2O and DIN deficit were observed in the ETNP (18.5°N – Equator), with an increased slope toward deeper isopycnals where highest ΔN_2O were
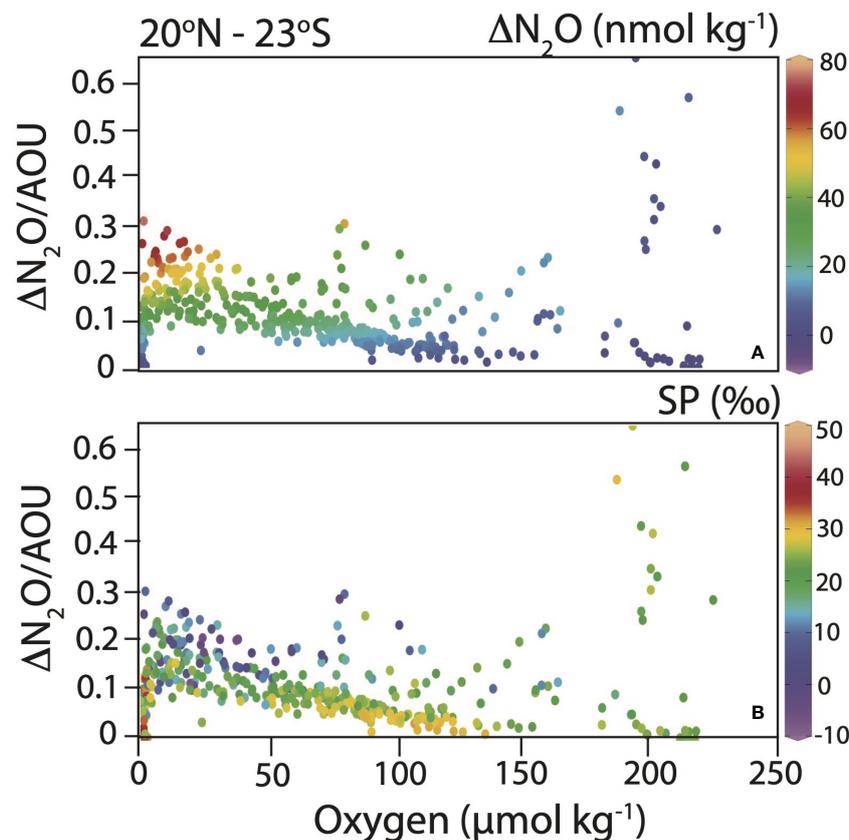
FIGURE 6
ΔN_2O/AOU versus O_2 concentration showing increased N_2O production yield (associated with low SP) under low O_2 conditions. ΔN_2O **(A)** and SP **(B)** are shown as the color of the symbols (see color bars).

FIGURE 7
DIN deficit versus ΔN_2O for the ETNP (18°N – Equator) (blue dots and lines) and ETSP (Equator – 13S) (red dots and line). In the ETNP, the light and dark blue dots and lines represent different isopycnal ranges.
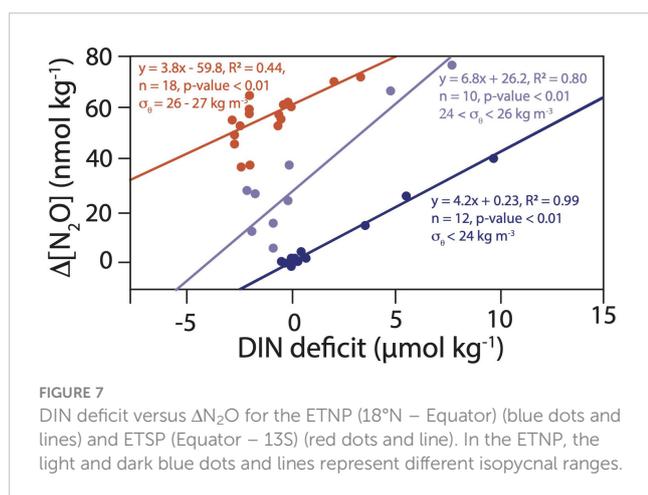
observed. A significant positive relationship between ΔN_2O and DIN deficit was also observed in the ETSP (Equator – 13°S) for the isopycnal range $26 < \sigma_\theta < 27$ kg m^{-3}. No such relationship was observed in the ETSP surface waters ($\sigma_\theta < 26$ kg m^{-3}) (Figure 7).

4 Discussion

4.1 Sources of the highest ΔN_2O accumulations near the oxycline in the ETNP and ETSP

We observed relatively high N_2O concentrations of up to 84 nmol kg^{-1} (940% supersaturation) near the upper oxycline in offshore waters along the P18 section crossing the ETNP ODZ and the fringe of the ETSP ODZ. High N_2O concentrations of up to ~100 nmol kg^{-1} were also observed offshore near the oxycline in the ETNP ODZ (Trimmer et al., 2016; Kelly et al., 2021). At offshore stations in the ETSP ODZ, Casciotti et al. (2018) observed N_2O concentrations were up to ~70 nmol kg^{-1}, comparable to observations along the P18 line. In contrast, Bourbonnais et al. (2017) observed much higher N_2O concentrations of up to ~190 nmol kg^{-1} in coastal surface waters off Peru in the ETSP ODZ. Differences in N_2O accumulation observed between these different studies appear to mostly relate to productivity (e.g., coastal versus offshore), but there is also evidence for dynamic N_2O cycling at offshore stations. For instance, the El Niño-Southern Oscillation and mesoscale processes such as eddies have been shown to influence N_2O distribution (e.g., Arévalo-Martínez et al., 2016; Ji et al., 2019; Babbin et al., 2020; Monreal et al., 2022)

Yield of N_2O from nitrification increased toward lower [O_2], as also observed in previous studies (e.g., Ryabenko et al., 2012; Ji et al., 2015; Ji et al., 2018; Frey et al., 2020) (Figure 6). If we assume that 138 moles of O_2 are reduced for 16 moles of NH_4^+ nitrified (Ward, 2008), a yield of up to 0.5% N-N_2O mol produced per mol NH_4^+ oxidized is obtained considering the highest ΔN_2O/AOU slope of 0.3. A 0.5% yield falls in the range observed in the Pacific Ocean (Yoshida et al., 1989), but is lower than the maximum N_2O production yield for nitrification reported for ^{15}N-labeled incubation studies (up to ~3% in the ETSP ODZ) (Ji et al., 2018;

Frey et al., 2020). However, while nitrification is clearly occurring in surface waters along the P18 transect, as shown by correlations between ΔN_2O and AOU, the data deviate from this relationship on isopycnals where the highest ΔN_2O were observed near the ODZ oxycline (Supplementary materials, Figure S2).

Two different sources of N_2O were observed from the Keeling plot analysis in both the ETNP and ETSP for [O_2] > 5 μmol kg^{-1} and $\sigma_\theta <$ 27 kg m^{-3} (Figures 8, 9). A break-point analysis was performed in R using the package "segmented" (Muggeo, 2003; Muggeo and Muggeo, 2017) as described in Kelly et al. (2021). The breakpoint was around 33 nmol kg^{-1} (1/[N_2O] = 0.03) in both regions. Coincidentally, this analysis divided the data into two main O_2 regimes: 5 μmol kg^{-1} < [O_2] < 100 μmol kg^{-1} and [O_2] > 100 μmol kg^{-1} (Table 1). It should be noted that highest ΔN_2O in the ETNP were observed at [O_2] below 5 μmol kg^{-1} and were thus not included in the Keeling plot analysis. Kelly et al. (2021) found a clear relationship between distinct N_2O sources and [NO_2^-], with highest N_2O accumulation at elevated [NO_2^-] (up to ~1 μmol kg^{-1}) in the ETNP. In contrast to Kelly et al. (2021), no such relationship was observed when restricting the dataset to the same isopycnal range ($\sigma_\theta \leq 25$ kg m^{-3}).

The $\delta^{15}N^{bulk}$ of source N_2O varied from 4.0 to 7.2‰ (Table 1). $\delta^{15}N^{bulk}$ is affected by the $\delta^{15}N$ of the substrate and fractionation effects during N_2O production (e.g., Sutka et al., 2006; Frame et al., 2014; Bourbonnais et al., 2017). Similar $\delta^{15}N^{bulk}$ values for source N_2O were reported in the ETSP (Casciotti et al., 2018). These values fall within the expected range for the $\delta^{15}N$ of remineralized NH_4^+, the substrate for nitrification, assuming a $\delta^{15}N$ of sinking particulate matter of 3-15‰ (Altabet et al., 1999; Altabet, 2001; Berelson et al., 2015) in the eastern Pacific and a modest isotope effect during ammonification (Altabet, 1988). N_2O could also be produced by denitrification as the $\delta^{15}N$ of NO_3^- observed in this study (4.5-15.4‰) was also similar to observed $\delta^{15}N^{bulk}$-N_2O values. However, the upper range in $\delta^{15}N$ values for these substrates is needed to reproduce the observed $\delta^{15}N^{bulk}$-N_2O during nitrification and denitrification as both processes are expected to add a lower $\delta^{15}N$ due to isotopic fractionation, unless the substrate is completely consumed, or the reaction is diffusion limited (see Table 1 in Bourbonnais et al., 2017 for a compilation of ε associated with these processes and Ostrom and Ostrom, 2012; Frame et al., 2014).

A high $\delta^{18}O$ (52.3‰) and relatively low $\delta^{15}N^\alpha$ (2.7‰) and SP (-9.0‰) were estimated for source N_2O in the ETNP just above the ODZ corresponding to highest ΔN_2O accumulations. In contrast, $\delta^{15}N^{bulk}$, $\delta^{15}N^\alpha$, $\delta^{15}N^\beta$, $\delta^{18}O$ and SP of source N_2O in surface waters ([O_2] higher than 100 μmol kg^{-1}) were more comparable to atmospheric signatures (Table 1). In the ETSP, the $\delta^{15}N^\alpha$ (11.0‰), $\delta^{18}O$ (55.7‰) and SP (9.5‰) of source N_2O were higher than in the ETNP near the oxycline and the SP in oxic surface waters (11.3‰) was significantly lower. The isotopic values of source N_2O observed in surface waters overlying the ODZ in the ETSP contrasted with the values estimated by Casciotti et al. (2018). For instance, the $\delta^{18}O$ was significantly lower and the SP was higher than the values reported by Casciotti et al. (2018) (Table 1). These differences were likely caused by spatial and/or temporal heterogeneity. Moreover, P18 only crossed the fringe of the ETSP ODZ, with [O_2] generally higher than 5 μmol kg^{-1}, except at a few stations/depths.
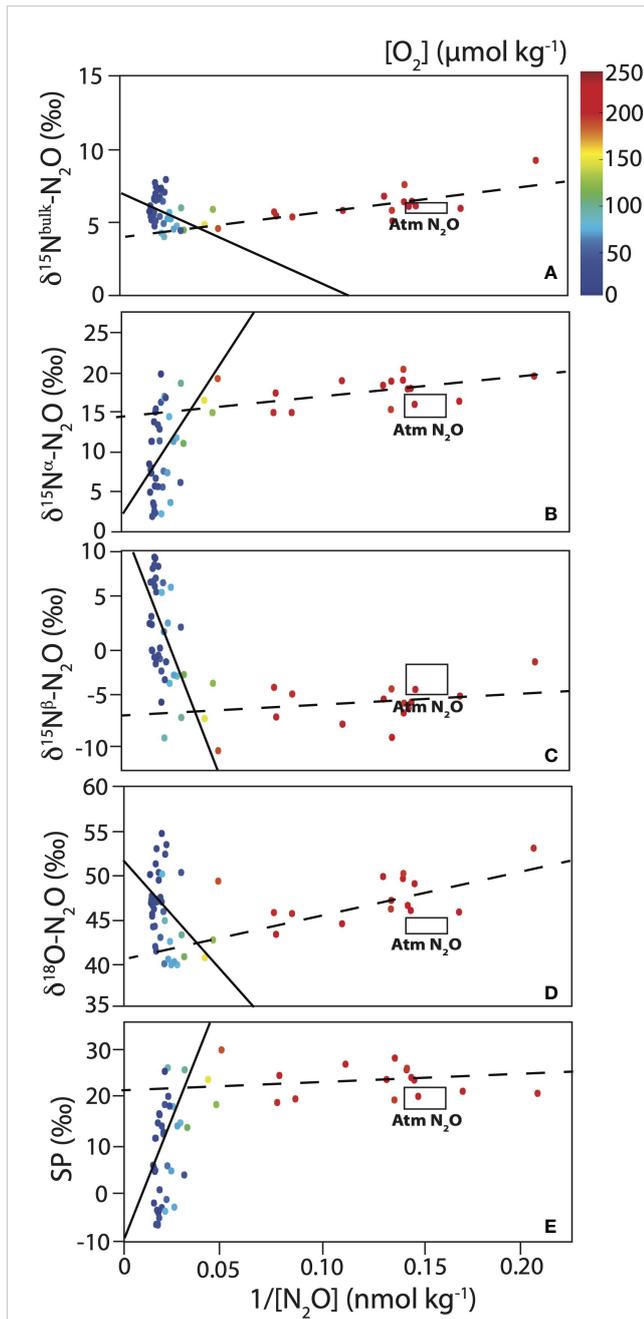
FIGURE 8
Keeling plot analysis for the ETNP (18°N − Equator) for **(A)** $\delta^{15}N^{bulk}$, **(B)** $\delta^{15}N^{\alpha}$, **(C)** $\delta^{15}N^{\beta}$, **(D)** $\delta^{18}O$ and **(E)** SP of N_2O (in ‰). Black lines are linear regressions (full line: 5 µmol kg^{-1} < [O_2] < 100 µmol/kg and dashed line: [O_2] > 100 µmol kg^{-1}). O_2 concentrations are shown as the color of the symbols (see color bar in top panel). Atmosphere N_2O isotopic values are shown as a rectangle (from Kelly et al., 2021). See Table 1 for intercept, R^2 and p-value for the linear regressions.

FIGURE 9
Keeling plot analysis for the ETSP (Equator − 13°S) for **(A)** $\delta^{15}N^{bulk}$, **(B)** $\delta^{15}N^{\alpha}$, **(C)** $\delta^{15}N^{\beta}$, **(D)** $\delta^{18}O$ and **(E)** SP of N_2O (in ‰). Black lines are linear regressions (full line: 5 µmol kg^{-1} < [O_2] < 100 µmol kg^{-1} and dashed line: [O_2] > 100 µmol kg^{-1}). O_2 concentrations are shown as the color of the symbols (see color bar in top panel). Atmosphere N_2O isotopic values are shown as a rectangle (from Kelly et al., 2021). See Table 1 for intercept, R^2 and p-value for the linear regressions.

The stable isotopic and isotopomer signatures observed at the highest ΔN_2O and lowest [O_2] near the oxycline in both the ETNP and ETSP are consistent with production from denitrification or nitrifier-denitrification. SP is particularly useful for differentiating N_2O production processes as it is mainly pathway dependent and independent of the isotopic composition of the substrate (Schmidt et al., 2004; Sutka et al., 2004). For instance, formation of N_2O

during bacterial denitrification occurs by combining two NO molecules following a *trans* mechanism (Toyoda et al., 2005; Magyar, 2017). This mechanism involves the asymmetrical intermediate *trans*-hyponitrite (-ONNO-) bridging two iron centers; the α N atom and the O comes from NO bonded to Fe_{heme} and the β N atom comes from NO bonded to Fe_B. If the precursor NO molecules are derived from the same substrate, this

TABLE 1 Intercept (in ‰), R^2, and p-values of linear regressions for Keeling plot analysis under different O_2 regimes and regions/water masses along the P18 line.

	$\delta^{15}N^{bulk}$-N_2O	$\delta^{15}N^{\alpha}$-N_2O	$\delta^{15}N^{\beta}$-N_2O	$\delta^{18}O$-N_2O	SP
ETNP (18.5°N – EQ)					
100 µmol kg^{-1} > [O_2] > 5 µmol kg^{-1} (27 > σ_θ > 23.3 kg m^{-3}) (n = 37)					
Intercept	7.2 ± 0.8	2.7 ± 4.0	11.7 ± 3.5	52.3 ± 3.2	-9.0 ± 7.3
R^2	0.079	0.086	0.19	0.10	0.14
p-value	0.09	0.08	**<0.01**	0.06	0.02
[O_2] > 100 µmol kg^{-1}(surface, σ_θ < 25.7 kg m^{-3}) (n = 18)					
Intercept	4.0 ± 0.4	12.3 ± 14.7	-6.5 ± 1.4	40.8 ± 1.4	20.7 ± 2.5
R^2	0.59	0.038	0.045	0.54	0.06
p-value	**<0.01**	0.4	0.4	**<0.01**	0.3
Deep waters, σ_θ > 27.3 kg m^{-3} (n = 47)					
Intercept	9.1 ± 5.0	16.8 ± 1.1	1.4 ± 0.9	52.7 ± 1.7	15.4 ± 2.0
R^2	0.01	0.39	0.46	0.020	0.45
p-value	0.6	**<0.01**	**<0.01**	0.4	**<0.01**
ETSP (13°S – EQ)					
100 µmol kg^{-1} > [O_2] > 5 µmol kg^{-1}(27 > σ_θ > 26 kg m^{-3}) (n = 34)					
Intercept	6.3 ± 0.9	11.0 ± 2.6	1.6 ± 2.0	55.7 ± 3.3	9.5 ± 4.3
R^2	0.0056	0.0084	0.035	0.17	0.020
p-value	0.7	0.6	0.3	**0.01**	0.4
[O_2] > 100 µmol kg^{-1} (surface waters, σ_θ < 26 kg m^{-3}) (n = 26)					
Intercept	4.9 ± 0.4	10.6 ± 1.2	-0.7 ± 1.2	38.3 ± 1.2	11.3 ± 2.2
R^2	0.12	0.54	0.37	0.59	0.49
p-value	0.08	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Deep waters, σ_θ > 27 kg m^{-3} (n = 26)					
Intercept	7.0 ± 0.2	17.0 ± 0.9	-3.0 ± 1.0	45.5 ± 0.7	20.0 ± 1.8
R^2	0.67	0.34	0.0068	0.41	0.14
p-value	**<0.01**	**<0.01**	0.5	**<0.01**	**<0.01**
AAIW					
Intercept	9.4 ± 0.3	18.1 ± 0.8	0.8 ± 0.6	52.5 ± 0.6	17.2 ± 1.4
R^2	0.42	0.020	0.42	0.67	0.19
p-value	**<0.01**	0.2	**<0.01**	**<0.01**	**<0.01**
Casciotti et al. (2018) - ETSP					
Surface	6.1 ± 1.4	na	na	53.0 ± 3.1	5.4 ± 4.4
Oxycline	7.5 ± 2.2	na	na	50.7 ± 3.6	9.3 ± 2.4
Deep	6.6 ± 0.6	na	na	54.3 ± 0.6	20.1 ± 0.5
Kelly et al. (2021) – ETNP					
Surface	na	6.6 ± 0.5	0.6 ± 0.5	46.3 ± 0.5	6.6 ± 2.3
Oxycline	na	2.9 ± 1.4	-3.8 ± 0.9	53.1 ± 1.3	5.9 ± 0.8

(Continued)

TABLE 1 Continued

	$\delta^{15}N^{bulk}$-N_2O	$\delta^{15}N^{\alpha}$-N_2O	$\delta^{15}N^{\beta}$-N_2O	$\delta^{18}O$-N_2O	SP
Deep	6.2 ± 1.0	16.8 ± 0.	-4.5 ± 0.6	57.3 ± 0.8	21.3 ± 1.0
Atmosphere-equilibrated seawater (Kelly et al., 2021)	6.2 ± 0.4	15.8 ± 1.4	-3.4 ± 1.6	44.3 ± 0.8	19.2 ± 2.9

Standard error of the intercept is reported. P-value associated with a confidence level >90% are in bold. Atmospheric values are from Kelly et al. (2021). na means non available. AAIW temperature and salinity ranges are defined as in Emery (2001) (section 3.1).

mechanism causes little difference between the $\delta^{15}N$ of the α and β N atoms, resulting in a low SP (see Magyar, 2017 for more detail). In contrast, during N_2O formation according to a *cis* mechanism, a first NO molecule binds to one or the other iron center with the second NO molecule binding directly to the first NO, forming a symmetrical intermediate. Cleavage of ^{14}N-O bond is preferred over ^{15}N-O bond, leading to enrichment of the α position and a higher SP. Thus, N_2O produced by nitrification, either by archaea and bacteria, is generally associated with a high SP of 30-38‰ consistent with a *cis*-formation mechanism (Sutka et al., 2003; Sutka et al., 2004; Frame and Casciotti, 2010; Santoro et al., 2011; Löscher et al., 2012) whereas denitrification and nitrifier-nitrification are associated with much lower SPs (~ - 10 to 0‰) (Sutka et al., 2003; Sutka et al., 2004; Toyoda et al., 2005; Frame and Casciotti, 2010).

N_2O produced by denitrification is associated with a low SP of ~ -5 to 0‰ and adds a relatively low $\delta^{15}N$ (especially at the α position) with an isotope effect ($^{15}\epsilon$) ranging from 13-37‰ (Barford et al., 1999; Sutka et al., 2003; Sutka et al., 2004; Toyoda et al., 2005; Sutka et al., 2006; Frame and Casciotti, 2010). On the other hand, branching fractionation, i.e., the preferential loss of ^{16}O relative to ^{18}O during NO_3^- reduction to N_2O, is expected to lead to high $\delta^{18}O$-N_2O values (Casciotti et al., 2002; Frame et al., 2014). The $\delta^{18}O$-N_2O is affected by both the branching isotope effects ($^{18}\epsilon$) of 25-30‰ for NO_3^- reduction to NO_2^- and 10-12‰ during NO_2^- reduction to N_2O during denitrification as well as the equilibration of NO_2^- O isotope with water (Casciotti et al., 2007; Casciotti and Buchwald, 2012). Nitrifier-denitrification is associated with a SP of -10-0‰ and adds a lower $\delta^{15}N$ compared to denitrification due to a larger $^{15}\epsilon$ ranging from 31-58‰. Furthermore, an effective O isotope effect ($^{18}\epsilon$) of 8-12‰ was reported for NO_2^- reduction to N_2O during nitrifier denitrification (Sutka et al., 2003; Sutka et al., 2004; Sutka et al., 2006; Frame and Casciotti, 2010). Newly produced $\delta^{18}O$-N_2O values during nitrification and nitrifier denitrification are thus generally lower (13-35‰; Snider et al., 2012). Accordingly, low source $\delta^{18}O$-N_2O values were observed at $[O_2]$ > 100 μmol kg^{-1} in the ETNP and ETSP whereas higher values were observed at deeper isopycnal ranges overlying the ODZs (Table 1), consistent with the dominance of N_2O production by nitrification in surface waters and denitrification at lower O_2 concentrations near the oxycline.

Kelly et al. (2021) similarly observed two distinctive sources of N_2O in the ETNP above the σ_θ < 25 kg m^{-3} isopycnal. SPs of 6-8‰ were observed. Based on an isotopic mass balance and assuming that the SPs for N_2O produced during nitrification and denitrification are 30-38‰ and 0‰, respectively (Sutka et al., 2003; Sutka et al., 2004; Toyoda et al., 2005; Frame and Casciotti, 2010; Santoro et al., 2011; Löscher et al., 2012), about 80% of the

N_2O production was attributed to denitrification or nitrifier-denitrification. Bourbonnais et al. (2017) and Casciotti et al. (2018) also observed low SPs (-3 to 10‰) corresponding to large ΔN_2O accumulation near the oxycline above the ETSP ODZ. The much lower SP observed in the ETNP in this study (-9‰) preclude any contribution from nitrification. The higher SP (9.5‰) for source N_2O observed at low $[O_2]$ near the fringe of the ETSP ODZ suggests a relatively minor contribution from nitrification (~1/3) and is consistent with the value reported by Casciotti et al. (2018). However, hybrid N_2O formation by archaea, where one atom is derived from nitric oxide (NO; from NO_2^-) and the other from hydroxylamine (NH_2OH; from NH_4^+) was shown to be an important production pathway in marine environments and could possibly contribute to endmember signatures estimated from the Keeling plot analysis (Stieglmeier et al., 2014; Kozlowski et al., 2016; Trimmer et al., 2016; Frame et al., 2017). Frey et al. (2020) showed that hybrid N_2O production accounts for 70-85% of the total N_2O production from NH_4^+ oxidation using ^{15}N-labeled incubation experiments in the ETSP. SP may, in part, reflect the relative $\delta^{15}N$ of the substrates for the α and β positions during hybrid archaeal N_2O production rather than being indicative of a particular pathway (Casciotti et al., 2018). Therefore, if lower SP could be produced by archaeal hybrid N_2O production, the fraction of denitrification needed to explain low SP signatures associated with high N_2O supersaturations observed above or in the ODZs in this and previous studies would further decrease (Bourbonnais et al., 2017; Casciotti et al., 2018; Kelly et al., 2021).

The observed relationships between ΔN_2O and DIN deficit at isopycnals σ_θ < 26 kg m^{-3} and the absence of clear relationships between ΔN_2O and $[NO_2^-]$ further support a role for denitrification (rather than nitrifier-denitrification) as the dominant N_2O production pathway in both the ETSP and the ETNP. The decreased slope for the ΔN_2O versus DIN deficit relationship toward surface isopycnal ranges observed in the ETNP suggests decreased N_2O yield at higher $[O_2]$ concentrations or mixing/dilution of N_2O produced by denitrification advected from below, especially for upwelling waters near the equator. The $\delta^{15}N$ of NO_3^- provides more insights into N_2O sources yet these measurements were mostly available at ETSP stations. A plot of SP versus $\Delta^{15}N$ ($\delta^{15}N$-NO_3^- - $\delta^{15}N$-N_2O) showed that some of the highest ΔN_2O datapoint indeed fell within the expected compositional fields for N_2O production by bacterial denitrification ($\Delta\delta^{15}N$= 0-35‰, SP: -5-0‰) (Wankel et al. (2017) and references therein, Supplementary Figure S3). Some high ΔN_2O were associated with relatively high SPs (> 10‰), showing the overprinting effect of N_2O consumption during denitrification (Ostrom et al., 2007). Compositional field analysis (Figure S3) also suggests production by either archaeal and bacterial ammonia oxidation at higher $[O_2]$

and lower ΔN_2O. Bourbonnais et al. (2017) also invoked incomplete denitrification as a major pathway for extreme N_2O accumulation in newly upwelled surface waters off Peru based on the absence of a relationship between ΔN_2O and apparent O_2 utilization and significant relationships between NO_3^- and N_2O isotopes. The dominance of denitrification for N_2O production near the oxycline is also supported by ^{15}N-tracer incubation studies in the ETSP ODZ. N_2O production rates were indeed one order of magnitude higher for denitrification compared to ammonia oxidation under low-O_2 conditions at the ΔN_2O maximum just above the ETSP ODZ (Ji et al., 2015; Frey et al., 2020).

4.2 Sources of N_2O below the ETNP and ETSP ODZs and in AAIW

N_2O sources in deep ETNP and ETSP were also investigated using Keeling plot analysis (Supplementary materials, Figure S4 and Table 1). A deeper isopycnal range ($\sigma_\theta > 27.3$ kg m^{-3}was selected for the ETNP due to the deeper ODZ at this location as in a previous study (Kelly et al., 2021). An isopycnal range with $\sigma_\theta > 27$ kg m^{-3} was selected for the ETSP comparable to the σ_θ range in Casciotti et al. (2018). N_2O source values of 9.1‰ ($\delta^{15}N^{bulk}$), 16.8‰ ($\delta^{15}N^\alpha$), 1.4‰ ($\delta^{15}N^\beta$), 52.7‰ ($\delta^{18}O$) and 15.4‰ (SP) were estimated below the ETNP ODZ (Table 1). In the ETSP, the Keeling plot analysis revealed values of 7.0‰ ($\delta^{15}N^{bulk}$), 17.0‰ ($\delta^{15}N^\alpha$), -3.0‰ ($\delta^{15}N^\beta$), 45.5‰ ($\delta^{18}O$) and 20.0‰ (SP). The generally higher $\delta^{15}N^\beta$ as well as lower $\delta^{18}O$ and SP compared to prior studies (Casciotti et al., 2018; Kelly et al., 2021) might be the result of a lesser influence from N_2O production and consumption in the ODZ since our analysis also included equatorial waters. Overall, these isotopic signatures suggest the dominance of N_2O production by nitrification in deeper waters below the ETNP and ESTP ODZs. In fact, our results are more in line with a N_2O source from nitrification as a relatively low $\delta^{18}O$ is expected for this process (i.e., 13-35‰; Snider et al., 2012).

The sources of N_2O were investigated by restricting the Keeling plot analysis for the absolute salinity/potential temperature ranges characteristic of AAIW as described in section 3.1. The analysis was restricted to 60°S to 20°S, even if the northernmost extent of AAIW is found below the ETNP, to eliminate possible effects of the ETNP and ETSP ODZs on N_2O production in this water mass.

ΔN_2O clearly increased from -0.89 nmol kg^{-1} (-7.4% supersaturation) at 52°S to up to 20.6 nmol kg^{-1} (180% supersaturation) in the intermediate water mass AAIW (Figure 5), which is comparable to values observed by Carrasco et al. (2017). Keeling plot analysis revealed a N_2O source with a $\delta^{15}N^{bulk}$ = 9.4‰, $\delta^{15}N^\alpha$ = 18.1‰, $\delta^{15}N^\beta$ = 0.8‰, $\delta^{18}O$ = 52.5‰, and SP = 17.2‰ (Table 1). The relatively high SP suggests that N_2O is mainly derived from nitrification, consistent with previous studies (Casciotti et al., 2018; Toyoda et al., 2019; Kelly et al., 2021). The positive significant relationship observed between ΔN_2O and AOU in the AAIW water mass observed in this study and Carrasco et al. (2017) further supports this interpretation (Supplementary materials, Figure S2). The lower $\delta^{15}N^\beta$ and higher $\delta^{18}O$ and SP might reflect the influence of N_2O consumption in the ODZs in these prior studies.

4.3 Isotopic signatures of N_2O consumption in the ETNP

High values of $\delta^{15}N^\alpha$, $\delta^{18}O$ and SP and low or negative ΔN_2O were observed at $[O_2] < 5$ µmol kg^{-1} in the ETNP ODZ, which are clear signatures of N_2O consumption (Figure 5). During N_2O reduction to N_2 gas, the N-O bond is broken, leaving the remaining substrate (N_2O) enriched in ^{15}N and ^{18}O. The α position in N_2O is preferentially enriched in ^{15}N compared to the β position since it is directly attached to the O atom being cleaved (e.g., Popp et al., 2002; Toyoda et al., 2002). Thus, ε for the α N atom (6.6-9.1‰) and $\delta^{18}O$ (10.9-15‰) are relatively large with only a small or negligible ε for β N atom (Ostrom et al., 2007). Notably, while $\delta^{15}N^\alpha$ and $\delta^{18}O$ increased in the ODZ, $\delta^{15}N^\beta$ generally decreased, as also observed in previous ETNP and ETSP ODZ studies (Bourbonnais et al., 2017; Casciotti et al., 2018; Kelly et al., 2021) (Figure 5). This trend is not expected during pure N_2O consumption.

The slope for the relationship between $\delta^{18}O$ versus and $\delta^{15}N^\alpha$ (1.6 ± 0.1) was indistinguishable from the expected slope (i.e., 1.7) during N_2O consumption in soils and pure denitrifier cultures (Ostrom et al., 2007). However, the observed slope for $\delta^{18}O$-N_2O versus SP (0.8 ± 0.1) clearly deviated from the expected value of 2.2 for pure N_2O reduction (Ostrom et al., 2007) (Figure 10). A wider
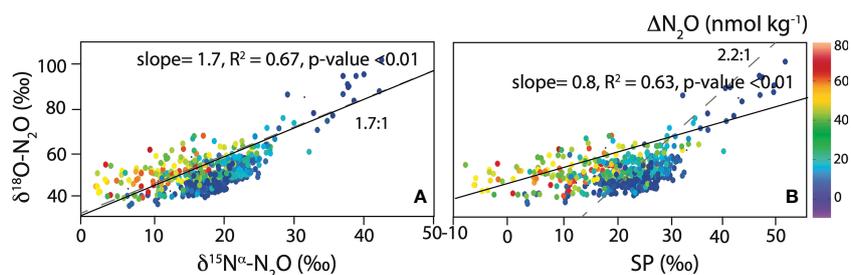
FIGURE 10
$\delta^{18}N$-N_2O versus **(A)** $\delta^{15}N^\alpha$-N_2O, and **(B)** SP. The slope expected for pure denitrification is indicated with dashed grey lines (Ostrom et al., 2007). ΔN_2O concentrations are shown as the color of the symbols (see color bar in top panel). Linear regressions for $O_2 < 5$ µmol kg^{-1} are shown with black lines.

range of 1.3-3.3 for $\delta^{18}O$ versus SP during consumption by denitrification was also reported for soil mesocosms, which is still higher than the slope observed in this study (Lewicka-Szczebak et al., 2017). This observation is consistent with previous studies in marine ODZs, reporting slopes for $\delta^{18}O$ versus SP ranging between (0.9-1.8) (Bourbonnais et al., 2017; Casciotti et al., 2018; Kelly et al., 2021). For instance, in the ODZ off Peru, the slope for increase in $\delta^{18}O$-N_2O versus SP deviates from what is expected during pure denitrification, mostly due to a decrease in $\delta^{15}N^\beta$ within the ODZ (Bourbonnais et al., 2017; Casciotti et al., 2018). This trend showing a decreasing $\delta^{15}N^\beta$ associated with high $\delta^{18}O$-N_2O has also been observed in other ODZs and marine anoxic environments (Yamagishi et al., 2005; Westley et al., 2006; Yamagishi et al.,

2007; Farías et al., 2009; Kelly et al., 2021). The cause for the decreasing $\delta^{15}N^\beta$ in the ODZ will be further discussed below.

In this study, apparent isotope effects (ε_{app}) were derived for all isopycnal ranges within the ETNP ODZ at $[O_2]$ < 5 µmol kg^{-1}, where N_2O consumption is known to occur (Dalsgaard et al., 2014). This approach was used in Casciotti et al. (2018) to further investigate if a decrease in $\varepsilon^{18}O$ or increase in εSP could explain the deviation from the expected slope for $\delta^{18}O$-N_2O versus SP during pure N_2O consumption in the ETSP ODZ. Apparent isotope effects calculated for all isopycnal ranges were 3.6‰ for $^{15}N^{bulk}$, 9.4‰ for $^{15}N^\alpha$, -2.3‰ for $^{15}N^\beta$, 12.0‰ for ^{18}O and 11.7‰ for SP (Table 2 and Figure 11). The ε_{app} for $^{15}N^{bulk}$ was slightly lower than the range reported for pure culture by Ostrom et al. (2007). Yet, the

TABLE 2 Apparent isotope effects for N$_2$O consumption (in ‰) calculated using a closed system Rayleigh model for $^{15}N^{bulk}$, $^{15}N^\alpha$, $^{15}N^\beta$, ^{18}O and SP for the ETNP.

	This study	Ostrom et al. (2007) Pure culture	Casciotti et al. (2018) ETSP ODZ	Kelly et al. (2021) ETNP ODZ
$\varepsilon^{15}N^{bulk}$	3.6 ± 0.4 R^2 = 0.65, P < 0.01	4.1 – 6.6	na	na
$\varepsilon^{15}N^\alpha$	9.4 ± 0.9 R^2 = 0.66, P < 0.01	6.6 – 9.1	na	11.8 ± 2.5
$\varepsilon^{15}N^\beta$	-2.3 ± 0.6 R^2 = 0.20, P < 0.01	1.6 – 2.2	na	-2.0 ± 2.0
$\varepsilon^{18}O$	12.0 ± 1.3 R^2 = 0.60, P < 0.01	10.9 – 15.0	14.5 – 25.6	20.2 ± 6.1
εSP	11.7 ± 1.4 R^2 = 0.57, P < 0.01	5.0 - 6.8	11.6 – 17.5	na
$\varepsilon^{18}O/\varepsilon^{15}N^{bulk}$	3.4 ± 0.5 R^2 = 0.91, P < 0.01	2.5 ± 0.2	na	na
$\varepsilon^{18}O/\varepsilon$SP	1.0 ± 0.2	2.2	0.9 – 1.5	na

Only samples with $[O_2]$ < 5 µmol kg^{-1} were considered. Isotope effects observed in pure laboratory culture and field studies in the ETNP and ETSP ODZs are also listed (Ostrom et al., 2007; Casciotti et al., 2018; Kelly et al., 2021). Standard error of the slope is shown. na means non available.
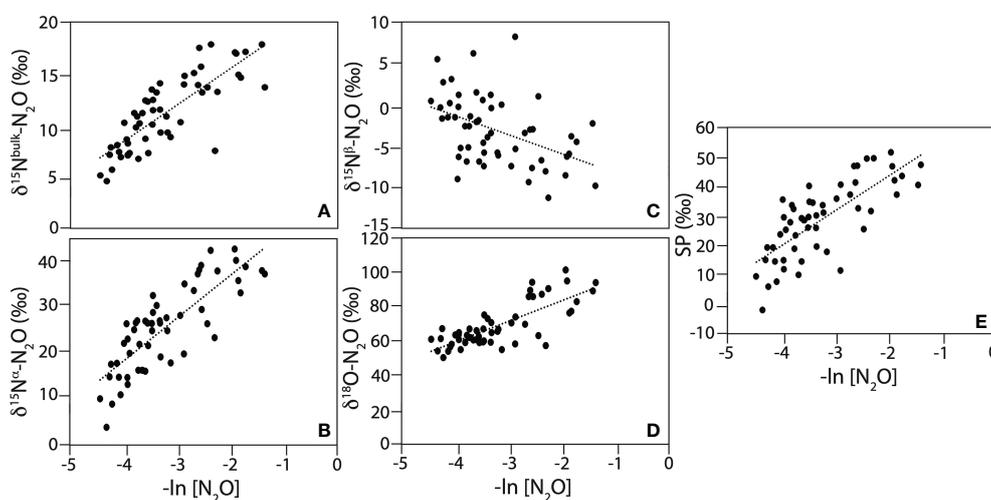
FIGURE 11
Apparent isotope effects for (A) $\delta^{15}N^{bulk}$-N_2O, (B) $\delta^{15}N^\alpha$-N_2O, (C) $\delta^{15}N^\beta$-N_2O, (D) $\delta^{18}O$-N_2O, and (E) SP calculated for $[O_2]$ < 5 µmol kg^{-1}. Black lines are linear regressions. Linear regression outputs are reported in Table 2.

ε_{app} for $^{15}N^{\alpha}$ and $^{15}N^{\beta}$ were comparable to values (11.8‰ and -2‰, respectively) estimated by Kelly et al. (2021) in the ETNP ODZ. Notably, the negative isotope effect for $^{15}N^{\beta}$ indicates a decrease in $\delta^{15}N^{\beta}$ as N_2O is reduced to N_2 within the ODZ. The ε for ^{18}O calculated in this study was significantly lower than the values (14.5 – 20.2‰) estimated in other ODZ marine field studies (Casciotti et al., 2018; Kelly et al., 2021), but comprised within the range observed for pure culture (10.9 – 15‰) (Ostrom et al., 2007). The ε for SP was higher than for pure culture (5.0 – 6.8‰) but comparable to values estimated in the ETSP ODZ (11.6 – 17.5‰) (Casciotti et al., 2018). Casciotti et al. (2018) observed different apparent isotope effects at different isopycnal ranges within the ETSP ODZ. They observed a decrease in the $\varepsilon^{18}O$ within the ODZ (from 25.6‰ in the upper, to 19.6‰ in the middle and 14.5‰ in the lower ODZ) while the εSP increased slightly and then decreased (17.5‰ in the upper to 21.7‰ in the middle to 11.6‰ in the lower ODZ). A more detailed analysis by isopycnal ranges (upper, middle, and lower ODZ) could however not reproduce observed trends in Casciotti et al. (2018) for the P18 transect. For instance, ε_{app} for ^{18}O (13.9‰ in the upper ODZ to 11.4‰ in the middle, and 16.2‰ in the lower ODZ) and SP (14.2‰ in the upper ODZ to 7.5‰ in the middle, and 17.9‰ in the lower ODZ) both decreased toward the middle ODZ and then increased in the deeper ODZ (Supplementary materials, Figure S5). The slope for $\varepsilon^{18}O$ versus εSP also gradually decreased from 1.6 to 0.65 instead of being lowest in the middle ODZ. Irrespective of the observed trends, using a modeling approach and isotope values of substrates (NO_3^- and $N^{18}O_2^-$) during denitrification, previous studies attributed the lower $\varepsilon^{18}O$:εSP to an increase in SP due to a decrease in $\delta^{15}N^{\beta}$ rather than a decrease in $\varepsilon^{18}O$ or increase in εSP in both the ETNP and ESTP ODZs (Casciotti et al., 2018; Kelly et al., 2021).

Several hypotheses have been proposed to explain the decreasing $\delta^{15}N^{\beta}$ in ODZ waters, including concurrent N_2O production via denitrification of NO_3^- with a site preference >0‰ (Schmidt et al., 2004; Casciotti et al., 2018; Kelly et al., 2021). Rate experiments as well as prior stable abundance isotopic studies suggest concurrent N_2O production and consumption as well as a rapid N_2O turnover in the ODZ (Farías et al., 2009; Babbin et al., 2015; Ji et al., 2015; Bourbonnais et al., 2017; Casciotti et al., 2018; Frey et al., 2020; Kelly et al., 2021). Casciotti et al. (2018) modeled N_2O cycling at steady-state in the ODZ using both $\delta^{15}N$ and $\delta^{18}O$ of substrate molecules (NO_2^- and NO_3^-) during denitrification. N_2O isotopic signatures were only reproduced when NO_3^- was used as the substrate and implying a site preference >0‰ (i.e., greater fractionation at the $^{15}N^{\beta}$ position relative to the $^{15}N^{\alpha}$) during N_2O production by denitrification. This suggests that an internal (rather than ambient) pool of NO_2^- is used during denitrification as further discussed in Casciotti et al. (2018). Accordingly, a relatively long residence time was estimated for ambient NO_2^- (in the order of months) in the primary NO_2^- maximum and low-O_2 waters based on rates of abiotic O isotope exchange between nitrite and water (Buchwald and Casciotti, 2013; Bourbonnais et al., 2015). In another modeling study, a non-steady-state N_2O cycling as well as an ambient NO_2^- substrate source (with a $\delta^{15}N$ as low as -30‰) or a SP of ~25‰ during concurrent N_2O production by denitrification were required to explain the observed low $\delta^{15}N^{\beta}$

values in the ETNP ODZ (Kelly et al., 2021). The low $\delta^{15}N^{\beta}$ of N_2O could also result from an alternate mechanism in the ODZ, such as N_2O production from AO archaea and bacteria, which is associated with a high SP (30-38‰; Sutka et al., 2003; Sutka et al., 2004; Frame and Casciotti, 2010; Santoro et al., 2011; Löscher et al., 2012). For instance, an archaeal AO isolated from ODZs (*Nitrosopumilus maritimus*) produced both N_2 and O_2 under dark anaerobic conditions, following a pathway that involves N_2O as an intermediate (Kraft et al., 2022). Yet, N_2O production rates from AO based on ^{15}N-labeled experiments were relatively low (up to 0.1 nmol L^{-1} d^{-1}) and generally at least one order of magnitude lower than N_2O production rates from NO_3^- (denitrification) under anoxic conditions in the ETSP ODZ (Frey et al., 2020).

The idea of denitrification with a site preference >0‰ is not new (i.e., see Schmidt et al., 2004). Several studies invoked N_2O production during denitrification with a site preference >0‰ in the ETNP and ETSP ODZs and Canadian Arctic bottom waters influenced by sedimentary processes (Casciotti et al., 2018; Lehmann et al., 2019; Kelly et al., 2021). Some variability in the SP of N_2O produced by denitrification was reported for different bacterial strains. For instance, Toyoda et al. (2005) observed a high SP of 22 to 24‰ for N_2O production during denitrification by *Pseudomonas fluorescens*, suggesting a symmetrical intermediate (i.e., *cis* formation mechanism). These observations for denitrifying bacteria are analogous to the wide range of SPs (15.8 – 37.1‰) that have been reported for different fungal species and strains (Maeda et al., 2015; Lazo-Murphy et al., 2022). These data challenge the conventional view that fungal denitrification fits into narrow compositional fields with relatively high SP values. Similarly, it is possible that denitrifying microbial consortia thriving under different O_2 regimes are associated with variable SPs, but this hypothesis remains to be verified.

5 Concluding remarks

This study presents oceanic N_2O concentration, stable isotope and isotopomer data of unprecedently high spatial resolution along the P18 line in the eastern Pacific sampled in 2016/2017. Highest ΔN_2O accumulations (up to 940% supersaturation) were observed close to the oxycline in both the ETNP and the fringe of the ETSP ODZ. N_2O yield from AOU increased at lower $[O_2]$, consistent with previous studies. Keeling plot analysis identified two distinct sources of N_2O at different $[O_2]$ regimes. At lower $[O_2]$ concentrations close to the oxycline in both ODZs, where highest ΔN_2O were observed, SP of source N_2O was relatively low, suggesting production from denitrification (or nitrifier-denitrification). Relationships between ΔN_2O and DIN deficit and the isotopic composition of $\delta^{15}N$ of the substrate (NO_3^-) further suggest that denitrification is the dominant process at low $[O_2]$ concentrations. SP generally increased in more oxygenated surface waters, suggesting a greater contribution from nitrification. The isotopic composition of source N_2O in deeper waters of the ETNP and ETSP as well as in AAIW also suggested that nitrification was the main pathway for N_2O formation.

The $\delta^{18}O$ versus $\delta^{15}N^{\alpha}$ relationship showed a slope characteristic of N_2O consumption during denitrification in low O_2 waters. Isotope

effects calculated for N_2O consumption were consistent with previous field and laboratory studies (Ostrom et al., 2007; Casciotti et al., 2018; Kelly et al., 2021). Yet, $\delta^{15}N^\beta$ decreased (rather than the predicted no change or slight increase during denitrification) in the ODZ, which was also observed by previous studies (e.g., Bourbonnais et al., 2017; Casciotti et al., 2018; and Kelly et al., 2021). These signatures are best explained by concurrent N_2O production (from NO_3^- or NO_2^-) with a site preference >0‰, an ambient NO_2^- source with a low $\delta^{15}N$ and non-steady-state conditions (e.g., Kelly et al., 2021). Clearly, more research is needed to elucidate the SP and its variability for diverse denitrifying bacterial strains living in marine ODZs as current values are mostly from terrestrial environments (e.g., Toyoda et al., 2005). Some contribution from an alternative N_2O production pathway is also possible, for example anaerobic AO (see Kraft et al., 2022).

This study establishes a benchmark against which to evaluate changes in N_2O cycling for future decadal occupations of the P18 line. ODZ are currently expanding (Stramma et al., 2013), with unknown impacts on N_2O cycling. The vertical expansion of ODZs not only increases the volume of low-O_2 waters where N_2O is potentially produced but also increases N_2O exchange with the atmosphere. However, co-occurring warming causes stronger stratification, which could reduce mixing and N_2O outgassing to the atmosphere. At this point, it is unclear which mechanism will dominate. Thus, more observational data is needed to evaluate the impacts of ODZs expansion on marine N_2O cycling and atmospheric emissions.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://cchdo.ucsd.edu/cruise/33RO20161119.

Author contributions

AB, SD, and MA designed the study. AB analyzed stable isotope and isotopomer samples. BC analyzed N_2O concentration and CFC/SF_6 samples and RS calculated the transit time distributions. AB wrote the manuscript with input from all co-authors. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1137064/full#supplementary-material

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