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Mechanisms of nitrous oxide production in the subtropical North Pacific based on determinations of the isotopic abundances of nitrous oxide and di-oxygen

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Importance of this paper: Nitrous oxide is an important greenhouse gas that has been rising steadily in the last 100 years. The oceans are recognized as an important source of nitrous oxide to the atmosphere, however, the biochemical pathways resulting in its formation are not well known. In this study, we determined the stable isotopic composition of nitrous oxide, di-oxygen and water at a station in the subtropical North Pacific Ocean. Our stable isotope data confirmed that nitrification is the principal means by which nitrous oxide is produced in di-oxygen replete waters but also revealed two pathways of formation; one of which predominated between 350 and 500 m depth. Knowledge of the pathways of nitrous oxide formation will enable a better understanding of the conditions resulting in its formation and fluxes out of the ocean.

Abstract

In this study, we compare stable isotopic compositions of di-oxygen (O_2) and nitrous oxide (N_2O) in two depth profiles at the well-characterized deep water station ALOHA (A Long-term Oligotrophic Habitat Assessment) in the subtropical North Pacific gyre to attain an understanding of the mechanisms of N_2O production. The $\delta^{18}O$ of O_2 varied from values indicative of an atmospheric origin near the surface (24.7‰), to minimum values reflective of a predominance of photosynthesis over respiration between the surface and 200 m (as low as 22.2‰), to maximum values as high as 36.6‰ in association with the O_2 minimum near 800 m. A similar pattern of isotopic variation was evident in the $\delta^{18}O$ of N_2O, however, values were enriched by approximately 20‰. The similar pattern of variation in $\delta^{18}O$ with depth is consistent with an origin of O in N_2O from dissolved O_2 via the nitrification of intermediate compounds NH_2OH or NO. Between the depths of 350 and 500 m, however, the distinction in the isotopic composition of N_2O and O_2 was reduced to as little as 12‰. This decrease in the difference between the $\delta^{18}O$ of N_2O and that of O_2 with depth indicates either a reduction in the magnitude of isotopic discrimination during nitrification or a contribution of O in N_2O from water via the reduction of NO_2^-. Two mechanisms of N_2O production via nitrification may, therefore, occur in the subtropical Pacific; release from the nitrification of NH_2OH or NO at most depths and reduction of NO_2^- between 350 and 500 m. In that, the carbon flux decreases markedly over a similar depth interval at this locale (Karl, D.M., Knauer, G.A., Martin, J.H., 1988. Downward flux of particulate organic matter in the ocean: A particle decomposition paradox. Nature 332, 438–441), this distinct mechanism of N_2O production between 350 and 500 m may be associated with the mineralization of organic matter from sinking particles. Low O_2 or anoxic micro-environments within particles within this depth interval may be maintained by lower ambient O_2 than at the surface and high rates of microbial activity

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supported by the mineralization of organic matter. Such conditions may facilitate an environment conducive to N_2O production via NO_2^- reduction. © 2000 Elsevier Science Ltd. All rights reserved.
Keywords: Nitrification; Denitrification; Nitrogen isotopes; Oxygen isotopes; Nitrous oxide; Subtropical North Pacific

1. Introduction

Observations of steadily increasing concentrations of N_2O in the atmosphere have raised questions regarding the origins, sinks and mechanisms that produce this greenhouse gas (Rasmussen and Khalil, 1986; Prinn et al., 1990). Insight into the origins of N_2O in the ocean and atmosphere has recently been provided by stable isotope data (Kim and Craig, 1990, 1993; Yoshinari et al., 1997; Dore et al., 1998; Naqvi et al., 1998), however, less is known about the relative importance of different microbial pathways of N_2O production. Although the production of N_2O from denitrification and nitrification has long been recognized, the exact chemical mechanism of N_2O formation from nitrification is uncertain and likely proceeds by more than one pathway (Ritchie and Nicholas, 1972; Groffman, 1991). The magnitude and direction of isotopic discrimination for each pathway may be distinct, therefore, in addition to providing information on sources, stable isotope data can yield insight into the predominant biochemical mechanism of production or consumption.

Denitrification is the reduction of oxidized forms of inorganic nitrogen to nitrogen gases, primarily N_2O and N_2, and is described by the pathway indicated in Fig. 1 (Knowles, 1981; Zafiriou et al., 1989; Goretski and Hollocher, 1990). The presence of O_2 is generally considered to have an inhibitory effect on denitrification (Knowles, 1981; Payne, 1981). In oxic ocean waters, however, the interior of particles has been proposed as a site that may provide the conditions suitable for denitrification (Kaplan and Wofsy, 1985; Yoshinari and Koike, 1994). Denitrification produces and consumes N_2O and markedly high $\delta^{15}N$ [1] and $\delta^{18}O$ values for this gas in anoxic water suggests that the isotope effect during consumption predominates in these areas (Yoshinari et al., 1997; Naqvi et al., 1998).

The exact biochemical pathway of N_2O generation via nitrification is not clear, however, a series of mechanisms have been proposed (Fig. 1) (Ritchie and Nicholas, 1972; Naqvi and Noronha, 1991). Formation of N_2O can occur from the oxidation of NH_4^+ via the intermediate compounds NH_2OH (pathway 1) or NO (pathway 2). These compounds rarely accumulate during nitrification and are present in the ocean at extremely low concentrations (Von Breyman et al., 1982; Ward and Zafirou, 1988). Pathway 3, here termed nitrifier–denitrification, has generally been considered to be the predominant pathway in soils and oceans but is likely to be restricted to low oxygen conditions (Poth and Focht, 1985; Remde and Conrad, 1990). The potential exists that some of the transformations described by these chemical pathways may be carried out by unique microbial metabolisms including aerobic denitrification, heterotrophic nitrification, and anaerobic ammonium oxidation (Jetten et al., 1997, 1999; Strous et al., 1999). In marine environments, support for the predominance of pathway 3 has been based on the observation of an inverse relationship between the concentrations of N_2O and O_2 (Goreau et al., 1980; Hynes and Knowles, 1984). Anoxic microsites in soils and suspended or sinking particles in the ocean are the most likely environments providing the reducing conditions favoring this process (Alldredge and Cohen, 1987; Paerl and Prufert, 1987).

Recent studies of the stable isotopic composition of N_2O have suggested that the N_2O in oxic waters is

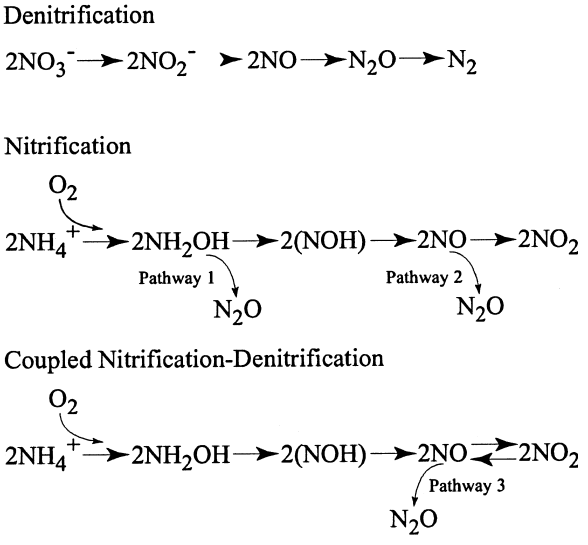


Fig. 1. Biochemical pathways of N_2O production from denitrification and nitrification.

[1] N and O stable isotope ratios are expressed in per mil (‰) notation: $\delta^{15}N$ or $\delta^{18}O = [(R_{sample}/R_{standard}) - 1] * 1000$ where R is the abundance ratio of the heavy to light isotope. The internationally recognized standard for N and O are atmospheric N gas and V-SMOW, which, by definition, have per mil values of 0.

produced mainly by nitrification, however, the importance of production by the intermediate compounds NH_2OH or NO or by coupled nitrification–denitrification is uncertain (Kim and Craig, 1990; Naqvi and Noronha, 1991; Naqvi et al., 1998). The purpose of this paper is to expand on these earlier studies and use the $\delta^{18}O$ of N_2O, H_2O and O_2 to better define the mechanisms of N_2O production in the oligotrophic subtropical North Pacific. During the nitrification sequence, the first O atom added is derived from O_2 whereas the second is derived from water (Dua et al., 1979; Hollocher et al., 1981; Andersson and Hooper, 1983; Kumar et al., 1983). The $\delta^{18}O$ of water and O_2 in the ocean differ by greater than 20‰ (Kroopnick and Craig, 1976). Therefore, knowledge of the source of O in N_2O and an understanding of the direction and magnitude of shifts in isotopic abundances may provide an indication of the relative importance of denitrification and nitrification in the production of N_2O as well as insight into the biochemical pathways utilized in nitrification. This study provides the first detailed comparison between the isotopic composition of N_2O, H_2O and O_2 within the water column of the oligotrophic subtropical North Pacific as a means to better understand the dynamics of N_2O production in the ocean.

2. Methods

Samples were collected from the oligotrophic subtropical North Pacific at Hawaii Ocean Time-series (HOT) station ALOHA (A Long-term Oligotrophic Habitat Assessment) (22°45′N, 158°00′W; Karl and Lukas, 1996), during HOT 98 (10/98 R/V Moana Wave) and HOT 101 (1/99; R/V Moana Wave) research cruises. Station ALOHA is located 100 km north of the island of Oahu, Hawaii and has a water column depth of 4500 m.

Samples for determination of the $\delta^{18}O$–O_2 were collected in pre-evacuated glass vessels following the procedure of Emerson et al. (1991, 1999). The $\delta^{18}O$ of O_2 was accomplished using gas chromatography interfaced to a Micromass Prism stable isotope ratio mass spectrometer (Roberts et al., 2000). The sample vessel was connected to an inlet system on the gas chromatograph that consists of, in series, an ascarite trap to remove water, a LiOH trap to remove CO_2, a 3-ml gas sampling loop with two 6-port sampling valves, a vacuum isolation valve, and a vacuum pump. Initially, the inlet system was completely evacuated and then isolated from vacuum. The sample was then released and immediately expanded throughout the inlet system. During this process water and CO_2 were absorbed. After 10–15 s of equilibration, the valves on the gas sampling loop were rotated to initiate He flow through the loop and push sample gases onto the GC column. A 5 m by 1/8″ OD molecular sieve 5 Å column (Alltech) was used with a He head pressure of 50 PSI and constant temperature of 50°C to separate N_2 and O_2. The outflow of the gas chromatograph was allowed to enter the mass spectrometer (via an open split to reduce over-pressurizing the mass spectrometer) and the isotope ratio was determined in comparison to a reference gas. Precision of replicate samples is 0.2‰ or better.

Subsamples for isotopic analyses of N_2O were taken from the PVC water sample bottles in 250 ml serum vials, preserved with $HgCl_2$, and capped with a butyl rubber stopper for later laboratory analysis. The analytical system used for stable isotopic analysis of N_2O is a modification of that described by Sansone et al. (1997) which was originally designed for analysis of the $\delta^{13}C$ of dissolved CH_4 in seawater and is described in detail in Dore et al. (1998). Briefly, seawater was transferred directly from a serum vial to a sparging column and the dissolved gases stripped from seawater using ultra-pure He. Excess water vapor and CO_2 were removed from the sparged gases and the remaining condensable gases trapped. These gases were cryofocused (see Popp et al., 1995) prior to separating N_2O from other trapped gases using a PoraPLOT-Q analytical column. N_2O concentration, $\delta^{15}N$, and $\delta^{18}O$ were measured simultaneously by monitoring the ion currents of masses 44, 45 and 46 using a Finnigan MAT 252 mass spectrometer. $\delta^{15}N$ values were corrected for ^{17}O using the method following Brand (1995). Analysis of triplicate samples yielded reproducibility better than 0.5‰ for $\delta^{15}N$ and 0.8‰ for the $\delta^{18}O$ of dissolved N_2O in 250 ml of near-surface waters (~6 nM). The isotopic composition of our laboratory standard gas was characterized using the traditional analytical methods of Yoshida and Matsuo (1983) and Yoshinari (1990). Calibration of N_2O concentration is achieved using a commercial gas mixture.

Water samples were collected in 20 ml crimp seal vials from the upper 500 m (HOT 101 only) for determination of the $\delta^{18}O$ of H_2O. Samples were prepared for isotopic analysis following the procedure of Epstein and Mayeda (1953). Precision of the measurement is better than 0.15‰.

For dissolved O_2 determinations, seawater from predetermined depths was collected in PVC sample bottles and drawn into calibrated iodine flasks, fixed with standard Winkler reagents (Carpenter, 1965) and stored in the dark. The $Mn(OH)_3$ floc was dissolved with acid and the I_3^- was quantitatively titrated with $S_2O_3^{2-}$ to a potentiometric endpoint using a Dosimat-based, computer-assisted titration system. Analysis of replicate samples has yielded a precision of 0.1% or better.

3. Results

The depth profiles of O_2 and N_2O concentrations and isotopic abundances at station ALOHA for cruises HOT

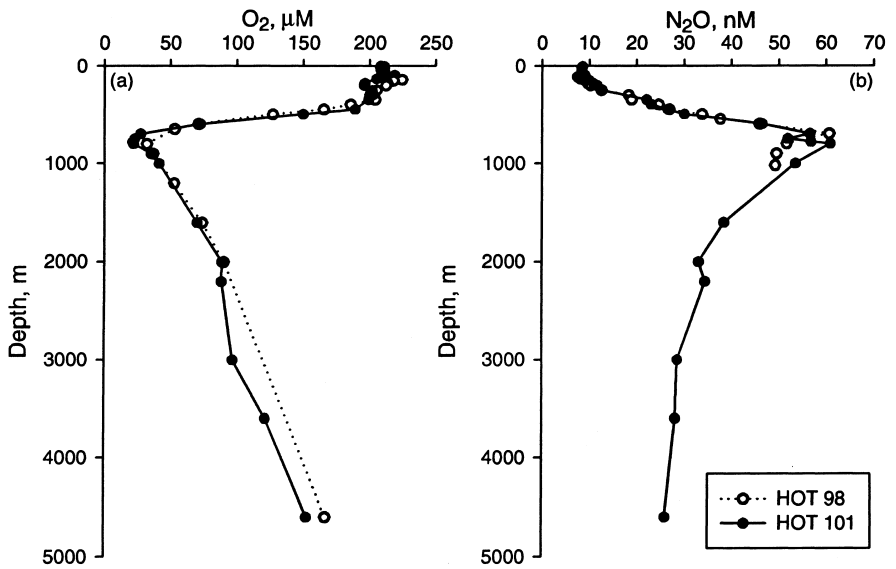


Fig. 2. The concentration of (a) O_2 in μM (10^{-6}M) and (b) N_2O in nM (10^{-9}M) at station ALOHA for research cruises HOT 98 and HOT 101.

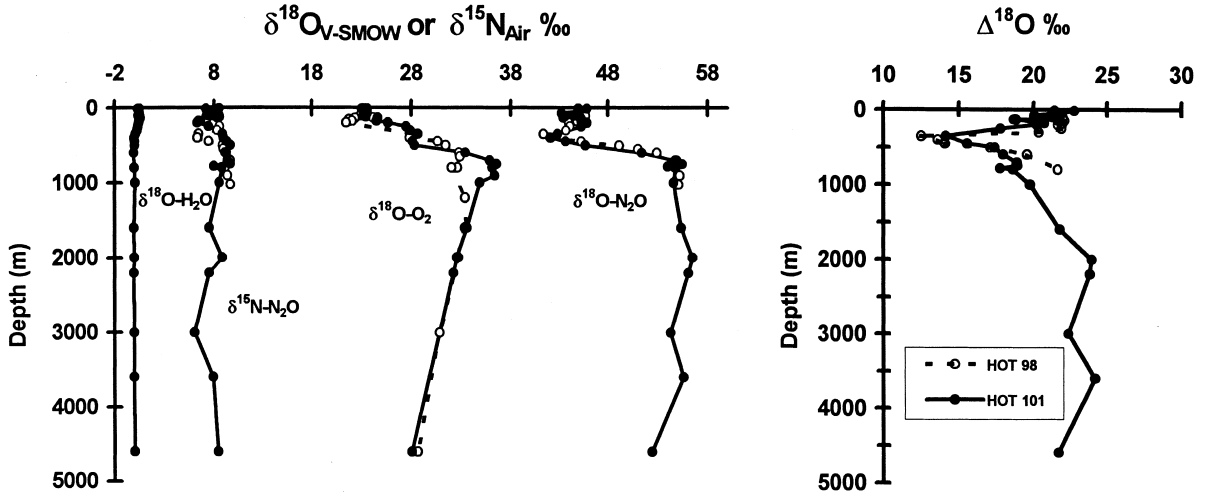


Fig. 3. The isotopic composition O_2 and N_2O at station ALOHA for research cruises HOT 98 and HOT 101 (a) and the difference between N_2O and O_2 in $\delta^{18}O$ (b).

98 and HOT 101 are similar to those reported in previous studies at this location (Figs. 2 and 3) (Bender and Grande, 1987; Emerson et al., 1993, 1995; Dore et al., 1998). Concentrations of O_2 ranged between 21.4 and 224.6 μM and obtained minimum values for both sampling dates at a depth of approximately 800 m. The $\delta^{18}O$ of O_2 varied between 22.2‰ and 36.6‰ with maximum values associated with the O_2 minimum region. Isotope values in surface waters of approximately 24.7‰ are in equilibrium with atmospheric O_2 (23.5‰) after accounting for the small isotope effect during gas exchange (Kroopnick and Craig, 1972; Knox et al., 1992; Quay et al., 1993). In near surface waters (0–200 m) $\delta^{18}O$ values less than 24.7‰ reflect a contribution of O_2 from photosynthesis (Bender and Grande, 1987; Quay et al., 1993). During photosynthesis, O_2 is released with an isotopic composition similar to that of the surrounding waters which at station ALOHA is approximately 0‰ (Fig. 3). Increases in $\delta^{18}O$ values with depth result from fractionation associated with respiratory consumption of O_2 (Bender, 1990; Kiddon et al., 1993; Quay et al., 1993). The two profiles of $\delta^{18}O$–O_2 values differ slightly; lower values are present between 130 and 200 m during the HOT 98 collection. These lower $\delta^{18}O$ values are

consistent with a greater supply of O_2 from photosynthesis, and with the observation that primary production at station ALOHA is generally restricted to the upper 175 m (Karl et al., 1996). Concentrations of N_2O increased markedly with depth at station ALOHA from values of 8.5 nM at the surface to values as high as 60.9 nM within the O_2 minima (Fig. 2). A significant inverse relationship exists between the concentrations of N_2O and O_2 ($r^2 = 0.94$; $n = 41$; $P > 0.05$). A similar relationship between N_2O concentration and apparent oxygen utilization has previously been interpreted as suggesting a nitrification source for N_2O (Yoshinari, 1976; Cohen and Gordon, 1979; Cline et al., 1987; Oudot et al., 1990). Profiles of the $\delta^{15}N$ and $\delta^{18}O$ of N_2O were similar between the two sample dates and ranged from 6.0‰ to 11.5‰ and 40.0‰ and 56.5‰, respectively (Fig. 3).

4. Discussion

The isotopic composition of N_2O is largely controlled by kinetic isotope effects in which ^{14}N or ^{16}O is preferentially transferred to the product of a reaction (Kim and Craig, 1990, 1993). For example, the isotopic composition of N_2, the primary product of denitrification, can be depleted in ^{15}N relative to the substrate, NO_3^- by as much as 40‰ (Cline and Kaplan, 1975). N_2O is a product of nitrification and denitrification and marked depletions in ^{15}N have been observed in this gas relative to the initial isotopic composition of NH_4^+ and NO_3^-, respectively (Yoshida et al., 1984; Yoshinari and Wahlen, 1985; Yoshida, 1988; Wada et al., 1996; Barford et al., 1999). Denitrification also results in the consumption of N_2O in which ^{15}N and ^{18}O are preferentially concentrated in the residual N_2O (Yoshida et al., 1984; Wahlen and Yoshinari, 1985; Yamazaki et al., 1987; Barford et al., 1999). Consequently, depletions in ^{15}N and ^{18}O are generally indicative of the production of N_2O by nitrification or denitrification and enrichments may be attributed to consumption by denitrification. The observation of $\delta^{15}N$ and $\delta^{18}O$ values in near surface oxic waters similar or less than those of atmospheric N_2O, for example, is strong evidence of a nitrification source (Dore et al., 1998; Naqvi et al., 1998). Furthermore, in the oligotrophic North Pacific these depleted values were associated with substantial nitrification activity and maxima in NO_2^- concentrations (Dore and Karl, 1996a,b). Consumption of N_2O by denitrification in anoxic waters has clearly been recognized by dramatic enrichments in ^{15}N and ^{18}O (Yoshinari et al., 1997; Naqvi et al., 1998). Although denitrification may also yield N_2O depleted in the ^{15}N and ^{18}O within oceanic regions with low or undetectable O_2 concentrations, the isotope effect associated with the consumption of N_2O from denitrification generally overwhelms that associated with its production and markedly high $\delta^{15}N$ and $\delta^{18}O$ values have been observed (Wada et al., 1996; Yoshinari et al., 1997; Naqvi et al., 1998).

Beyond this simple interpretation of the relative importance of denitrification and nitrification in N_2O production and consumption, the stable isotope data have several puzzling attributes that demand attention. First, $\delta^{18}O$ values for N_2O are exceptionally high and show dramatic shifts with depth even in oxic waters in which, presumably, denitrification is not important (Kim and Craig, 1990; Dore et al., 1998). Second, the large shift in $\delta^{18}O$ with depth, of greater than 15‰, is suggestive of an isotope effect associated with the consumption of N_2O during denitrification, however, other evidence is consistent with a source from nitrification. Previously, a minimum in the nitrogen and oxygen isotopic composition of N_2O between 200 and 300 m and values lower than those expected from atmospheric equilibration have been demonstrated at station ALOHA (Dore et al., 1998). Given that the consumption of N_2O by denitrification involves a large nitrogen isotope effect of 27‰ to 39‰ (Yoshida et al., 1984; Yamazaki et al., 1987), $\delta^{15}N$ values less than those of N_2O in the atmosphere are not expected if this process is predominant. The $\delta^{15}N$ of N_2O does increase with depth (by 6‰; Fig. 3) which is suggestive of denitrification, however, this is a small increase relative to that in $\delta^{18}O$ (15‰) and a small shift in comparison to the $\delta^{18}O/\delta^{15}N$ of approximately 0.5 reported for the denitrification of NO_3^- (Böttcher et al., 1990). Evidence for the predominance of denitrification has been provided by increases in the ^{15}N content of NO_3^- in the water column, particularly in anoxic waters (Cline and Kaplan, 1975; Liu and Kaplan, 1989). Such shifts in the isotopic composition of NO_3^- within the O_2 minimum zone were not evident in nearby waters (Cline and Kaplan, 1975; Liu and Kaplan, 1989) and at station ALOHA (Ostrom, unpublished data). These lines of reasoning strongly suggest that denitrification is not the predominant process controlling the isotopic character of N_2O in the subtropical North Pacific.

While the isotopic evidence is not consistent with the consumption of N_2O by denitrification as a control on its isotopic composition, the mechanisms responsible for the observed isotopic trends are still not clear. Reduction of NO_2^- via nitrifier–denitrification (pathway 3) is a likely mechanism to explain low isotope values relative to atmospheric N_2O in surface waters. Pathway 3, however, is unlikely as a mechanism in deep waters because this process should yield N_2O depleted in ^{15}N and ^{18}O and enrichments are observed. Such unique isotope behavior is best understood by recognizing that N_2O produced via nitrification can potentially occur at two branch points in the NO_2^- production sequence yielding N_2O from the intermediate compounds

NH_2OH (pathway 1) or NO (pathway 2). If these intermediate compounds are primarily oxidized to NO_2^-, then ^{15}N and ^{18}O will be concentrated in the residual NH_2OH and/or NO. Upon production of N_2O, the isotopically enriched signal within the intermediary compounds will be transferred to N_2O (Kim and Craig, 1990). Evidence in support of this mechanism was provided by the observation of ^{15}N enriched NO produced by nitrification in incubation studies (Yoshida, 1988). Naqvi and Noronha (1991) suggested that coupled nitrification and denitrification might be possible whereby the residual isotopically enriched NO partially consumed by denitrification might then be transformed to N_2O by nitrification. The observation, however, of isotopic enrichments in NO does not eliminate the possibility of only a nitrification control. Furthermore, denitrification is unlikely in the oxic water column of ALOHA (Dore et al., 1998).

Insight into the relative importance of the biochemical pathways of N_2O production via nitrification can be provided by an understanding of the source of O in N_2O. The first O atom added to NH_4^+ within the nitrification sequence to yield NH_2OH and NO is derived from dissolved O_2, whereas the second O atom required to yield NO_2^- is derived from water (Dua et al., 1979; Hollocher et al., 1981; Andersson and Hooper, 1983; Kumar et al., 1983). Water is depleted in ^{18}O by 23.5‰ relative to O_2 because of fractionation associated with respiration (Kroopnick and Craig, 1976). Consequently, pathways 1 and 2 may potentially be distinguished from pathway 3 by a comparison of the $\delta^{18}O$ of N_2O with that of water and dissolved O_2. The $\delta^{18}O$ of water at station ALOHA was found to be close to 0‰ and varied by less than 1‰ throughout the water column (Fig. 3). Larger variation in the $\delta^{18}O$ of water has not been reported for other locations in the North Pacific (Craig, 1961; Craig and Weiss, 1970). The trend in the $\delta^{18}O$ of water with depth does not correlate with the isotope data for N_2O shown in Fig. 3. A significant relationship between the $\delta^{18}O$ of O_2 and that of N_2O ($F_{1,37} = 40.6$; $P < 0.1$) and shifts in $\delta^{18}O$ in each of these species with depth of approximately the same magnitude is strong evidence that the primary source of O in N_2O is dissolved O_2 and not water. A source of O in N_2O from O_2 is evidence that the production of N_2O occurs primarily from either NH_2OH or NO. At this time which of these intermediate compounds yields N_2O is unknown, however, this might be resolved in incubations with isotopically labeled NH_2OH or NO. The $\delta^{18}O$ of N_2O is still, nonetheless, enriched in ^{18}O by approximately 20‰ relative to that of O_2. This enrichment is consistent with the idea that the production of NO_2^- leaves a small residual intracellular pool of either NH_2OH or NO that is isotopically enriched in both ^{15}N and ^{18}O and it is from a portion of this residual pool that N_2O is produced. The magnitude of isotopic discrimination during nitrification must be greater for O than it is for N in that the range of $\delta^{15}N$ values in N_2O is substantially less than that of $\delta^{18}O$.

Despite the relationship between the $\delta^{18}O$ of N_2O and O_2, the $\Delta^{18}O$ ($\delta^{18}O$–N_2O–$\delta^{18}O$–O_2) is not constant as a function of depth and the interval between 350 and 500 m depth is characterized by values as much as 9‰ less than those at other depths (Fig. 3). Lower $\Delta^{18}O$ values between 350 and 500 m compared to those above and below this depth interval indicates that the source of O in N_2O or the magnitude of isotopic discrimination must be distinct. We have no evidence or reason to believe that isotopic discrimination during the production of N_2O via nitrification is unique within this depth interval. A greater contribution of O from water, however, is consistent with the direction of this isotope shift and suggests that over this depth interval N_2O may be derived, at least in part, from the reduction of NO_2^- via nitrification. The reducing conditions that might favor nitrifier–denitrification are not readily apparent based on O_2 concentrations. Concentrations of O_2 between 350 and 500 m are lower than at the surface but not nearly as low as those present within the O_2 minimum near 800 m (Fig. 2). The depth interval between 150 and 500 m is however, characterized by a dramatic decrease in the flux of sinking particles at station ALOHA and elsewhere in the North Pacific (Karl et al., 1988, 1996; Christian et al., 1997). Karl et al. (1988) indicate that microbial growth associated with sinking particles is not sufficient to explain the entire reduction in carbon flux, consequently, the release of suspended particles and dissolved organic matter from sinking particles may promote microbial activity over this depth interval including the production of NO_3^- (Karl et al., 1988). The decrease in $\Delta^{18}O$ values over the same depth interval where reduction in carbon flux has been observed suggests that the liberation of dissolved or suspended matter may encourage nitrification using a pathway, nitrifier–denitrification, that is unique from the rest of the water column. The reduction of NO_2^- via nitrifier-dentrification is not expected in oxic waters, hence, it is likely that low O_2 or anoxic microsites within sinking or suspended particles are the site of N_2O production within this depth interval.

In summary, our data support the conclusion of others (Kim and Craig, 1990; Dore and Karl, 1996b; Dore et al., 1998) that nitrification is the primary source of N_2O in oxic Pacific waters. We do, however, conclude that there exists more than one mechanism of N_2O formation via nitrification in these waters. Within the mixed layer (~0–120 m) O_2 and N_2O are strongly influenced by exchange with the atmosphere as indicated by concentrations near saturation levels and isotope values similar to these gases in the troposphere (Kim and Craig, 1990). Nitrification at these depths is likely inhibited by high light levels (Olson, 1981; Ward et al., 1989). Within the depth interval of 120 and 350 m, N_2O

at supersaturation levels clearly indicates production and the similarity in $\Delta^{18}O$ to waters below 500 m is evidence for a pathway of formation similar to that in deep waters. The correlation between the $\delta^{18}O$ of O_2 and that of N_2O suggests that N_2O production occurs primarily via the intermediate compounds NH_2OH or NO. The $\Delta^{18}O$ minimum between 350 and 500 m clearly reflects a unique pathway of N_2O production that is consistent with the reduction of NO_2^- via nitrifier–denitrification. Our results and the observation of a $\Delta^{18}O$ minimum agrees well with data collected at three other locations in the Pacific (Kroopnick and Craig, 1976; Kim and Craig, 1990) (Fig. 4) which demonstrates that the unique pathway of N_2O production in this depth interval is likely to be a ubiquitous phenomena throughout the Pacific and possibly other oceans as well.

The question remains as to what environmental conditions favor a unique pathway of N_2O formation within this depth interval. Concentrations of O_2 have been clearly recognized as an important control on N_2O production by both nitrification and denitrification as evidenced by increased yields of N_2O relative to NO_2^- by nitrifiers as O_2 declines and increased production of N_2O by denitrification at very low O_2 levels (Goreau et al., 1980; Lipschultz et al., 1981; Jorgensen et al., 1984; Seitzinger et al., 1984). Kaplan and Wofsy (1985) discuss the possibility of anoxic interiors of particles in the ocean as a function of particle sizes, ambient O_2 concentrations, and rates of microbial activity. Within the euphotic zone, given the high concentrations of O_2 in relation to the typical sizes of particles (Sheldon et al., 1972; Bishop et al., 1977), anoxic interiors are unlikely. In the deep ocean, O_2 consumption is limited by low temperatures and a supply of labile organic matter, consequently, anoxic interiors are also unlikely. Nitrification does not require anoxic conditions, however, production of N_2O by nitrifiers is favored at low O_2. Furthermore, the reduction of NO_2^- between 350 and 500 m as suggested by our data is most favorable under low O_2. We suggest that the presence of this unique pathway of N_2O production in the Pacific Ocean results from a lowered ambient O_2 relative to the surface, and an enhanced supply of labile organic matter from sinking particles that are clearly mineralized within this depth interval (see Karl et al., 1988). The mineralization of organic matter from sinking particles serves (1) as a supply of NH_4^+ to support nitrification and (2) to enhance microbial activity and O_2 consumption within particles. Other possible conditions that may favor a unique pathway of N_2O production within the 350–500 m interval include activity by a novel assemblage of microbes or the enhancement of heterotrophic nitrification supported by the availability of labile organic matter. Further studies focused on nitrification in field and in the laboratory setting in which environmental conditions can be readily regulated will greatly clarify our understanding of nitrification pathways and associated N_2O production.

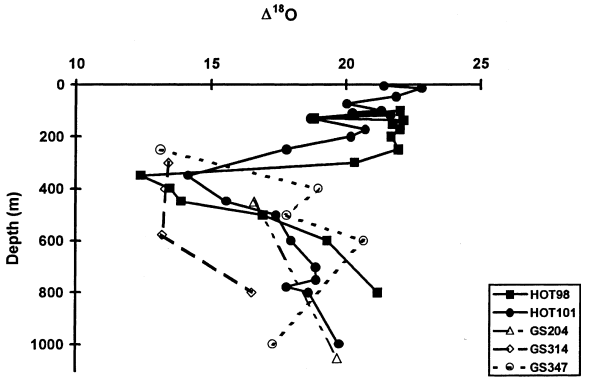


Fig. 4. The difference in $\delta^{18}O$ between N_2O and O_2 ($\Delta^{18}O$) in the upper 1000 m for cruises HOT 98 and HOT 101 to station ALOHA and GEOSECS stations GS204 (31°22′N, 150°02′W), GS314 (23°44′N, 153°37′W) and GS347 (28°30′N, 121°29′W). $\Delta^{18}O$ for the GEOSECS stations also show minimum values approximately between 350–500 m that is evident for the ALOHA results. Data for the GEOSECS stations are from Kroopnick and Craig (1976) and Kim and Craig (1990).

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