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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Abstract

In this study, we compare stable isotopic compositions of di-oxygen (O2) and nitrous oxide (N2O) 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 N2O production. The δ18O of O2 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 O2 minimum near 800 m. A similar pattern of isotopic variation was evident in the δ18O of N2O, however, values were enriched by approximately 20‰. The similar pattern of variation in δ18O with depth is consistent with an origin of O in N2O from dissolved O2 via the nitrification of intermediate compounds NH2OH or NO. Between the depths of 350 and 500 m, however, the distinction in the isotopic composition of N2O and O2 was reduced to as little as 12‰. This decrease in the difference between the δ18O of N2O and that of O2 with depth indicates either a reduction in the magnitude of isotopic discrimination during nitrification or a contribution of O in N2O from water via the reduction of NO2. Two mechanisms of N2O production via nitrification may, therefore, occur in the subtropical Pacific; release from the nitrification of NH2OH or NO at most depths and reduction of NO2 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 N2O production between 350 and 500 m may be associated with the mineralization of organic matter from sinking particles. Low O2 or anoxic micro-environments within particles within this depth interval may be maintained by lower ambient O2 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 \( \text{N}_2\text{O} \) production via \( \text{NO}_2^- \) 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 \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) production. Although the production of \( \text{N}_2\text{O} \) from denitrification and nitrification has long been recognized, the exact chemical mechanism of \( \text{N}_2\text{O} \) formation from nitrification is uncertain and likely proceeds by more than one pathway (Ritchie and Nicholas, 1972; Grofman, 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 \( \text{N}_2\text{O} \) and \( \text{N}_2 \), and is described by the pathway indicated in Fig. 1 (Knowles, 1981; Zafiriou et al., 1989; Goretski and Hollocher, 1990). The presence of \( \text{O}_2 \) 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 \( \text{N}_2\text{O} \) and markedly high \( \delta^{15}\text{N} \) and \( \delta^{18}\text{O} \) 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 \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) can occur from the oxidation of \( \text{NH}_4^+ \) via the intermediate compounds \( \text{NH}_2\text{OH} \) (pathway 1) or \( \text{NO} \) (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 Zafiriou, 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 \( \text{N}_2\text{O} \) and \( \text{O}_2 \) (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 \( \text{N}_2\text{O} \) have suggested that the \( \text{N}_2\text{O} \) in oxic waters is...
produced mainly by nitrification, however, the importance of production by the intermediate compounds NH₂OH 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 δ¹⁸O of N₂O, H₂O and O₂ to better define the mechanisms of N₂O production in the oligotrophic subtropical North Pacific. During the nitrification sequence, the first O atom added is derived from O₂ whereas the second is derived from water (Dua et al., 1979; Hollocher et al., 1981; Andersson and Hooper, 1983; Kumar et al., 1983). The δ¹⁸O of water and O₂ in the ocean differ by greater than 20‰ (Kroopnick and Craig, 1976). Therefore, knowledge of the source of O in N₂O 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 N₂O as well as insight into the biochemical pathways utilized in nitrification. This study provides the first detailed comparison between the isotopic composition of N₂O, H₂O and O₂ within the water column of the oligotrophic subtropical North Pacific as a means to better understand the dynamics of N₂O 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 δ¹⁸O–O₂ were collected in pre-evacuated glass vessels following the procedure of Emerson et al. (1991, 1999). The δ¹⁸O of O₂ 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 ascarrite trap to remove water, a LiOH trap to remove CO₂, 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 CO₂ 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 A column (Alltech) was used with a He head pressure of 50 PSI and constant temperature of 50°C to separate N₂ and O₂. 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 N₂O were taken from the PVC water sample bottles in 250 ml serum vials, preserved with HgCl₂, and capped with a butyl rubber stopper for later laboratory analysis. The analytical system used for stable isotopic analysis of N₂O is a modification of that described by Sansone et al. (1997) which was originally designed for analysis of the δ¹³C of dissolved CH₄ 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 CO₂ were removed from the sparged gases and the remaining condensable gases trapped. These gases were cryofocused (see Popp et al., 1995) prior to separating N₂O from other trapped gases using a PoraPLOT-Q analytical column. N₂O concentration, δ¹⁵N, and δ¹⁸O were measured simultaneously by monitoring the ion currents of masses 44, 45 and 46 using a Finnigan MAT 252 mass spectrometer. δ¹⁵N values were corrected for ¹⁷O using the method following Brand (1995). Analysis of replicate samples yielded reproducibility better than 0.5‰ for δ¹⁵N and 0.8‰ for the δ¹⁸O of dissolved N₂O 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 N₂O 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 δ¹⁸O of H₂O. 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 O₂ 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 flocc was dissolved with acid and the I₃⁻ was quantitatively titrated with S₂O₃²⁻ 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 O₂ and N₂O concentrations and isotopic abundances at station ALOHA for cruises HOT
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\textsubscript{2} ranged between 21.4 and 224.6 \textmu M and obtained minimum values for both sampling dates at a depth of approximately 800 m. The \( \delta^{18}O \) of O\textsubscript{2} varied between 22.2\permil and 36.6\permil with maximum values associated with the O\textsubscript{2} minimum region. Isotope values in surface waters of approximately 24.7\permil are in equilibrium with atmospheric O\textsubscript{2} (23.5\permil) 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\permil reflect a contribution of O\textsubscript{2} from photosynthesis (Bender and Grande, 1987; Quay et al., 1993). During photosynthesis, O\textsubscript{2} is released with an isotopic composition similar to that of the surrounding waters which at station ALOHA is approximately 0\permil (Fig. 3). Increases in \( \delta^{18}O \) values with depth result from fractionation associated with respiratory consumption of O\textsubscript{2} (Bender, 1990; Kiddon et al., 1993; Quay et al., 1993). The two profiles of \( \delta^{18}O \)–O\textsubscript{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\textsubscript{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\textsubscript{2}O 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\textsubscript{2} minima (Fig. 2). A significant inverse relationship exists between the concentrations of N\textsubscript{2}O and O\textsubscript{2} ($r^2 = 0.94$; $n = 41$; $P > 0.05$). A similar relationship between N\textsubscript{2}O concentration and apparent oxygen utilization has previously been interpreted as suggesting a nitrification source for N\textsubscript{2}O (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\textsubscript{2}O were similar between the two sample dates and ranged from 6.0‰ to 11.5‰ and 40.0‰ to 56.5‰, respectively (Fig. 3).

4. Discussion

The isotopic composition of N\textsubscript{2}O is largely controlled by kinetic isotope effects in which $^{14}$N or $^{18}$O is preferentially transferred to the product of a reaction (Kim and Craig, 1990, 1993). For example, the isotopic composition of N\textsubscript{2}, the primary product of denitrification, can be depleted in $^{15}$N relative to the substrate, NO\textsubscript{3} by as much as 40‰ (Cline and Kaplan, 1975). N\textsubscript{2}O 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\textsubscript{3} and NO\textsubscript{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\textsubscript{2}O in which $^{15}$N and $^{18}$O are preferentially concentrated in the residual N\textsubscript{2}O (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\textsubscript{2}O 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\textsubscript{2}O, 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\textsubscript{3} concentrations (Dore and Karl, 1996a,b). Consumption of N\textsubscript{2}O 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\textsubscript{2}O depleted in the $^{15}$N and $^{18}$O within oceanic regions with low or undetectable O\textsubscript{2} concentrations, the isotope effect associated with the consumption of N\textsubscript{2}O 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\textsubscript{2}O production and consumption, the stable isotope data have several puzzling attributes that demand attention. First, $\delta^{18}$O values for N\textsubscript{2}O 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\textsubscript{2}O during denitrification, however, other evidence is consistent with a source from nitrification. Previously, a minimum in the nitrogen and oxygen isotopic composition of N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O in the atmosphere are not expected if this process is predominant. The $\delta^{15}$N of N\textsubscript{2}O 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\textsubscript{3} (Böttcher et al., 1990). Evidence for the predominance of denitrification has been provided by increases in the $^{15}$N content of NO\textsubscript{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\textsubscript{3} within the O\textsubscript{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\textsubscript{2}O in the subtropical North Pacific.

While the isotopic evidence is not consistent with the consumption of N\textsubscript{2}O by denitrification as a control on its isotopic composition, the mechanisms responsible for the observed isotopic trends are still not clear. Reduction of NO\textsubscript{3} via nitrifier–denitrification (pathway 3) is a likely mechanism to explain low isotope values relative to atmospheric N\textsubscript{2}O in surface waters. Pathway 3, however, is unlikely as a mechanism in deep waters because this process should yield N\textsubscript{2}O depleted in $^{15}$N and $^{18}$O and enrichments are observed. Such unique isotope behavior is best understood by recognizing that N\textsubscript{2}O produced via nitrification can potentially occur at two branch points in the NO\textsubscript{3} production sequence yielding N\textsubscript{2}O from the intermediate compounds.
NH₂OH (pathway 1) or NO (pathway 2). If these intermediate compounds are primarily oxidized to NO₂, then ¹⁵N and ¹⁸O will be concentrated in the residual NH₂OH and/or NO. Upon production of N₂O, the isotopically enriched signal within the intermediary compounds will be transferred to N₂O (Kim and Craig, 1990). Evidence in support of this mechanism was provided by the observation of ¹⁵N 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 N₂O 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 N₂O production via nitrification can be provided by an understanding of the source of O in N₂O. The first O atom added to NH₂OH within the nitrification sequence to yield NH₂OH and NO is derived from dissolved O₂, whereas the second O atom required to yield NO₂ is derived from water (Dua et al., 1979; Hollocher et al., 1981; Andersson and Hooper, 1983; Kumar et al., 1983). Water is depleted in ¹⁸O by 23.5‰ relative to O₂ 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 δ¹⁸O of N₂O with that of water and dissolved O₂. The δ¹⁸O 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 δ¹⁸O of water has not been reported for other locations in the Pacific (Craig, 1961; Craig and Weiss, 1970). The trend in the δ¹⁸O of water with depth does not correlate with the isotope data for N₂O shown in Fig. 3. A significant relationship between the δ¹⁸O of O₂ and that of N₂O (F₁,₃⁷ = 40.6; P < 0.1) and shifts in δ¹⁸O in each of these species with depth of approximately the same magnitude is strong evidence that the primary source of O in N₂O is dissolved O₂ and not water. A source of O in N₂O from O₂ is evidence that the production of N₂O occurs primarily from either NH₂OH or NO. At this time which of these intermediate compounds yields N₂O is unknown, however, this might be resolved in incubations with isotopically labeled NH₂OH or NO. The δ¹⁸O of N₂O is still, nonetheless, enriched in ¹⁸O by approximately 20‰ relative to that of O₂. This enrichment is consistent with the idea that the production of NO₂ leaves a small residual intracellular pool of either NH₂OH or NO that is isotopically enriched in both ¹⁵N and ¹⁸O. 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 N₂O in oxic Pacific waters. We do, however, conclude that there exists more than one mechanism of N₂O formation via nitrification in these waters. Within the mixed layer (~0–120 m) O₂ and N₂O are strongly influenced by exchange with the atmosphere as indicated by concentrations near saturation levels and isotope values similar to those 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, N₂O...
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 \( NO \) by 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 \( NO \), 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 \( NO \) 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 \( NH_2 \) 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.

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