The microbial nitrogen (N) cycle involves a variety of redox processes that control the availability and speciation of N in the environment and that are involved with the production of nitrous oxide (N₂O), a climatically important greenhouse gas. Isotopic measurements of ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), and N₂O can now be used to track the cycling of these compounds and to infer their sources and sinks, which has lead to new and exciting discoveries. For example, dual isotope measurements of NO₂⁻ and NO₃⁻ have shown that there is NO₃⁻ regeneration in the ocean’s euphotic zone, as well as in and around oxygen deficient zones (ODZs), indicating that nitrification may play more roles in the ocean’s N cycle than generally thought. Likewise, the inverse isotope effect associated with NO₂⁻ oxidation yields unique information about the role of this process in NO₂⁻ cycling in the primary and secondary NO₃⁻ maxima. Finally, isotopic measurements of N₂O in the ocean are indicative of an important role for nitrification in its production. These interpretations rely on knowledge of the isotope effects for the underlying microbial processes, in particular ammonia oxidation and nitrite oxidation. Here we review the isotope effects involved with the nitrification process and the insights provided by this information, then provide a prospectus for future work in this area.

Keywords: nitrification, isotopic fractionation, oxygen, nitrogen, nitrate, nitrous oxide

NITRIFICATION IN THE OCEAN—ROLES IN NO₃⁻ SUPPLY AND N₂O PRODUCTION

Nitrification comprises a key link in the marine nitrogen (N) cycle converting the most reduced form of N (ammonia, NH₃) to the most oxidized (nitrate, NO₃⁻). Although sunlight appears to partly inhibit nitrification (Olson, 1981a; Guerrero and Jones, 1996; Merbt et al., 2012), there are many indications that nitrification occurs in the euphotic zone (Ward, 1985, 2005; Wankel et al., 2007; Yool et al., 2007; Clark et al., 2008). Therefore, when reduced organic N is released into solution through cell lysis, grazing and digestion, it can be either reassimilated or oxidized back to NO₃⁻ in the sunlit surface waters. Also, when particulate organic matter (in the form of detritus, fecal pellets, or marine snow) sinks out of the euphotic zone, it is gradually broken down into its component parts and remineralized into its inorganic forms: CO₂, NH₄⁺, and PO₄³⁻. In oxic water columns, the NH₄⁺ released from organic matter remineralization below the euphotic zone is rapidly oxidized to NO₃⁻. The distribution of nitrification rates in the ocean is therefore expected to follow the distribution of NH₄⁺ supply from organic matter remineralization, which decreases exponentially with depth (Ward and Zafiriou, 1988).

Nitrification is carried out through the combination of two microbial processes: ammonia oxidation to NO₂⁻ and nitrite oxidation to NO₃⁻. Ammonia oxidation is a chemoautotrophic process carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). These organisms use NH₃ as their source of reducing power for CO₂ fixation and energy production. Nitrite oxidation is also a chemoautotrophic process and is carried out by nitrite-oxidizing bacteria (NOB). These bacteria use nitrite (NO₂⁻) as their source of reducing power for CO₂ fixation and energy production (Watson, 1965; Bock et al., 1989). Most ammonia and nitrite oxidizers are obligate chemoautotrophs (Watson and Waterbury, 1971), although a few are able to grow mixotrophically (Watson et al., 1986).

Although NO₂⁻ is an intermediate in the nitrification process, it rarely accumulates in the ocean. NO₃⁻ can be found at the base of the euphotic zone in a feature termed the primary nitrite maximum (PNM; Wada and Hattori, 1971). The processes contributing to NO₂⁻ accumulation in the PNM are still debated, but most likely include a combination of ammonia oxidation and nitrite oxidation, as well as assimilatory nitrate and nitrite reduction by phytoplankton (Ward et al., 1982, 1989; Dore and Karl, 1996; Lomas and Lipschultz, 2006; Mackey et al., 2011). The relative contributions of these processes to NO₂⁻ cycling have different implications for N biogeochemistry and the links between C and N cycling. Net production of NO₂⁻ through nitrification (decoupling of ammonia and nitrite oxidation) can also have implications for the production of nitrous oxide (N₂O), a climatically important greenhouse gas. It is therefore important to know how the processes contributing to the production and maintenance of the PNM vary in space and time.

NO₂⁻ also accumulates in oxygen deficient regions of the water column in a feature termed the secondary nitrite maximum.
(SNM; Brandhorst, 1959). The SNM is generally assumed to reflect active denitrification in oxygen deficient zones (ODZs), as SNM features are only found in the absence of dissolved oxygen (Brandhorst, 1959; Cline and Richards, 1972; Codispoti and Christensen, 1985). However, recent studies have shown that the presence of a SNM feature may not coincide with the most intense NO$_2^-$ cycling, as active NO$_2^-$ reduction occurs in the Omani upwelling region in the absence of NO$_2^-$ accumulation (Jensen et al., 2011; Lam et al., 2011). NO$_2^-$ consumption in the SNM may occur through many processes, including denitrification (reduction of NO$_2^-$ to N$_2$), anaerobic ammonia oxidation (reduction of NO$_2^-$ to N$_2$ and oxidation to NO$_3^-$), and nitrite oxidation (oxidation of NO$_2^-$ to NO$_3^-$). Recent studies using natural abundance isotopes (Casciotti, 2009), profile modeling (Lam et al., 2011), isotope tracers (Lipschultz et al., 1990; Füssel et al., 2012), and gene markers (Füssel et al., 2012) suggest that a significant fraction of NO$_2^-$ produced within the SNM may be consumed through oxidation to NO$_3^-$.

Several questions remain about the roles of AOB and AOA in marine nitrification, the controls on their distribution and activity, and the rates of these processes. These questions relate to the cycling of NO$_3^-$, NO$_2^-$, and NH$_4^+$ in the water column, and the production of N$_2$O linked to nitrification. These questions can be addressed with a variety of complementary approaches, including molecular community analysis and quantification, instantaneous rate measurements, natural abundance stable isotope measurements, and geochemical modeling.

Examples of applications involving the use of natural abundance stable isotopes to study nitrification include: (1) the role of eutrophic zone nitrification in supplying NO$_3^-$ for photosynthetic growth (Wankel et al., 2007; DiFiore et al., 2009), (2) the contributions of nitrification and nitrate reduction to NO$_3^-$ accumulation in the PNM (Buchwald and Casciotti, unpublished), (3) the role of nitrification in near-surface N$_2$O production (Dore et al., 1998; Santoro et al., 2010, 2011), and (4) the role of nitrate oxidation in recycling NO$_3^-$ in and around ODZs (Sigman et al., 2005; Casciotti and Mcllvin, 2007; Casciotti, 2009). Understanding the isotopic systematics for nitrification is also important for tracking the balance of high-latitude and low-latitude productivity and N budget processes (N fixation and denitrification) through NO$_3^-$ isotope distributions in the deep ocean (Sigman et al., 2009). In order to understand these applications we first review the N and O isotopic systematics of the nitrification process, including both ammonia and nitrite oxidation.

**ISOTOPE SYSTEMATICS FOR AMMONIA OXIDATION**

The $\delta^{18}$O value of NO$_3^-$ produced during ammonia oxidation ($\delta^{18}$O$_{NO_3}$, init = $(^{18}$O/$^{16}$O)$_{NO_3}$ ÷ $(^{18}$O/$^{16}$O)$_{VSMOW}$ – 1) × 1000) is dependent on the $\delta^{18}$O values of the oxygen atom sources (O$_2$ and H$_2$O), isotopic fractionation during their incorporation (18$\text{O}_{\text{H}_2\text{O}}, \text{H}_2\text{O}$, and 18$\text{O}_{\text{NO}_3}$) and the corresponding equilibrium isotope effect (18$\text{O}_{\text{eq}}$) (Equation 1; Casciotti et al., 2011). Throughout this review, kinetic isotope fractionation factors are defined as $a_{\text{eq}} = R_1/R_2$ where R$_1$ and R$_2$ are the isotope ratios of two species in equilibrium. Kinetic and equilibrium isotope effects are defined by $\epsilon = (\alpha - 1) \times 1000$.

$$\delta^{18}\text{O}_{\text{NO}_3,\text{init}} = \left[ \frac{1}{2} (\delta^{18}\text{O}_{\text{O}_2} - \delta^{18}\text{O}_{\text{H}_2\text{O}}) + \frac{1}{2} (\delta^{18}\text{O}_{\text{H}_2\text{O}} - \delta^{18}\text{O}_{\text{NO}_3}) \right] \times (1 - a_{\text{eq}}) + (\delta^{18}\text{O}_{\text{H}_2\text{O}} + 18^{\text{O}}_{\text{eq}}) (x_{\text{AO}}) \quad (1)$$

Even though oxygen is incorporated enzymatically from O$_2$ to H$_2$O in a 1:1 ratio during ammonia oxidation (Andersson and Hooper, 1983), early studies of AOB found that a large amount of oxygen atom exchange with water could be associated with ammonia oxidation (Dua et al., 1979; Andersson et al., 1982; Andersson and Hooper, 1983). The conditions favoring oxygen atom exchange included high cell densities and high NO$_3^-$ concentrations. These findings, as well as the low variation of deep ocean $\delta^{18}$O$_{\text{NO}_3}$ (Casciotti et al., 2002; Sigman et al., 2009) led researchers to assume that the O atoms in oceanic NO$_3^-$ derive primarily from H$_2$O with little residual signal from dissolved O$_2$. In more recent studies, however, the amount of biologically-catalyzed exchange has been determined under lower cell densities and substrate concentrations and found to be much lower for marine AOB (Casciotti et al., 2010; Buchwald et al., 2012) and AOA (Santoro et al., 2011). Exchange levels were particularly low (5%) when NO$_3^-$ concentrations were held near 1 μm by co-cultivation with NOB (Buchwald et al., 2012). These results suggested that oxygen isotopic exchange during nitrification may be quite low where ammonia and nitrite oxidation are tightly coupled, but may play a role when ammonia and nitrite oxidation become decoupled, such as in the PNM.

Given low amounts of biologically-catalyzed oxygen atom exchange with H$_2$O, the low $\delta^{18}$O values of NO$_3^-$ in seawater may be surprising given the high $\delta^{18}$O values of dissolved O$_2$ (Kroopnick and Craig, 1976). However, oxygen atom incorporation from O$_2$ and/or H$_2$O during ammonia oxidation is associated with isotopic fractionation, such that the $^{18}$O/16O of oxygen atoms incorporated into NO$_3^-$ is significantly lower than the ambient pools of O$_2$ and H$_2$O (Casciotti et al., 2010; Santoro et al., 2011). This leads to production of NO$_3^-$ from ammonia oxidation with $\delta^{18}$O values between $-3\%$ and $5\%$ rather than near $12\%$, which would be expected from average $\delta^{18}$O$_{\text{H}_2\text{O}}$ and $\delta^{18}$O$_{\text{O}_2}$ values (Casciotti et al., 2010). Furthermore, since oxygen atom exchange occurs with an equilibrium isotope effect ($\delta_{\text{eq}}$) of 11–14% (Casciotti et al., 2007; Buchwald and Casciotti, unpublished), this equilibration would tend to raise the $\delta^{18}$O value of NO$_3^-$ relative to the initial $\delta^{18}$O$_{\text{NO}_3}$ produced by ammonia oxidation.

Nitrogen isotopic fractionation during ammonia oxidation ranges from 14% to 38% for AOB (Mariotti et al., 1981; Yoshida, 1988; Casciotti et al., 2003) and 20–22% for AOA (Santoro and Casciotti, 2011). These values represent the isotope effect expressed under non-limiting concentrations of NH$_4^+$. In the ocean NH$_4^+$ consumption generally goes to completion, so the isotope effect for ammonia oxidation may not be expressed. It may, however, be expressed at the branch point between ammonia assimilation and oxidation in the euphotic zone (Wankel et al., 2007; DiFiore et al., 2009).
et al., 2007; DiFiore et al., 2009) or in the production of N₂O by ammonia oxidizers (Yoshida, 1988; Frame and Casciotti, 2010).

**ISOTOPE SYSTEMATICS FOR N₂O PRODUCTION**

Production of N₂O by AOB occurs through two separate pathways: hydroxylamine decomposition and nitrite reduction, so-called “nitrifier denitrification” (Figure 1; Poth and Focht, 1985; Hooper et al., 1990). The isotopic compositions (δ¹⁸O, δ¹⁵N, δ¹³N, and δ¹⁵Νbulk), and site preference (SP) = δ¹⁵Ν − δ¹⁵Nbulk) of the N₂O produced through these pathways may provide insight into the mechanisms of N₂O production under different growth conditions (Frame and Casciotti, 2010; Sutka et al., 2003, 2004). For example, N₂O production through nitrifier denitrification (enhanced by high cell densities, high NO₂ concentrations, and low O₂ concentrations; Frame and Casciotti, 2010) has low δ¹⁵Nbulk and low SPs relative to that produced by hydroxylamine decomposition (Figure 2). This is most likely due to the additional steps involved with the production of N₂O from NO₂ and accumulation of the main product, NO₂, which enables fractionation associated with NO₂ reduction to be expressed.

Oxygen isotopes have been underutilized in determining N₂O sources, primarily because the isotopic systematics are less well understood, but knowledge of the mechanism of nitrification is increasing (Frame and Casciotti, 2010; Snider et al., 2012). The N₂O produced via nitrifier denitrification has a slightly lower δ¹⁸O value than that produced from hydroxylamine decomposition (Figure 2; Frame and Casciotti, 2010). This is most likely because H₂O is incorporated into NO₂, leading to lower δ¹⁸O values in NO₂ relative to NH₂OH. However, going from either NH₂OH or NO₂ to N₂O involves the loss of O atoms, which can occur with fractionation. This fractionation leads to preferential loss of ¹⁸O and retention of ¹⁶O in the residual N oxides transferred to N₂O. The net isotopic fractionation for oxygen isotopes in the hydroxylamine decomposition pathway (¹⁸O in NH₂OH), including both incorporation of O₂ into NH₂OH and production of N₂O from NH₂OH, was 2.9 ± 0.8‰ indicating that N₂O produced from this pathway had a lower ¹⁸O:¹⁶O than the ambient O₂ (Frame and Casciotti, 2010). The net isotope effect for N₂O production from NO₂ via nitrifier denitrification (¹⁸Oexp) was −8.4 ± 1.4‰ (Frame and Casciotti, 2010). The negative value indicates that the N₂O produced from NO₂ is enriched in ¹⁸O relative to NO₂, consistent with branching of O atoms and preferential loss of ¹⁶O during this reaction (Casciotti et al., 2007).

The N₂O site preference (SP) is determined mainly by the enzymatic mechanism, rather than the substrate δ¹⁵N value (Toyoda and Yoshida, 1999; Yoshida and Toyoda, 2000; Schmidt et al., 2004). The SP of N₂O produced during nitrification is +30‰ to +38‰ (Figure 2; Sutka et al., 2003, 2004; Frame and Casciotti, 2010), while N₂O produced from denitrification and nitrifier denitrification has a SP of −10% to +5% (Sutka et al., 2003, 2004; Toyoda et al., 2005; Frame and Casciotti, 2010). The large difference between the SP values of these two primary mechanisms for N₂O production provides a large signal with which to distinguish their contributions. The interpretation of SP values is therefore somewhat simplified relative to bulk δ¹⁵N and δ¹⁸O values that reflect both mechanism and substrate isotopic ratios, which change over time. This seemingly simple distinction is complicated, however, by the fact that N₂O consumption during denitrification increases SP (Ostrom et al., 2007; Yamagishi et al., 2007; Koba et al., 2009). Therefore, a high SP value may arise through production of N₂O via nitrification or net N₂O consumption during denitrification. However, the δ¹⁸O signature of these two scenarios is quite different and can enable the scenarios to be distinguished (Figure 2).

Recently, the isotopic compositions of N₂O produced by AOA were found to be distinct from AOB (Santoro et al., 2011). In particular, N₂O produced by AOA is enriched in ¹³N and ¹⁸O relative to that produced by AOB, which may explain some of the elevated δ¹⁵N and δ¹⁸O values observed in oceanic N₂O (Santoro et al., 2011). The reasons for the isotopic distinction between AOA and AOB is not known, but may involve a different mechanism of N₂O production involving a unique intermediate or enzymatic pathway. However, the SP of N₂O produced by AOA is similar to that of N₂O produced by hydroxylamine decomposition by AOB (Santoro et al., 2011; Loescher et al., 2012). While it is not yet clear whether N₂O production (or nitrification in general) by AOA involves hydroxylamine, isotopic evidence to date shows that the N₂O produced aerobically by AOA does not have a SP consistent with denitrification or nitrifier-denitrification. δ¹⁸O data also show that the N₂O produced by AOA incorporates O primarily from O₂, rather than from H₂O, which supports production by decomposition of an intermediate, rather than from NO₂, under the conditions tested (Santoro et al., 2011). It is still unknown whether AOA are able to produce N₂O through a second pathway similar to nitrifier denitrification and thus produce N₂O with a lower SP. Genetic analyses currently suggest that nitrification in AOA may proceed via a NO or HNO intermediate (Walker et al., 2010), which could potentially be converted to N₂O. Further work
Casciotti and Buchwald Isotopic fractionation during microbial nitrification

FIGURE 2 | Isotopic signatures for nitrous oxide sources and sinks. Isotope-isotope plots for N\textsubscript{2}O sources from ammonia-oxidizing archaea (AOA; Santoro et al., 2011), nitrification and nitrifier-denitrification by ammonia-oxidizing bacteria (AOB; Frame and Casciotti, 2010), and production by denitrification of NO\textsuperscript{-15} or NO\textsuperscript{-18} (Barford et al., 1999; Casciotti et al., 2007). Also shown are average tropospheric air (Kim and Craig, 1990; Yoshida and Toyoda, 2000; Croteau et al., 2010) and the estimated near-surface source at Station ALOHA in the North Pacific Subtropical Gyre (Popp et al., 2002). The isotopic trends for N\textsubscript{2}O consumption by denitrification are based on the Arabian Sea data (McIlvin and Casciotti, 2010), ETNP data (Yamagishi et al., 2007), and culture studies (Ostrom et al., 2007). Sources and sinks are distinguished by their effects on \(\delta^{18}\)O-N\textsubscript{2}O vs. SP\textsubscript{(A)}, \(\delta^{18}\)O-N\textsubscript{2}O vs. \(\delta^{15}\)N bulk-N\textsubscript{2}O\textsubscript{(B)}, and SP vs. \(\delta^{15}\)N bulk-N\textsubscript{2}O\textsubscript{(C)}.

is required to determine the pathway and intermediates of nitrification and N\textsubscript{2}O production by AOA, and to further study its isotope systematics under a variety of growth conditions.

**ISOTOPE SYSTEMATICS FOR NITRITE OXIDATION**

The isotopic systematics for nitrite oxidation to nitrate have also been studied recently, and were found to occur with extremely unique inverse kinetic isotope effects for N (Casciotti, 2009) and O isotopes (Buchwald and Casciotti, 2010). Because of these inverse isotope effects, when nitrite oxidation is active, the \(\delta^{15}\)N\textsubscript{NO\textsubscript{2}} and \(\delta^{18}\)O\textsubscript{NO\textsubscript{2}} values are expected to be lower than the NO\textsubscript{2} initially produced by ammonia oxidation or nitrate reduction. As discussed below, this appears to occur in both primary and secondary nitrite maxima (Casciotti, 2009; Buchwald and Casciotti, unpublished). In most parts of the ocean, however, NO\textsubscript{2} does not accumulate and the isotope effects associated with nitrite oxidation can only be expressed through a branch point (Figure 3). Isotopic separation can occur at a branch point because there is more than one fate for NO\textsubscript{2} (e.g., NO\textsubscript{2} is either oxidized to NO\textsubscript{3} or assimilated into particulate N, PN) and the heavy isotope can be preferentially shunted in one direction vs. the other. This is analogous to the branch point that has been described during the oxidation or assimilation of ammonium (Sigman et al., 2005; Wankel et al., 2007; DiFiore et al., 2009). The
Equations that describe the steady state N isotopic partitioning between $\text{NO}_2^-$ and $\text{NO}_3^-$ when nitrite oxidation and assimilation occur concurrently are:

$$\delta^{15}\text{N}_{\text{NO}_2} = \delta^{15}\text{N}_{\text{NO}_2, \text{produced}} + f_{\text{NA}} \times \delta^{15}\text{N}_{\text{NA}} + f_{\text{NXR}} \times \delta^{15}\text{N}_{\text{NXR}}$$

$$\delta^{15}\text{N}_{\text{NO}_3, \text{produced}} = \delta^{15}\text{N}_{\text{NO}_3} - \delta^{15}\text{N}_{\text{NXR}}$$

where $f_{\text{NA}}$ and $f_{\text{NXR}}$ are the fractions of $\text{NO}_3^-$ consumed by assimilation and oxidation, respectively, and $\delta^{15}\text{N}_{\text{NA}}$ and $\delta^{15}\text{N}_{\text{NXR}}$ are the respective isotope effects. In general, nitrite oxidation will transfer $\text{NO}_2^-$ with an elevated $^{15}\text{N}/^{14}\text{N}$ ratio to the $\text{NO}_3^-$ pool, while nitrite assimilation transfers the residual $\text{NO}_2^-$ with a lower $^{15}\text{N}/^{14}\text{N}$ ratio into the PN pool. If $\delta^{15}\text{N}_{\text{NA}}$ is 1‰ (Waser et al., 1998), $\delta^{15}\text{N}_{\text{NXR}}$ is $-15‰$ (Buchwald and Casciotti, 2010), $\delta^{15}\text{N}_{\text{NO}_3}$ at steady state will be lower than the source of $\text{NO}_2^-$, unless nitrite assimilation is >95% of the $\text{NO}_2^-$ sink. This has the opposite sense of the ammonia oxidation/assimilation branching where ammonia oxidation transfers low $^{15}\text{N}/^{14}\text{N}$ material into the $\text{NO}_2^-$ and $\text{NO}_3^-$ pools and higher $^{15}\text{N}/^{14}\text{N}$ material into the PN pool.

When nitrite oxidation is tightly coupled to ammonia oxidation and $\text{NO}_2^-$ does not accumulate, the $\delta^{18}\text{O}$ value of the $\text{NO}_3^-$ produced primarily reflects the $\delta^{18}\text{O}$ values of the O atom sources (H$_2$O and O$_2$; Kumar et al., 1983) and the incorporation isotope effects for ammonia and nitrite oxidation (Buchwald et al., 2012). The oxygen isotope systematics of nitrite oxidation can be described by Equation 4, while the full oxygen isotope systematics of nitrification starting from NH$_4^+$, assuming no biologically-catalyzed oxygen atom exchange during nitrite oxidation ($\chi_{\text{NO}_2} = 0$; DiSpirito and Hooper, 1986; Friedman et al., 1986; Buchwald and Casciotti, 2010), is described by Equation 5.

$$\delta^{18}\text{O}_{\text{NO}_3, \text{final}} = \frac{2}{3} \left[ (1-x_{\text{NO}_2}) \delta^{18}\text{O}_{\text{NO}_2} + x_{\text{NO}_2} (\delta^{18}\text{O}_{\text{H}_2\text{O}} + \delta^{18}\text{O}_{\text{eq}}) \right]$$

$$+ \frac{1}{3} \left( \delta^{18}\text{O}_{\text{H}_2\text{O}} - \delta^{18}\text{O}_{\text{H}_2\text{O}, 2} \right)$$

Equation 5 indicates that the $\delta^{18}\text{O}_{\text{NO}_3}$ produced by tightly-coupled ammonia and nitrite oxidation should reflect variations in both $\delta^{18}\text{O}_{\text{O}_2}$ and $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ in a ratio of 1 to 2, with slight modification of this stoichiometry by biologically-catalyzed oxygen atom exchange during ammonia oxidation (Casciotti et al., 2010; Buchwald et al., 2012). As discussed below, when ammonia and nitrite oxidation are not tightly coupled, abiotic equilibration can affect $\delta^{18}\text{O}_{\text{NO}_3}$ and the final $\delta^{18}\text{O}_{\text{NO}_3}$ produced. Regardless of whether $\text{NO}_2^-$ accumulates, isotopic fractionation during oxygen atom incorporation should lead to an isotopic offset between the substrates (O$_2$ and H$_2$O) and the produced $\text{NO}_3^-$. The expected $\delta^{18}\text{O}_{\text{NO}_3}$ value produced in oxygenated seawater with little exchange is $-1%$ to $+1%$ (similar to $\delta^{18}\text{O}_{\text{H}_2\text{O}}$), resulting from a complex series of fractionation factors rather than the unfractionated incorporation of and exchange with H$_2$O (Buchwald et al., 2012).

### ABBIOTIC EQUILIBRATION OF OXYGEN ATOMS IN NITRITE

As introduced above, abiotic equilibration of oxygen atoms between $\text{NO}_2^-$ and H$_2$O is likely to play a role in setting $\delta^{18}\text{O}_{\text{NO}_2}$ and $\delta^{18}\text{O}_{\text{NO}_2}$ values observed in the ocean. This process does not change the concentration of $\text{NO}_2^-$ nor its $\delta^{15}\text{N}$ value, only its $\delta^{18}\text{O}$ value. Oxygen atom equilibration shifts a $\delta^{18}\text{O}_{\text{NO}_2}$ value from its biological starting point or “end member,” set by the isotopic systematics for biological production and consumption, toward the equilibrated $\delta^{18}\text{O}_{\text{NO}_2}$ value, dictated by ambient $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ and the equilibrium isotope effect for the exchange ($\delta^{18}\text{O}_{\text{eq}}$), which is dependent on temperature (Mclvln and Casciotti, 2006; Buchwald and Casciotti, unpublished). The relevance of abiotic exchange depends on the rates of biological turnover of nitrite relative to the rate of oxygen atom exchange with water. Where nitrite turns over quickly and does not accumulate, there is little opportunity for abiotic exchange to occur. Where nitrite turns over more slowly (several weeks-months), abiotic exchange can play an important role in $\delta^{18}\text{O}_{\text{NO}_2}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ (Buchwald et al., 2012).

The tendency of $\text{NO}_2^-$ to exchange oxygen atoms abiotically with H$_2$O at typical seawater pH and temperature conditions suggests a utility of $\text{NO}_2^-$ oxygen isotopes as a tracer for determining the rate of biological turnover of $\text{NO}_2^-$ (Buchwald and Casciotti, unpublished). This provides a unique approach to determining rates of biological processes based on static isotope measurements, without bottle incubation and associated perturbations.

---

**Euphotic Zone Nitrite Branch Point**

![Diagram of the nitrogen cycle](image)

**Equation 3** | Schematic of euphotic zone nitrite branch point. A schematic of the fluxes and isotope effects involved with $\text{NO}_2^-$ consumption in the euphotic zone. $\text{NO}_2^-$ is produced from ammonium oxidation and/or nitrate reduction, the mixture of which sets the incoming flux and $\delta^{15}\text{N}$ value. $\text{NO}_2^-$ consumption can occur through nitrite oxidation ($F_{\text{NXR}}, \delta^{15}\text{N}_{\text{NXR}}$) or nitrite assimilation by phytoplankton ($F_{\text{NA}}, \delta^{15}\text{N}_{\text{NA}}$). The relative rates of uptake vs. oxidation dictate the partitioning between $\text{NO}_2^-$ and $\text{NO}_3^-$ relative to the source(s) of $\text{NO}_2^-$. [Image 54x588 to 281x711]
of the system. Applications such as this move us from laboratory studies of isotope effects to a deeper understanding of the cycling of N in the environment. There are many additional examples of how knowledge of the isotope effects for nitrification has enabled advances in our understanding of the marine N cycle, and we highlight a few below.

**IMPLICATIONS FOR UNDERSTANDING N CYCLING IN OXYGEN DEFICIENT ZONES**

As mentioned above, processes that occur in ODZs are important for the marine N budget. Both denitrification and anammox can occur in these regions, producing N₂ gas from dissolved inorganic nitrogen (DIN) compounds thereby removing them from the nutrient inventory. The magnitudes of these fluxes have been estimated in many different ways: through isotope tracer experiments (Kuypers et al., 2005; Thamdrup et al., 2006; Hamersley et al., 2012), as well as geochemical techniques based on NO₃ deficit calculations (Cline and Richards, 1972; Naqvi et al., 1982; Codispoti and Christensen, 1985; Naqvi and Sen Gupta, 1985; Gruber and Sarmiento, 1997; Deutsch et al., 2001) and biogenic N₂ production (Devol et al., 2006; Chang et al., 2010). The ¹⁵N experiments in particular showcase a complex series of interacting processes cycling N in and around ODZs that can vary sporadically in space and time. What controls the overall rate of N₂ production is not known with certainty, although it is most likely tied directly or indirectly to organic carbon supply (Ward et al., 2008). Natural abundance stable isotopes provide an integrative longer-term view of the average rates of the major fluxes of N that can be used to complement short-term incubation studies. For example, natural abundance δ¹⁵NNO₃ and δ¹⁸ONO₃ measurements have been used to estimate the relative rates of N cycle processes such as N fixation and denitrification (Brandes et al., 1998; Sigman et al., 2005).

Another aspect of N cycling in ODZs that is of great interest is the fate of NO₃ that is produced in ODZs. Once produced, NO₃ can be consumed through oxidation, regenerating NO₂⁻ or reduction to N₂ and loss from the nutrient inventory. Since nitrite oxidation is believed to be an oxygen requiring process, the fate of NO₂⁻ in the oxygen deficient zone has generally been assumed to be through nitrite reduction. However, it has been shown though a variety of approaches that NO₂⁻ can also be oxidized to NO₃ in and around ODZs. For example, early 1-D modeling studies suggested that a large fraction of NO₂⁻ produced by nitrate reduction is reoxidized to NO₃, likely on the fringes of the oxygen deficient zone (Anderson et al., 1982). More recent nutrient profile modeling suggests that NO₂⁻ could be oxidized to NO₃ within the oxygen deficient zone itself (Lam et al., 2011). Furthermore, direct evidence for NO₂⁻ oxidation to NO₃ within the ODZ comes from short-term ¹⁵N incubation experiments (Lipschultz et al., 1990; Füssel et al., 2012).

The importance of nitrite oxidation as a sink of NO₂⁻ in and around ODZs is supported by natural abundance isotope measurements of NO₃ and NO₂⁻, which integrate over longer periods. Sigman et al. (2005) and Casciotti and McIlvin (2007) found that nitrite oxidation could be an important sink for NO₂⁻ at the top of the SNM based on δ¹⁵NNO₃ and δ¹⁸ONO₃ measurements. Casciotti (2009) also showed the need for nitrite oxidation to explain the large δ¹⁵N differences between NO₃ and NO₂⁻ (Δδ¹⁵N = δ¹⁵NNO₃ - δ¹⁵NNO₂) observed within ODZs (Casciotti and McIlvin, 2007). Although the isotope effect for NO₃ reduction to NO₂⁻ is approximately 25% (Brandes et al., 1998; Voss et al., 2001), Δδ¹⁵N values within the SNM ranged from 25‰ to 40‰ (Casciotti and McIlvin, 2007). At steady state, Δδ¹⁵N is given by equation 6:

\[
\Delta \delta^{15}N = \delta^{15}N_{NO_3} - \delta^{15}N_{NO_2} = 15 \epsilon_{k_{NIR}} - F_{NXR}/F_{NAR} \\
\times 15 \epsilon_{k_{NXR}} - F_{NIR}/F_{NAR} \times 15 \epsilon_{k_{NAR}} \tag{6}
\]

where F_NAR, F_NXR, and F_NIR are the fluxes from nitrate reduction, nitrite oxidation, and nitrite reduction, respectively, and 15  \epsilon_{k_{NAR}}, 15  \epsilon_{k_{NXR}}, and 15  \epsilon_{k_{NIR}} are the respective N isotope effects. At steady state, the large Δδ¹⁵N values cannot be explained by reductive processes alone since nitrite reduction would be expected to increase δ¹⁵NNO₂, thereby decreasing Δδ¹⁵N below 25‰. The only known mechanism for increasing Δδ¹⁵N above 25‰ is through NO₂⁻ consumption with an inverse kinetic isotope effect, such as observed in nitrite oxidation (Casciotti, 2009; Buchwald and Casciotti, 2010). If all NO₂⁻ consumption occurs through oxidation (F_NXR/F_NAR = 1) with a kinetic isotope effect of −15‰, then Δδ¹⁵N at steady state should approach 40‰. If all NO₂⁻ consumption occurs through nitrite reduction (F_NXR/F_NAR = 0) with a kinetic isotope effect of +15‰, then Δδ¹⁵N would be expected to approach 10‰ at steady state. The δ¹⁵N difference between NO₃ and NO₂⁻ may therefore be diagnostic of NO₂⁻ sinks in ODZs (Casciotti, 2009).

While nitrite oxidation is generally considered to be an oxygen requiring process, O₂ is not required as an enzymatic substrate for nitrite oxidation. Rather, O₂ is used as an electron acceptor to support the oxidation of NO₂⁻ to NO₃. Therefore, if an alternative electron acceptor could be substituted, nitrite oxidation may proceed in the absence of O₂. The alternate electron acceptors that can be used by NOB for nitrite oxidation remain to be determined, but oxidation of NO₂⁻ by species such as iodate (IO₃⁻), Fe(III), and Mn(IV) would be thermodynamically feasible. Moreover, as mentioned above, there is independent evidence based on ¹⁵N incubations for nitrite oxidation occurring within the ODZs in the ETSP (Lipschultz et al., 1990) and Namibian upwelling (Füssel et al., 2012). The presence of nitrite oxidizing bacteria from the genera *Nitrospina* and *Nitrooccus* comprising up to 9% of the microbial community in the Namibian upwelling (Füssel et al., 2012) also gives strong support to their success even in low oxygen environments.

Of course, even if nitrite oxidation is occurring in ODZs, more than one process may contribute, as both bacterial nitrite oxidizers and anammox bacteria can oxidize NO₂⁻ to NO₃. The contribution of anammox to nitrite oxidation can be estimated by comparison of F_{NXR}/F_{NIR} required to explain the isotopic data with that observed during anammox (0.26:1.06; Strous et al., 2006). This ratio places an upper limit on the amount of nitrite oxidation that could be catalyzed by anammox. If the ratio of nitrite oxidation to nitrite reduction necessary to explain
observed \( \Delta \delta^{15}N \) values is greater than this, then contributions from bacterial nitrite oxidation would be inferred (Casciotti, 2009). If the ratio of nitrite oxidation to nitrite reduction required to explain the isotopic data is less than this, then nitrite oxidation could potentially all be catalyzed by anammox, although denitrification may be required to explain the additional nitrite reduction. This analysis thus provides a new constraint on the relative rates of anammox and denitrification, integrated over long time periods. However, it assumes that the isotope effects for anammox are similar to denitrification for nitrite reduction and similar to nitrite oxidation for that step. Thus, the approach can be refined with additional information about the isotopic systematics of anammox.

**IMPLICATIONS FOR UNDERSTANDING NO\(_3^-\) CYCLING AND BUDGETS: \( \Delta(15, 18) \) REVISITED**

Knowing the isotopic systematics of nitrification is critical for interpreting \( \delta^{18}O_{NO_3}, \delta^{18}O_{NO_2}, \) and \( \delta^{18}O_{NO}, \) measurements from the ocean. The culture studies described above have advanced our understanding of the oxygen isotope systematics of nitrification; however, there are also constraints from field data (Casciotti et al., 2002; Sigman et al., 2009). Casciotti et al. (2002) used the nitrate \( \delta^{18}O \) data to put the first constraints on the \( \delta^{18}O \) value of NO\(_3^-\) produced in the ocean. These estimates showed that NO\(_3^-\) is most likely produced with \( \delta^{18}O \) values close to those of seawater (0\%e) and were used by Sigman et al. (2005) to constrain the rates of N\(_2\) fixation and nitrite reoxidation from \( \delta^{15}N_{NO_3} \) to \( \delta^{18}O_{NO_3} \) data. In order to do this, Sigman et al. (2005) introduced a NO\(_3^-\) isotope anomaly based on expected enrichments of \( \delta^{15}N_{NO_3} \) and \( \delta^{18}O_{NO_3} \) due to nitrate assimilation or nitrate reduction during denitrification:

\[
\Delta(15, 18) = (\delta^{15}N_{NO_3} - \delta^{15}N_{NO_3,\text{deep}}) - 18.5\delta^{15}N_{\text{NAR}}/15\delta^{15}N_{\text{NAR}}
\times (\delta^{18}O_{NO_3} - \delta^{18}O_{NO_3,\text{deep}})
\]

where \( \delta^{15}N_{NO_3} \) and \( \delta^{18}O_{NO_3} \) are the measured isotopic values of the sample, \( \delta^{15}N_{NO_3,\text{deep}} \) and \( \delta^{18}O_{NO_3,\text{deep}} \) are the isotopic values of unaltered deep seawater, which define the starting point for fractionation. \( \delta^{15}N_{\text{NAR}} \) and \( \delta^{15}N_{\text{NAR}} \) are the isotope effects for O and N isotopes, respectively, during nitrate reduction. While there is a wide range in the absolute values of \( \delta^{15}N_{\text{NAR}} \) and \( \delta^{15}N_{\text{NAR}} \), their ratio is very close to 1 (Granger et al., 2004, 2008, 2010). Therefore, NO\(_3^-\) consuming processes generally lead to \( \delta^{15}N_{NO_3} \) and \( \delta^{18}O_{NO_3} \) values that fall along a 1:1 line and produce samples with \( \Delta(15, 18) = 0\%e \). (Figure 4). Non-zero \( \Delta(15, 18) \) values correspond to an enrichment of \( \delta^{18}O_{NO_3} \) relative to \( \delta^{15}N_{NO_3} \) or a depletion in \( \delta^{15}N_{NO_3} \) relative to \( \delta^{18}O_{NO_3} \), generally arising from production of NO\(_3^-\) with anomalous isotopic signatures. The most likely cause for depletion in \( \delta^{15}N \), especially in the nitracline of oligotrophic oceanic provinces, is through remineralization of newly fixed N with a \( \delta^{15}N \) value near -1\%e (Capone et al., 1997; Karl et al., 1997; Meador et al., 2007). The particulate organic N produced by N fixation is remineralized to NO\(_3^-\) in the subsurface, gaining O atoms from nitrification, the same process that sets the oxygen isotopic signature of NO\(_3^-\) produced from other N sources. In scenario, the magnitude of \( \Delta(15, 18) \) would be proportional to the N fixation flux (Sigman et al., 2005).

A relative enrichment in \( \delta^{18}O \), especially in the vicinity of oceanic ODZs, could represent the cycling of NO\(_3^-\) through the reduction/reoxidation cycle, where the NO\(_3^-\) consumed by denitrification has a similar \( \delta^{15}N_{NO_3} \) but a lower \( \delta^{18}O_{NO_3} \) value than that returned to the NO\(_3^-\) pool from nitrite oxidation (Sigman et al., 2005). This formulation was successful at simulating data from regions of the ETNP where NO\(_2^-\) did not accumulate (Sigman et al., 2005) and where NO\(_2^-\) goes to zero at the top of the SNM (Casciotti and McLivin, 2007). However, where NO\(_2^-\) accumulates, its isotopic composition can vary dramatically within the oxygen deficient zone itself (Casciotti and McLivin, 2007), and an interpretation including NO\(_2^-\) isotope constraints is needed. The relationship between \( \delta^{18}O \) enrichment in NO\(_3^-\) and the magnitude of the nitrite reoxidation flux depends critically on the N and O isotope systematics of nitrite oxidation, which we reviewed above. Here we revisit the implications of this new knowledge for interpretations of \( \Delta(15, 18) \) in euphotic zone and oxygen deficient zones.

Using a simple time-dependent 1-box model of the ODZ N cycle, we have reevaluated the impact of nitrite reoxidation on \( \delta^{15}N_{NO_3} \) and \( \delta^{18}O_{NO_3} \) in a hypothetical ODZ (Figure 5) and
show that nitrite oxidation can either raise or lower \( \Delta^{15} \)N, depending on the relative \( \delta^{15} \)N and \( \delta^{18} \)O values of \( \text{NO}_2^- \) and \( \text{NO}_3^- \). Our model focuses on determining the relative rates of \( \text{NO}_2^- \) reoxidation to \( \text{NO}_3^- \) (FNXR) and reduction (to NO or \( \text{NH}_4^+ \); FNIR) from \( \text{NO}_2^- \) and \( \text{NO}_3^- \), respectively.

The oxidative flux is assumed to have the N and O isotopic systematics of bacterial nitrite oxidation (Buchwald and Casciotti, 2010). Regardless of whether it is carried out by bacterial nitrite oxidizers or anammox bacteria, or some mixture of the two. The reductive processes are assumed to have \( \delta^{15} \)N and \( \delta^{18} \)O values of \( \text{NO}_2^- \) and \( \text{NO}_3^- \) (Table 1) regardless of whether \( \text{NO}_2^- \) is reduced to \( \text{N}_2 \) (via anammox or denitrification) or \( \text{NH}_4^+ \) [via denitrification to ammonium (DNRA)]. Unfortunately, very little information is currently available on the N isotope effects for nitrite reduction by these processes (Bryan et al., 1983) and no information is available for the O isotope effects. In the absence of more specific information, we make the simplifying assumption that the different nitrite reductase enzymes have similar N and O isotope effects. Clearly, this is an important area of future research.

In our model, the processes are all represented as first order, and the rate constants (\( k \)'s) are given in units of day\(^{-1} \) to match measured rates of nitrate reduction, nitrite reduction, and nitrite oxidation in ODZs (Table 1). The isotope effects taken from the literature are also given in Table 1. We vary the relative rates of nitrite oxidation and nitrite reduction (\( F_{\text{NXR}}/F_{\text{NIR}} \) between 0 and 3 (\( F_{\text{NXR}} \) representing 0–75% of \( \text{NO}_2^- \) consumption) and the rate constant for exchange (\( k_{\text{EXCH}} \)) between 0 and 1 day\(^{-1} \) to evaluate the effects of changes in these parameters on simulated \( \delta^{15} \text{NO}_3 \) and \( \delta^{18} \text{NO}_3 \) (Figure 6). Maximum rate constants of exchange between \( \text{NO}_2^- \) and \( \text{H}_2\text{O} \) of 1 day\(^{-1} \) appear reasonable based on recent laboratory studies (Casciotti et al., 2007; Buchwald and Casciotti, unpublished). As \( F_{\text{NXR}}/F_{\text{NIR}} \) increases from 0 to 3, the amount of \( \text{NO}_3^- \), \( \text{NH}_4^+ \), and \( \text{N}_2 \) increases despite an unchanging rate constant for nitrate reduction. In fact, because the reaction is taken as first order, the higher concentrations of \( \text{NO}_2^- \) brought about by higher levels of \( F_{\text{NXR}} \) lead to higher overall rates of nitrate reduction. However, it is clear from the mass balances in the different scenarios that nitrite reoxidation helps buffer against excessive loss of \( \text{NO}_3^- \), accumulation of \( \text{NO}_2^- \), and production of \( \text{N}_2 \) (Figures 6A–D), and may help explain why \( \text{NO}_3^- \) is never fully removed in oceanic ODZs.

The magnitude of nitrite oxidation also affects the \( \delta^{15} \text{NO}_3 \) and \( \delta^{18} \text{NO}_3 \) patterns. When \( F_{\text{NXR}}/F_{\text{NIR}} = 0 \), the \( \delta^{15} \text{NO}_3 \) and \( \delta^{18} \text{NO}_3 \) data fall along the 1:1 line prescribed by the isotope effects for nitrate reduction (Figures 6E–G). As \( F_{\text{NXR}}/F_{\text{NIR}} \) increases, increasingly negative \( \Delta(15, 18) \) values are produced. The strength of this effect is also dependent on the rate of

| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| \( \delta^{15} \text{NO}_3 \) | Initial nitrate \( \delta^{15} \)N | 5% | Sigman et al., 2000 |
| \( \delta^{18} \text{O}_3 \) | Initial nitrate \( \delta^{18} \)O | 2% | Casciotti et al., 2002 |
| \( \delta^{18} \text{H}_2\text{O} \) | Water \( \delta^{18} \)O value | 0% | Craig and Gordon, 1965 |
| \( k_{\text{NAR}} \) | First order rate constant for nitrate reduction | 0.001 day\(^{-1} \) | Estimated to achieve a rate of 20 nM day\(^{-1} \); Lam et al., 2011 |
| \( k_{\text{NXR}} \) | First order rate constant for nitrite reduction | 0–0.003 day\(^{-1} \) | Estimated to achieve range of observed nitrite oxidation rates; Füssel et al., 2012; Lipschultz et al., 1990 |
| \( k_{\text{CH}} \) | First order rate constant for nitrate/water exchange | 0.001 day\(^{-1} \) | Estimated to achieve a rate of 5 nM day\(^{-1} \); Devol et al., 2006 |
| \( \delta^{15} \text{N}_{\text{NXR}} \) | N isotope effect for nitrite reduction | 1.019 | Deutsch et al., 2004; Granger et al., 2008 |
| \( \delta^{15} \text{N}_{\text{NIR}} \) | N isotope effect for nitrite oxidation | 0.985 | Casciotti, 2009; Buchwald and Casciotti, 2010 |
| \( \delta^{15} \text{N}_{\text{NXR}} \) | O isotope effect for nitrite reduction | 1.019 | Bryan et al., 1983 |
| \( \delta^{15} \text{N}_{\text{NIR}} \) | O isotope effect for nitrite oxidation | 1.019 | Granger et al., 2008 |
| \( \delta^{15} \text{N}_{\text{NXR}} \) | O isotope effect for nitrite reduction | 0.997 | Buchwald and Casciotti, 2010 |
| \( \delta^{15} \text{N}_{\text{NIR}} \) | O isotope effect for nitrite reduction | 1.015 | Sigman et al., 2005 |
| \( \delta^{15} \text{H}_2\text{O} \) | O isotope effect for \( \text{H}_2\text{O} \) incorporation | 1.010 | Buchwald and Casciotti, 2010 |
| \( \delta^{15} \text{O}_3 \) | Branching O isotope effect during nitrate reduction | 0.975 | Casciotti et al., 2007 |
| \( \delta^{15} \text{H}_2\text{O} \) | Equilibrium isotope effect for nitrite/water O exchange | 1.014 | Casciotti et al., 2007; (Buchwald and Casciotti, unpublished) |
Casciotti and Buchwald Isotopic fractionation during microbial nitrification

FIGURE 6 | Results of ODZ model for varying ratios of nitrite oxidation to nitrite reduction and rates of exchange. Results from the ODZ box model at different relative rates of nitrite oxidation and nitrite reduction (F_NXR/F_NIR), ranging from 0 to 3. Mass balance is maintained in the model between NO$_3^-$, NO$_2^-$ and excess N$_2$-N with F_NXR/F_NIR = 0 (panel A), 1 (panel B), 2 (panel C) and 3 (panel D). NO$_2^-$ accumulation and N$_2$ production decrease as F_NXR increases. The ODZ box model shows that NO$_2^-$ cycling can generate both positive and negative $\Delta^{15}$N, $\Delta^{18}$O values, depending on the extent of NO$_3^-$ consumption (increasing $\delta^{15}$N, $\delta^{18}$O values), the relative rates of nitrite oxidation and reduction (F_NXR/F_NIR), and the rate of oxygen atom exchange between NO$_2^-$ and H$_2$O (k_EXCH). In each case the slope of $\delta^{18}$ONO$_3$ vs. $\delta^{15}$NNO$_3$ is equal to 1 when F_NXR = 0. As F_NXR/F_NIR increases, the magnitude of the $\Delta^{15}$N, $\Delta^{18}$O anomaly increases at a given $\delta^{15}$N value. As NO$_2^-$/H$_2$O exchange increases (=0 in panel E, 0.5 in panel F, and 1.0 in panel G), the non-zero levels of nitrite oxidation generate positive $\Delta^{15}$N, $\Delta^{18}$O values, most likely due to the relative $\delta^{18}$O values of NO$_3^-$ produced and consumed under these scenarios. All parameters used in the model are reported in Table 1.

abiopic NO$_3^-$/H$_2$O exchange, with higher exchange rates partly diluting this effect and actually leading to positive $\Delta$(15, 18) values at high extents of NO$_3^-$ consumption (the highest $\delta^{15}$NNO$_3$ values; Figure 6). This interesting phenomenon is most likely due to reversal of the impact of nitrite reoxidation on $\delta^{18}$ONO$_3$ at high $\delta^{18}$ONO$_3$ values, with nitrite oxidation returning NO$_3^-$ with a lower $\delta^{18}$ONO$_3$ value than that removed by nitrite reduction. This would be exacerbated at high rates of exchange, which helps to maintain $\delta^{18}$ONO$_3$ values at a constant level regardless of $\delta^{18}$ONO$_3$. Tuning the model to match observed $\delta^{18}$ONO$_3$ data requires a high rate of exchange relative to biological fluxes, and therefore most closely follows the k_EXCH = 1 scenario.

Larger ratios of F_NXR/F_NIR could be imagined, but the model results from such simulations produce unrealistic $\Delta$(15, 18) anomalies at a given $\delta^{15}$NNO$_3$ value. Furthermore, because excess N$_2$ does accumulate in ODZs, we know that some NO$_3^-$ is ultimately reduced to N$_2$. Indeed, we could potentially use the stoichiometry of N$_2$ production in ODZs to interrogate the importance of nitrite oxidation. If nitrite oxidation is not important, the standard stoichiometry (Richards, 1965; Devol et al., 2006) of 106 CO$_2$: 55.2 N$_2$ would be expected, whereas higher amounts of CO$_2$ would be expected if a significant fraction of the produced NO$_2^-$ is reoxidized to NO$_3^-$. This may seem counterintuitive because autotrophic nitrite oxidation should fix CO$_2$ back into organic matter, but the excess NO$_3^-$ reduction required to supply the NO$_2^-$ in the first place should far outweigh the CO$_2$ fixed by nitrite oxidation.

It is interesting to note that the two scenarios for producing negative $\Delta$(15, 18) values (N$_2$ fixation and nitrite reoxidation) are each more effective at different points in NO$_3^-$ isotope space (Figure 7). N$_2$ fixation is most effective at generating negative $\Delta$(15, 18) signals at $\delta^{15}$NNO$_3$ and $\delta^{18}$ONO$_3$ values less than 10‰, near the base of the euphotic zone. In contrast, nitrite reoxidation is most effective at generating negative $\Delta$(15, 18) signals at intermediate $\delta^{15}$NNO$_3$ and $\delta^{18}$ONO$_3$ values and extents of NO$_3^-$. 

www.frontiersin.org

October 2012 | Volume 3 | Article 356 | 9
processes (N2 fixation and denitrification) and the ratio of low latitude productivity, where nutrient consumption goes to completion, to high latitude productivity, where nutrient uptake is incomplete. By comparing model results to δ15NNO3 and δ18O, data from a variety of oceanographic profiles representing the major ocean basins, the impacts of partial NO3 assimilation in polar regions on the N and O isotopes of NO3 in the ocean interior, and of low latitude productivity on the 18O enrichment in preformed NO3 was diagnosed. N budget processes (N2 fixation and denitrification) led to variations in subsurface δ15NNO3 and δ18O, but in their absence, the large scale steady state δ18O value of subsurface NO3 was set by nitrate assimilation in polar regions. Nitrate uptake in the southern ocean leads to heavy isotope enrichment in preformed NO3, while nitrate assimilation in low latitudes removes the δ18O signal of the preformed NO3 and replaces it with the nitrification signal (Sigman et al., 2009). Overall, when only internal processes were active in the model, the mean ocean δ18O value was 1.1‰ higher than the nitrification source. When the N budget was added to the model, the mean ocean δ18O value was 2.4‰ higher than the nitrification source value. This analysis provides additional constraints on the δ18O value of newly produced NO3 in the ocean to fall between −1‰ and +1‰ (Sigman et al., 2009), which is consistent with culture studies that illustrate how these values are controlled biochemically (Buchwald et al., 2012).

NITROGEN CYCLING IN THE EUPHOTIC ZONE

Several studies have now used N and O isotope ratio measurements to study the relative rates of N cycling in the euphotic zone. In particular, knowledge of the isotopic systematics of nitrate uptake (Granger et al., 2004, 2010) and nitrification (Buchwald and Casciotti, 2010; Casciotti et al., 2010, 2011; Buchwald et al., 2012) enables the assessment of the relative rates of nitrification and nitrate uptake from euphotic zone NO3 isotope data.

Wankel et al. (2007) used a steady-state box model to interpret the amount of nitrification contributing to nitrate uptake by phytoplankton in Monterey Bay, CA using δ15NNO3 and δ18O, variations. Assuming that nitrate assimilation leads to equivalent fractionation of N and O isotopes (Granger et al., 2004), and that δ18O = 2.9‰, they estimated that nitrification could supply up to 30% of NO3 assimilated by phytoplankton in Monterey Bay, consistent with intensive isotope tracer incubation studies (Ward, 2005). Because δ18O was uncertain at that time, they performed sensitivity studies to address the impact of different δ18O values on their interpretation. We now believe that δ18O is between −1‰ and +1‰ (Buchwald et al., 2012), and applying this to the model from Wankel et al. (2007), leads to a smaller increase in δ18O, for the same amount of nitrification. Thus, to achieve the same δ18O enrichment in their model requires more nitrification than originally estimated.

DiFiore and colleagues (2009) estimated the amount of nitrification contributing to nitrate uptake in the euphotic zone of the Polar Antarctic Zone using a time-dependent 1-box model. Like Wankel et al. (2007), they assumed that δ18O = 18O for nitrate
ammonia oxidation contributed to maintenance of the PNM. In
\[ \delta_{\text{N}} \text{NO}_3 \] positively small impact on
as discussed above, but they found that nitrification had a rela-
tively small impact on
\[ \delta_{\text{N}} \text{NO}_3 \] throughout the water column. In the transition from well mixed
uptake and allowed branching of \( \text{NH}_4^+ \) (and \( \text{NO}_3^- \)) between
nitrification and assimilation to partition isotopes between the
\( \text{NO}_3^- \) and particulate N pools. One important difference from
the Wankel et al. (2007) model is that they assumed \( \delta^{18}\text{O}_{\text{NO}_3} = +1.1\% \) based on more recent constraints on this value (Sigman et al., 2009). They inferred that \( \delta^{15}\text{N}_{\text{NO}_3} \) should be lowered
slightly due to nitrification (offsetting the isotopic fractionation
during uptake) and \( \delta^{18}\text{O}_{\text{NO}_3} \) should be raised (because the
\( \delta^{18}\text{O} \) of newly produced \( \text{NO}_3^- \) was higher than that removed).
Both of these factors should lead to negative \( \Delta(15, 18) \) values, as
discussed above, but they found that nitrification had a relatively
small impact on \( \delta^{15}\text{N}_{\text{NO}_3} \) and \( \delta^{18}\text{O}_{\text{NO}_3} \) values in the Polar
Antarctic Zone. They concluded that in the Polar Antarctic Zone
less than 1% of \( \text{NO}_3^- \) assimilated by phytoplankton is likely to
have been produced by nitrification in the euphotic zone (DiFiore
et al., 2009). This is consistent with other estimates from the
southern ocean (Olson, 1981b; Bianchi et al., 1997; Law and Ling,
2001) and quite a bit lower than other regions (Yool et al., 2007;
Wankel et al., 2007; Clark et al., 2008). This elegant study pro-
vides an excellent example of how \( \text{NO}_3^- \) isotopes can be used to
constrain N cycle processes in an appropriate model framework.

\( \text{NO}_3^- \) and \( \text{NO}_2^- \) isotopes have also been used to understand
the sources and cycling of \( \text{NO}_2^- \) in the PNM at the base of
the euphotic zone. Mackey et al. (2011) used natural abundance
\( \text{NO}_3^- + \text{NO}_2^- \) isotope data and isotope tracer experiments
to determine the sources of \( \text{NO}_3^- \) to the PNM in the Gulf of
Aqaba. They found active nutrient regeneration and nitrification
throughout the water column. In the transition from well mixed
to stratified conditions, \( \text{NO}_3^- \) was generated by incomplete \( \text{NO}_3^- \)
reduction by light-limited phytoplankton creating a broad band
of \( \text{NO}_2^- \). After stratification was established, \( \text{NO}_2^- \) generation by
ammonia oxidation contributed to maintenance of the PNM. In
both cases, \( \text{NO}_2^- \) was consumed by nitrite oxidation below the
PNM. Once again, nitrification was interpreted to play an impor-
tant role in \( \text{NO}_3^- \) isotope dynamics in the upper water column
where increases in \( \delta^{15}\text{N}_{\text{NO}_3} \) were much higher than increases in
\( \delta^{18}\text{O}_{\text{NO}_3} \).

In another recent study of PNM dynamics, natural abundance
\( \delta^{18}\text{O}_{\text{NO}_3} \), and \( \delta^{15}\text{N}_{\text{NO}_3} \) values were used to infer the sources and
average age of \( \text{NO}_2^- \) in the PNM of the Arabian Sea (Buchwald
and Casciotti, unpublished). Because the \( \delta^{15}\text{N}_{\text{NO}_3} \) and \( \delta^{18}\text{O}_{\text{NO}_3} \)
values produced from ammonia oxidation and nitrate reduc-
tion are distinct, the sources can be readily distinguished. Based
on natural abundance \( \delta^{15}\text{N}_{\text{NO}_3} \) and \( \delta^{18}\text{O}_{\text{NO}_3} \) data, ammonia oxidation was inferred to be the main source of \( \text{NO}_2^- \) to the PNM
in the Arabian Sea.

**IMPLICATIONS FOR INTERPRETING \( \text{N}_2\text{O} \) SOURCES**

Uncertainty in the isotopic composition of \( \text{N}_2\text{O} \) produced during
ammonia oxidation has hampered the interpretation of near-
surface \( \text{N}_2\text{O} \) production rates and fluxes using two-component
end member models (Dore et al., 1998; Popp et al., 2002; Santoro
et al., 2010). Better understanding of the oxygen isotopic system-
atics of nitrification can provide further insight into outstanding
questions in \( \text{N}_2\text{O} \) oxygen isotope variations, such as why \( \delta^{18}\text{O}_{\text{NO}_3} \)
in seawater is so high (Ostrom et al., 2000; Popp et al., 2002), what
mechanisms of \( \text{N}_2\text{O} \) production operate in oxyclines surrounding
oceanic ODZs (Codispoti and Christensen, 1985), and what
the mechanisms and controls on \( \text{N}_2\text{O} \) production are in the near-
surface ocean (Dore et al., 1998; Popp et al., 2002; Santoro et al.,
2011).

For example, \( \text{N}_2\text{O} \) production in the near-surface ocean is
largely believed to be the result of nitrification. However, the iso-
topic composition of \( \text{N}_2\text{O} \) in the near surface and the inferred
near surface source (Dore et al., 1998) have higher \( \delta^{15}\text{N} \) and
\( \delta^{18}\text{O} \) values than are characterized by bacterial ammonia oxida-
tion (Yoshida, 1988; Frame and Casciotti, 2010). Recent evidence
suggests that AOA are important for nitrification in such envi-
ronments (Wuchter et al., 2006; Beman et al., 2008; Mincer et al.,
2007; Church et al., 2010; Santoro et al., 2010) and that they
produce \( \text{N}_2\text{O} \) with bulk \( \delta^{15}\text{N} \) and \( \delta^{18}\text{O} \) values similar to the
near-surface source (Santoro et al., 2011). These data support
a role for them in near-surface \( \text{N}_2\text{O} \) production. As discussed
above, the mechanisms of \( \text{N}_2\text{O} \) production by AOA are cur-
rently unknown, and more work is needed to characterize the
\( \text{N}_2\text{O} \) production and isotopic composition of marine AOA under
a variety of growth conditions. For example, the SP of \( \text{N}_2\text{O} \)
produced by AOB varies widely with dissolved oxygen levels
(Frame and Casciotti, 2010) but so far the isotopic composition
of \( \text{N}_2\text{O} \) produced by AOA has only been examined under aer-
obic growth conditions (Santoro et al., 2011; Loescher et al., 2012).
Therefore, we do not know whether they are capable of produc-
ing \( \text{N}_2\text{O} \) with a SP similar to near surface \( \text{N}_2\text{O} \) (Popp et al.,
2002).

**CONCLUDING REMARKS**

Understanding the nitrogen and oxygen isotopic systematics of
nitrification can contribute greatly to our understanding of nitro-
gen cycling in the ocean, as nitrification is involved with trans-
formations between the major pools of DIN (\( \text{NH}_4^+ \), \( \text{NO}_2^- \), \( \text{NO}_3^- \),
and \( \text{N}_2\text{O} \)). Both ammonia and nitrite oxidation are involved with
large and distinctive isotope effects, leading to predictable pat-
terns in the isotope ratios of compounds that they transform.
The discovery of AOA and their importance in ocean biogeo-
chemistry necessitates renewed study of the isotopic systematics
of nitrification. In preliminary studies, the isotopic system-
atics of AOA appear similar to AOB for N isotope fractionation
and O atom incorporation into \( \text{NO}_2^- \) (Santoro and Casciotti,
2011; Santoro et al., 2011). However, the production of \( \text{N}_2\text{O} \)
and the isotopic systematics of this process need to be further
investigated.

**ACKNOWLEDGMENTS**

We would like to acknowledge the pioneering work of those cited
in this review. We have attempted to integrate studies of many
authors using various approaches to understand the importance
of nitrification in the marine environment, with a focus on the
use of natural abundance stable isotope measurements. We thank
two anonymous reviewers for their suggestions on an earlier draft
of this manuscript. Funding for this work has been provided by
NSF/OCE ETSP grants 05-26277, 07-48674, and 11-40404 to
Karen L. Casciotti.
REFERENCES
Anderson, J. J., Okubo, A., Robins, A. S., and Richards, F. A. (1982). A model for nitrite and nitrate distributions in oceanic oxygen min- imum zones. Deep Sea Res. 29, 1113–1140.
Anderson, K. K., and Hooper, A. B. (1983). O₂ and H₂O are each the source of one O in NO−_3 pro- duced from NH₃ by Nitrosomonas: ¹⁵N-NMR evidence. FEBS Lett. 164, 236–240.
Anderson, K. K., Philson, S. B., and Hooper, A. B. (1982). ¹⁵O iso- tope shift in ¹⁵N NMR analysis of biological N oxida- tions: H₂O-NO−_3 exchange in the ammonia-oxidizing bacterium Nitrosomonas. Proc. Natl. Acad. Sci. U.S.A. 79, 5871–5875.
Barford, C. C., Montoya, J. P., Altbel, M. A., and Mitchell, R. (1999). Steady-state nitrogen isotope effects of N₂ and N₂O production in Paracoccus denitrificans. Appl. Environ. Microbiol. 65, 899–904.
Beman, J. M., Pop, B. N., and Francis, C. A. (2008). Molecular and bio- geochemical evidence for ammonia oxidation by marine Crenarchaeota in the Gulf of California. ISME J. 2, 429–441.
Bianchi, M., Feliatra, F., Treguer, P., Vincen Mazda, M.-A., and Morvan, J. (1997). Nitrification rates, ammoxi- num and nitrate distribution in upper layers of the water column and in sediments of the Indian sec- tor of the Southern Ocean. Deep Sea Res. Part II Top. Stud. Oceanogr. 44, 1017–1032.
Bock, E., Koops, H.-P., and Harms, H. (1989). “Nitrifying bacteria,” in Autotrophic Bacteria, ed H. G. Schlegel and B. Bowien (Berlin: Springer-Verlag), 81–96.
Brandes, J. A., Devo, A. H., Yoshinari, T., Jayakumar, D. A., and Naik, H., et al. (1998). Isotopic composition of nitrate in the central Arabian sea and eastern tropical North Pacific: a tracer for mixing and nitro- gen cycles. Limnol. Oceanogr. 43, 1680–1689.
Brandhorst, W. (1959). Nitrification and denitrification in the Eastern Tropical North Pacific. Journal du Conseil Permanent International L’Exploration de la Mer 25, 3–20.
Bryan, B. A., Shearer, G., Skeeters, J. L., and Kohl, D. H. (1983). Variable expression of the nitrogen isotope equi- librium associated with denitriifica- tion of nitrite. J. Biol. Chem. 258, 8613–8617.
Buchwald, C., and Casciotti, K. L. (2010). Oxygen isotopic fractiona- tion and exchange during bacterial nitrite oxidation. Limnol. Oceanogr. 55, 1064–1074.
Chang, B. X., Devo, A. H., and Emerson, S. R. (2010). Denitrification and the nitrogen gas excess in the eastern tropical South Pacific oxygen deficient zone. Deep Sea Res. Part I Oceanogr. Res. Pap. 57, 1092–1101.
Chukh, M. I., Wal, B., Karl, D. M., and DeLong, E. F. (2010). Abundances of crenarchaeal amoA genes and transcripts in the Pacific Ocean. Environ. Microbiol. 12, 679–689.
Clark, D. R., Rees, A. P., and Joint, I. (2008). Ammonium regeneration and nitrification rates in the oligo- trophic Atlantic Ocean: implications for new production estimates. Limnol. Oceanogr. 53, 52–62.
Cline, J. D., and Richards, F. A. (1972). Oxygen deficient conditions and nitrate reduction in the eastern tropical North Pacific Ocean. Limnol. Oceanogr. 17, 885–900.
Codicispi, L. A., and Christensen, J. P. (1985). Nitrification, denitrifica- tion, and nitrous oxide cycling in the eastern tropical Pacific Ocean. Mar. Chem. 16, 277–300.
Craig, H., and Gordon, L. I. (1965). “Deuterium and oxygen 18 vari- ations in the ocean and marine atmosphere,” in Stable Isotopes in Oceanographic Studies and Paleotemperatures, ed E. Tioniogi (Speroleti, Italy), 9–130.
Croteau, P., Atlas, E. L., Schauffer, S. M., Blake, D. R., Diskin, G. S., and Boering, K. A. (2010). Effect of local and regional sources on the iso- topic composition of nitrous oxide in the tropical free troposphere and tropopause layer. J. Geophys. Res. 115, D20011.
Deutsch, C., Gruber, N., Key, R. M., Sarmiento, J. L., and Gananschau, A. (2001). Denitrification and N₂ fix- ation in the Pacific Ocean. Global Biogeochem. Cycles 15, 483–506.
Deutsch, C., Sigman, D. M., Thunnell, R. C., Meckler, A. N., and Haug, G. H. (2004). Isotopic constraints on glacial/interglacial changes in the oceanic nitrogen budget. Global Biogeochem. Cycles 18, GB012.
Devol, A. H., Ulenhopp, A. G., Naqui, S. W. A., Brandes, J. A., Jayakumar, D. A., Naik, H., et al. (2006). Denitrification rates and excess nitrogen gas concentrations in the Arabian Sea oxygen deficient zone. Deep Sea Res. Part I Oceanogr. Res. Pap. 53, 1533–1547.
DiFrico, P. J., Sigman, D. M., Dunbar, R. B., and Wang, Y. (2009). Upper ocean nitrogen fluxes in the Polar Antarctic zone: constraints from the nitrogen and oxygen isotopes of nitrate. Geochim. Geophys. Geosyst. 10. doi: 10.1029/2009GC002468. [Erratum in print].
D’Sposito, A. A., and Hooper, A. B. (1986). Oxygen exchange between nitrate molecules during nitrite oxida- tion by Nitrobacter. J. Biol. Chem. 261, 10534–10537.
Dore, J. E., and Karl, D. M. (1996). Nitrite distributions and dynamics at station ALOHA. Deep Sea Res. 43, 385–402.
Dore, J. E., Pop, B. N., Karl, D. M., and Sansone, E. J. (1998). A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters. Nature 396, 63–66.
Dua, R. D., Bhardari, B., and Nicholas, D. J. D. (1979). Stable isotopic studies of the oxidation of ammonia to hydroxylamine by Nitrosomonas europaea. FEBS Lett. 106, 401–404.
Frame, C. H., and Casciotti, K. L. (2010). Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. Biogosci. Discuss. 7, 3019–3059.
Friedman, S. H., Massetti, S., and Hollocher, T. C. (1986). Catalysis of intermolecular oxygen atom trans- fer by nitrite dehydrogenase of Nitrobacter agilis. J. Biol. Chem. 261, 10538–10543.
Füssel, J., Lam, P., Lavik, G., Jensen, M. M., Holttappels, M., Gunter, M., et al. (2012). Nitrite oxidation in the Namibian oxygen minimum zone. ISME J. 6, 1200–1209.
Granger, J., Sigman, D. M., Needoba, J. A., and Harrison, P. (2004). Coupled nitrogen and oxygen iso- tope fractionation of nitrate during assimilation by cultures of marine phytoplankton. Limnol. Oceanogr. 49, 1763–1773.
Granger, J., Sigman, D. M., Lehmann, M. F., and Tortell, P. D. (2008). Nitrogen and oxygen isotope fractionation during dissimilatory nitrate reduction by denitrifying bacteria. Limnol. Oceanogr. 53, 2533–2545.
Granger, J., Sigman, D. M., Rhode, M. M., Maldonado, M. T., and Tortell, P. D. (2010). N and O isotope effects during nitrate assimilation by unicellular prokaryotic and eukaryotic plankton cultures. Geochim. Cosmochim. Acta 74, 1030–1040.
Guerrero, N., and Sarmiento, J. L. (1997). Global patterns of marine nitrogen fixation and denitrification. Global Biogeochem. Cycles 11, 235–266.
Guerrero, M. A., and Jones, R. D. (1996). Photoinhibition of marine
nitrifying bacteria. 2. Dark recovery after monochromatic or polychromatic irradiation. Mar. Ecol. Prog. Ser. 141, 193–198.

Hamersley, R. M., Lavik, G., Woebken, D., Rattey, J. E., Lam, P., Hopmans, E. C., et al. (2007). Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone. Limnol. Oceanogr. 52, 923–933.

Hooper, A., Arciero, D., DiSpirito, A. F., Fuchs, J., Johnson, M., LaQuier, F., et al. (1990). “Production of nitrite and N\textsubscript{2}O by the ammonia-oxidizing nitrifiers,” in Nitrogen Fixation: Achievements and Objectives, eds R. Gresshoff, Stacey, and Newton (New York, NY: Chapman and Hall), 387–391.

Jensen, M. M., Lam, P., Revsbech, N. P., Nagel, B., Gaye, B., Jetten, M. S. M., et al. (2011). Intensive nitrogen loss over the Omani Shelf due to anammox coupled with dissimilatory nitrite reduction to ammonium. ISME J. 5, 1660–1670.

Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., and Hebel, D. (1997). The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature 388, 533–538.

Kim, K.-R., and Craig, H. (1990). Two-isotope characterization of N\textsubscript{2}O in the Pacific Ocean and constraints on its origin in deep water. Nature 347, 58–61.

Koba, K., Osaka, K., Tobari, Y., Toyoda, S., Ohte, N., Katsuyama, M., et al. (2009). Biogeochemistry of nitrous oxide in groundwater in a forested ecosystem elucidated in a forested nitrifier isotope fingerprint. Geochim. Cosmochim. Acta 73, 3115–3133.

Kroopnick, P., and Craig, H. (1976). Oxygen isotope fractionation in dissolved-oxygen in deep-sea. Earth Planet. Sci. Lett. 32, 375–388.

Kumar, S., Nicholas, J. D. J., and Williams, E. H. (1983). Definitive N-15 NMR evidence that water

Mincner, T. J., Church, M. J., Taylor, L. T., Preston, C., Karl, D. M., and DeLong, E. F. (2007). Quantitative distribution of presumptively archaeal and bacterial nitritifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environ. Microbiol. 9, 1162–1175.

Naqvi, S. W. A., Noronha, R. J., and Reddy, C. V. G. (1982). Denitrification in the Arabian Sea. Deep Sea Res. 29, 459–469.

Naqvi, S. W. A., and Sen Gupta, R. (1985). “NO\textsubscript{3}” a useful tool for the estimation of nitrate deficits in the Arabian Sea. Deep Sea Res. 32, 665–674.

Olson, R. J. (1981a). Differential photothionisation of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. J. Mar. Res. 39, 227–238.

Olson, R. J. (1981b). 15N tracer studies of the primary nitrite maximum. J. Mar. Res. 39, 203–226.

Ostrom, N. E., Pitt, A., Sut era, R., Ostrom, P. H., Grandy, A. S., Huizenga, K. M., et al. (2007). Isotopologue effects during N\textsubscript{2}O reduction in soils and in pure cultures of denitrifiers. J. Geophys. Res. 112, G02005.

Ostrom, N. E., Russ, M. E., Popp, B., Rust, T. M., and Karl, D. M. (2000). Mechanisms of nitrous oxide production in the subtropical North Pacific based on determinations of the isotopic abundances of nitrous oxide and di-oxygen. Chemosphere Global Change Sci. 2, 281–290.

Popp, B. N., Westley, M. B., Toyoda, S., Miwa, T., Dore, J. E., Yoshida, N., et al. (2002). Nitrogen and oxygen isotopometric constraints on the origins and sea-to-air-flux of N\textsubscript{2}O in the oligotrophic subtropical North Pacific gyre. Global Biogeochem. Cycles 16, 1064.

Poth, M., and Focht, D. D. (1983). 15N kinetic analysis of N\textsubscript{2}O produced by Nitrosomonas europaea: an examination of nitrifier denitrification. Appl. Environ. Microbiol. 49, 1134–1141.

Richards, F. A. (1965). “Anoxic basins and fjords,” in Chemical Oceanography, Vol. 1, ed J. P. Riley and G. Skirrow (New York, NY: Academic Press), 611–645.

Santero, A. E., Buchwald, C., Minclev, M. R., and Casciotti, K. L. (2011). Isotopic signature of N\textsubscript{2}O produced by marine ammonia-oxidizing archaea. Science 333, 1282–1285.

Santoro, A. E., and Casciotti, K. L. (2012). Activity, abundance, and diversity of nitrifying archaea and bacteria in the central California Current. Environ. Microbiol. 12, 1899.

Santoro, A. E., and Casciotti, K. L. (2011). Enrichment and characterization of ammonia-oxidizing archaea from the open ocean: phylogeny, physiology and stable isotope fractionation. ISME J. 5, 1796–1808.

Schmidt, H.-L., Werner, R. A., Yoshida, N., and Well, R. (2004). Is the isotopic composition of nitrous oxide an indicator for its origin from nitrification or denitrification? A theoretical approach from referred data and microbiological and enzyme kinetic aspects. Rapid Commun. Mass Spectrom. 18, 2036–2040.

Sigman, D. M., Altabet, M. A., McCorkle, D. C., Francois, R., and Fischer, G. (2000). The delta N-15 of nitrogen from the Southern Ocean: nitrogen cycling and circulation in the ocean interior. J. Geophys. Res. 105, 19599–19614.

Sigman, D. M., DiFiore, P. J., Hain, M. P., Deutsch, C., Wang, Y., Karl, D. M., et al. (2009). The dual isotope of deep nitrate as a constraint on the cycle and budget of oceanic fixed nitrogen. Deep Sea Res. Part I Oceanogr. Res. Pap. 56, 1419–1439.

Sigman, D. M., Granger, I., DiFiore, P. J., Lehmann, M. F., Ho, R., Cane, R., et al. (2005). Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin. Global Biogeochem. Cycles 19, GB4024.

Smid, D. M., Venkiteswaran, J. J., Schiff, S. L., and Spoelstra, J. (2012). Deciphering the oxygen isotope composition of nitrous oxide produced by nitrification. Global Change Biology 18, 356–370.

Strous, M., Pelletier, E., Mangenot, S., Rattie, T., Lehner, A., Taylor, M. W., et al. (2006). Deciphering the evolution and metabolism of an ammonia bacterium from a community genome. Nature 440, 799–794.

Sutka, R. L., Ostrom, E. N., Ostrom, P. H., Gandhi, H., and Breznak, J. A. (2003). Nitrogen isotopomer site preference of N\textsubscript{2}O produced by Nitrosomonas europaea and Methylcoccus capsulatus Bath. Rapid Commun. Mass Spectrom. 17, 738–743.

Sutka, R. L., Ostrom, E. N., Ostrom, P. H., Gandhi, H., and Breznak, J. A. (2004). Nitrogen isotopomer site preference of N\textsubscript{2}O produced by Nitrosomonas europaea and Methylcoccus capsulatus Bath.
Casciotti and Buchwald Isotopic fractionation during microbial nitrification

Wankel, S. D., Kendall, C., Pennington, W. A., and Clark, D. R. (2007). The significance of nitrification for oceanic new production. Nature 447, 999–1002.

Yoshida, N. (1988). $^{15}$N-depleted N$_2$O as product of nitrification. Nature 335, 528–529.

Yoshida, N., and Toyoda, S. (2000). Constraining the atmospheric N$_2$O budget from intramolecular site preference in N$_2$O isotopomers. Nature 405, 330–334.

Conflict of Interest Statement: 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.