Insights on the marine microbial nitrogen cycle from isotopic approaches to nitrification

Karen L. Casciotti1* and Carolyn Buchwald2

1 Department of Environmental Earth System Science, Stanford University, Stanford, CA, USA
2 MIT/WHOI Joint Program in Chemical Oceanography, Woods Hole, MA, USA

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 reasimilated 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 PO4−. 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 chemotrophic 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 chemotrophic 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 chemotrophs (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.

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 NO3− and NO2− 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
Isotopic fractionation during microbial nitrification

October 2012 | Volume 3 | Article 356

δe is that for reac-

concentrations were held near 1−11 consumption generally goes to completion, so the accumula-

e cycling, as active NO

18 produced within the SNM may be consumed to N−ε+Cl.

Santoro et al., 2010; Casciotti et al., 2010; Casciotti, 2009]

accumulation (cycling) and nitrite oxidation to and/or H(1)

where k

= (1)

2 to NO(2) produced during ammonia oxidation con-

sumption in the SNM may occur through many processes, including denitrification (reduc-

tion of NO2 to N2), anaerobic ammonia oxidation (reduction of NO2 to N2 and oxidation to NO3), and nitrite oxidation (oxidation of NO2 to NO3). 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 NO2 produced within the SNM may be consumed through oxidation to NO3.

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 NO3, NO2, and NH4 in the water column, and the production of N2O 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 NO3 for photosynthetic growth (Wankel et al., 2007; DiFiore et al., 2009), (2) the contributions of nitrification and nitrate reduction to NO3 accumulation in the PNM (Buchwald and Casciotti, unpublished), (3) the role of nitrification in near-surface N2O production (Dore et al., 1998; Santoro et al., 2010, 2011), and (4) the role of nitrite oxidation in recycling NO3 in and around ODZs (Sigman et al., 2005; Casciotti and McIlvin, 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 NO3 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.

ISO TOPE SY S T EMAT ICS FOR AMMONIA OX IDATION

The δ18O value of NO3 produced during ammonia oxidation (δ18O NO3,xmit = (18O/16O)NO3, oxidized / (18O/16O)H2O, unknown − 1) × 1000) is dependent on the δ18O values of the oxygen atom sources (O2 and H2O), isotopic fractionation during their incorporation (18O/16O in and 18O/16O in H2O, respectively), as well as any exchange of oxygen atoms between nitrite and water (XH2O) and the corresponding equilibrium isotope effect (18O/16O) (Equation 1; Casciotti et al., 2011). Throughout this review, kinetic isotope fractionation factors are defined as αeq = R1/R2 where R1 and R2 are the isotope ratios of two species in equilibrium. Kinetic and equilibrium isotope effects are defined by ε = (α − 1) × 1000.

δ18O NO3,xmit = \left[ \frac{1}{2} \left( \delta^{18}O_{O2} \cdot \delta^{18}O_{H2O} \cdot 1 \right) \right]

× (1 − XH2O) + \left( \delta^{18}O_{H2O} \cdot \delta^{18}O_{xH2O} \right).

(1)

Even though oxygen is incorporated enzymatically from O2 to H2O 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 NO3 concentrations. These findings, as well as the low variation of deep ocean δ18O NO3 (Casciotti et al., 2002; Sigman et al., 2009) led researchers to assume that the O atoms in oceanic NO3 derive primarily from H2O with little residual signal from dissolved O2. 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 (Santo et al., 2011). Exchange levels were particularly low (5%) when NO3 concentrations were held near 1 μM by co-cultivation with NOB (Buchwald et al., 2012). These results suggested that oxygen isotope 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 H2O, the low δ18O values of NO3 in seawater may be surprising given the high δ18O values of dissolved O2 (Kroopnick and Craig, 1976). However, oxygen atom incorporation from O2 and/or H2O during ammonia oxidation is associated with isotopic fractionation, such that the 18O/16O of oxygen atoms incorporated into NO3 is significantly lower than the ambient pools of O2 and H2O (Casciotti et al., 2010; Santoro et al., 2011). This leads to production of NO3 from ammonia oxidation with δ18O values between −3‰ and 5‰ rather than near 12‰, which would be expected from average δ18O H2O and δ18O O2 values (Casciotti et al., 2010). Furthermore, since oxygen atom exchange occurs with an equilibrium isotope effect (18O eq) of 11–14‰ (Casciotti et al., 2007; Buchwald and Casciotti, unpublished), this equilibration would tend to raise the δ18O value of NO3 relative to the initial δ18O NO3 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 (Santo et al., 2011). These values represent the isotope effect expressed under non-limiting concentrations of NH4+. In the ocean NH4+ 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
Isotopic fractionation during microbial nitrification

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 site preference (SP) = δ¹³Nα − δ¹³Nβ) 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 δ¹³Nbulk 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, NO₃⁻, 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 O isotope systematics 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 (¹⁸OₙH₂O), 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 (δ¹⁸O₁₈N) 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 isotope 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 δ¹⁵N and δ¹⁸O 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; Loecher 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\textsubscript{3} or NO\textsubscript{2} (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 (A), $\delta^{15}$Nbulk-N\textsubscript{2}O (B), and SP vs. $\delta^{15}$Nbulk-N\textsubscript{2}O (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 NO$_2^-$ and NO$_3^-$ when nitrite oxidation and assimilation occur concurrently are:

$$\delta^{15}N_{\text{NO}_2} = \delta^{15}N_{\text{NO}_2,\text{produced}} + f_{\text{NA}} \times 15\varepsilon_{k, \text{NA}} + f_{\text{NXR}} \times 15\varepsilon_{k, \text{NXR}} \tag{2}$$

$$\delta^{15}N_{\text{NO}_3,\text{produced}} = \delta^{15}N_{\text{NO}_3} - 15\varepsilon_{k, \text{NXR}} \tag{3}$$

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

When nitrite oxidation is tightly coupled to ammonia oxidation and NO$_2^-$ does not accumulate, the $\delta^{18}$O value of the NO$_3^-$ produced primarily reflects the $\delta^{18}$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 ($x_{\text{NO}_2} = 0$; DiSpirito and Hooper, 1986; Friedman et al., 1986; Buchwald and Casciotti, 2010), is described by Equation 5.

$$\delta^{18}O_{\text{NO}_3,\text{final}} = \frac{2}{3} \left[ (1-x_{\text{NO}_2}) \delta^{18}O_{\text{NO}_2} + x_{\text{NO}_2} (\delta^{18}O_{\text{H}_2\text{O}} + 18\varepsilon_{\text{eq}}) \right] + \frac{1}{3} (\delta^{18}O_{\text{H}_2\text{O}} - 18\varepsilon_{k, \text{H}_2\text{O}, 2}) \tag{4}$$

$$\delta^{18}O_{\text{NO}_3,\text{final}} = \left[ \frac{2}{3} + \frac{1}{3} \varepsilon_{\text{AO}} \right] \delta^{18}O_{\text{H}_2\text{O}} + \frac{1}{3} \left[ (\delta^{18}O_{\text{H}_2\text{O}} - 18\varepsilon_{k, \text{O}_2} - 18\varepsilon_{k, \text{H}_2\text{O}, 1}) (1-x_{\text{AO}}) - 18\varepsilon_{k, \text{H}_2\text{O}, 2} \right] + \frac{2}{3} 18\varepsilon_{\text{eq}} (x_{\text{AO}}) \tag{5}$$

Equation 5 indicates that the $\delta^{18}$O$_{\text{NO}_3}$ produced by tightly-coupled ammonia and nitrite oxidation should reflect variations in both $\delta^{18}$O$_{\text{H}_2\text{O}}$ and $\delta^{18}$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 $\delta^{18}$O$_{\text{NO}_3}$ and the final $\delta^{18}$O$_{\text{NO}_3}$ produced. Regardless of whether 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 NO$_3^-$ . The expected $\delta^{18}$O$_{\text{NO}_3}$ value produced in oxygenated seawater with little exchange is −1‰ to +1‰ (similar to $\delta^{18}$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 NO$_2^-$ and H$_2$O is likely to play a role in setting $\delta^{18}$O$_{\text{NO}_3}$ and $\delta^{15}$N$_{\text{NO}_3}$ values observed in the ocean. This process does not change the concentration of NO$_2^-$ nor its $\delta^{15}$N value, only its $\delta^{18}$O value. Oxygen atom equilibration shifts a $\delta^{18}$O$_{\text{NO}_3}$ value from its biological starting point or “end member,” set by the isotopic systematics for biological production and consumption, toward the equilibrated $\delta^{18}$O$_{\text{NO}_3}$ value, dictated by ambient $\delta^{18}$O$_{\text{H}_2\text{O}}$ and the equilibrium isotope effect for the exchange ($18\varepsilon_{\text{eq}}$), which is dependent on temperature (McIlvin 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 $\delta^{18}$O$_{\text{NO}_3}$ and $\delta^{18}$O$_{\text{NO}_2}$ (Buchwald et al., 2012).

The tendency of NO$_2^-$ to exchange oxygen atoms abiotically with H$_2$O at typical seawater pH and temperature conditions suggests a utility of NO$_2^-$ oxygen isotopes as a tracer for determining the rate of biological turnover of 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.
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 (Kuyipers et al., 2005; Thamdrup et al., 2006; Hamersley et al., 2007; Lam et al., 2009; Ward et al., 2009; Bulow et al., 2010; Jensen et al., 2011), 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 δ¹⁵N, and δ¹⁸O 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 nitrate oxidation could be an important sink for NO₂⁻ at the top of the SNM based on δ¹⁵N, 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:

\[
\Delta \delta^{15}N = \delta^{15}N_{NO_3} - \delta^{15}N_{NO_2} = \frac{F_{NIR} - F_{NXR}}{F_{NAR}} \times \frac{\delta^{15}N_{NO_2}}{\delta^{15}N_{NO_3}}
\]

where \(F_{NAR}, F_{NXR}, \text{ and } F_{NIR}\) are the fluxes from nitrate reduction, nitrite oxidation, and nitrite reduction, respectively, and \(\delta^{15}N_{NO_2}, \delta^{15}N_{NIR}, \text{ and } \delta^{15}N_{NXR}\) 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 (\(F_{NIR}/F_{NAR} = 0\)) 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 Nitrococcus 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 Δδ15N 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 NO3- CYCLING AND BUDGETS: Δ(15, 18) REVISITED**

Knowing the isotopic systematics of nitrification is critical for interpreting δ15ONO3, δ18ONO3, and δ18ON2O 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 δ18O data to put the first constraints on the δ18O value of NO3 produced in the ocean. These estimates showed that NO3 is most likely produced with δ18O values close to those of seawater (0‰) and were used by Sigman et al. (2005) to constrain the rates of N2 fixation and nitrite reoxidation from δ15NNO3 to δ18ONO3 data. In order to do this, Sigman et al. (2005) introduced a NO3− isotope anomaly based on expected enrichments of δ15NNO3 and δ18ONO3 due to nitrate assimilation or nitrate reduction during denitrification:

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

where δ15NNO3 and δ18ONO3 are the measured isotopic values of the sample, δ15NNO3,deep and δ18ONO3,deep are the isotopic values of unaltered deep seawater, which define the starting point for fractionation. 18N,NAR and 15N,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 18N,NAR and 15N,NAR, their ratio is very close to 1 (Granger et al., 2004, 2008, 2010). Therefore, NO3− consuming processes generally lead to δ15NNO3 and δ18ONO3 values that fall along a 1:1 line and produce samples with Δ(15, 18) = 0‰ (Figure 4). Non-zero Δ(15, 18) values correspond to an enrichment of δ18ONO3 relative to δ15NNO3, or a depletion in δ15NNO3 relative to δ18ONO3, generally arising from production of NO3− with anomalous isotopic signatures. The most likely cause for depletion in δ15N, especially in the nitracline of oligotrophic ocean provinces, is through remineralization of newly fixed N with a δ15N value near −1‰ (Capone et al., 1997; Karl et al., 1997; Meador et al., 2007). The particulate organic N produced by N fixation is remineralized to NO3− in the subsurface, gaining O atoms from nitrification, the same process that sets the oxygen isotopic signature of NO3− produced from other N sources. In scenario, the magnitude of

\[\Delta (15, 18) \text{ would be proportional to the N fixation flux (Sigman et al., 2005).} \]

A relative enrichment in 18O, especially in the vicinity of oceanic ODZs, could represent the cycling of NO3− through the reduction/reoxidation cycle, where the NO3− consumed by denitrification has a similar δ15NNO3 but a lower δ18ONO3 value than that returned to the NO3− pool from nitrite oxidation (Sigman et al., 2005). This formulation was successful at simulating data from regions of the ETNP where NO3− did not accumulate (Sigman et al., 2005) and where NO3− goes to zero at the top of the SNM (Casciotti and Mcllvyn, 2007). However, where NO3− accumulates, its isotopic composition can vary dramatically within the oxygen deficient zone itself (Casciotti and Mcllvyn, 2007), and an interpretation including NO3− isotope constraints is needed. The relationship between 18O enrichment in NO3− 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 Δ(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 δ15NNO3 and δ18ONO3 in a hypothetical ODZ (Figure 5)
show that nitrite oxidation can either raise or lower Δ(15, 18), depending on the relative 15N and 18O values of NO2- and NO3-. Our model focuses on determining the relative rates of NO2- reoxidation to NO3- (FNXR) and reduction (to NO or NH4+; FNIR) from NO3- and NO2- isotopic data. The oxidative flux is assumed to have the N and O isotopic systematics of bacterial nitrite oxidation (Buchwald and Casciotti, 2010; Table 1), 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 15E = 18E = 15% (Table 1) regardless of whether NO2- is reduced to N2 (via anammox or denitrification) or NH4+ [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 (FNXR/FNIR) between 0 and 3 (FNXR representing 0–75% of NO2- consumption) and the rate constant for exchange (kEXCH) between 0 and 1 day−1 to evaluate the effects of changes in these parameters on simulated 15NNO3 and 18ONO3 (Figure 6). Maximum rate constants of exchange between NO2- and H2O of 1 day−1 appear reasonable based on recent laboratory studies (Casciotti et al., 2007; Buchwald and Casciotti, unpublished). As FNXR/FNIR increases from 0 to 3, the amount of NO2- retained in the system increases despite an unchanging rate constant for nitrate reduction. In fact, because the reaction is taken as first order, the higher concentrations of NO2- brought about by higher levels of FNXR 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 NO3-, accumulation of NO2-, and production of N2 (Figures 6A–D), and may help explain why NO2- is never fully removed in oceanic ODZs.

The magnitude of nitrite oxidation also affects the 15NNO3 and 18ONO3 patterns. When FNXR/FNIR = 0, the 15NNO3 and 18ONO3 data fall along the 1:1 line prescribed by the isotope effects for nitrate reduction (Figures 6E–G). As FNXR/FNIR increases, increasingly negative Δ(15, 18) values are produced. The strength of this effect is also dependent on the rate of

---

**Table 1** Parameters used in oxygen deficient zone box model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15NNO3 initial</td>
<td>Initial nitrate 15N</td>
<td>5%</td>
<td>Sigman et al., 2000</td>
</tr>
<tr>
<td>18O NO3 initial</td>
<td>Initial nitrate 18O</td>
<td>2%</td>
<td>Casciotti et al., 2002</td>
</tr>
<tr>
<td>18O HzO</td>
<td>Water 18O value</td>
<td>0%</td>
<td>Craig and Gordon, 1965</td>
</tr>
<tr>
<td>kNAR</td>
<td>First order rate constant for nitrate reduction</td>
<td>0.001 day−1</td>
<td>Estimated to achieve a rate of 20 nM day−1; Lam et al., 2011</td>
</tr>
<tr>
<td>kNXR</td>
<td>First order rate constant for nitrite oxidation</td>
<td>0–0.003 day−1</td>
<td>Estimated to achieve range of observed nitrite oxidation rates; Füssel et al., 2012; Lipschultz et al., 1990</td>
</tr>
<tr>
<td>kEXCH</td>
<td>First order rate constant for nitrite/water exchange</td>
<td>0.01 day−1</td>
<td>Estimated to achieve a rate of 5 nM day−1; Devol et al., 2006</td>
</tr>
<tr>
<td>15Nex_NAR</td>
<td>N isotope effect for nitrate reduction</td>
<td>1.019</td>
<td>Deutsch et al., 2004; Granger et al., 2008</td>
</tr>
<tr>
<td>15Nex_NXR</td>
<td>N isotope effect for nitrite oxidation</td>
<td>0.985</td>
<td>Casciotti, 2009; Buchwald and Casciotti, 2010</td>
</tr>
<tr>
<td>15Nex_NXR</td>
<td>N isotope effect for nitrite reduction</td>
<td>1.015</td>
<td>Bryan et al., 1983</td>
</tr>
<tr>
<td>18Oex_NXR</td>
<td>O isotope effect for nitrite oxidation</td>
<td>1.019</td>
<td>Granger et al., 2008</td>
</tr>
<tr>
<td>18Oex_NXR</td>
<td>O isotope effect for nitrite reduction</td>
<td>0.997</td>
<td>Buchwald and Casciotti, 2010</td>
</tr>
<tr>
<td>18Oex_H2O.2</td>
<td>O isotope effect for H2O incorporation</td>
<td>1.015</td>
<td>Sigman et al., 2005</td>
</tr>
<tr>
<td>18Oex_BB</td>
<td>Branching O isotope effect during nitrate reduction</td>
<td>0.975</td>
<td>Casciotti et al., 2007</td>
</tr>
<tr>
<td>18Oex_BB</td>
<td>Equilibrium isotope effect for nitrite/water O exchange</td>
<td>1.014</td>
<td>Casciotti et al., 2007; (Buchwald and Casciotti, unpublished)</td>
</tr>
</tbody>
</table>
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 Δ15N, Δ18O values, depending on the extent of NO_3^- consumption (increasing δ15N, δ18O values), the relative rates of nitrite oxidation and reduction (F_NXR/F_NIR), and the rate of oxygen atom exchange between NO_3^- and H_2O (k_EXCH). In each case the slope of δ18O_NO3 vs. δ15N_NO3 is equal to 1 when F_NXR = 0. As F_NXR/F_NIR increases, the magnitude of the Δ15N, Δ18O anomaly increases at a given δ15N value. As NO_2^-/H_2O exchange increases (k_EXCH = 0 in panel E, 0.5 in panel F, and 1.0 in panel G), the non-zero levels of nitrite oxidation generate positive Δ15N, Δ18O values, most likely due to the relative δ18O values of NO_3^- produced and consumed under these scenarios. All parameters used in the model are reported in Table 1.

Abiotic NO_2^-/H_2O exchange, with higher exchange rates partly diluting this effect and actually leading to positive Δ(15N, 18O) values at high extents of NO_3^- consumption (the highest δ15N_NO3 values; Figure 6). This interesting phenomenon is most likely due to reversal of the impact of nitrite reoxidation on δ18O_NO3 at high δ18O_NO3 values, with nitrite oxidation returning NO_3^- with a lower δ18O_NO3 value than that removed by nitrite reduction. This would be exacerbated at high rates of exchange, which helps to maintain δ18O_NO3 values at a constant level regardless of δ18O_NO3. Tuning the model to match observed δ18O_NO3 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 Δ(15N, 18O) anomalies at a given δ15N_NO3 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^-.

It is interesting to note that the two scenarios for producing negative Δ(15N, 18O) 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 Δ(15, 18) signals at δ15N_NO3 and δ18O_NO3 values less than 10‰, near the base of the euphotic zone. In contrast, nitrite reoxidation is most effective at generating negative Δ(15, 18) signals at intermediate δ15N_NO3 and δ18O_NO3 values and extents of NO_3^-.
consumption by denitrification, where N\textsubscript{2} fixation has relatively little effect on the $\Delta$(15, 18). Therefore, we may be able to distinguish between the processes responsible for $\Delta$(15, 18) generation by where the anomaly lies in $\delta^{15}$N\textsubscript{NO\textsubscript{3}} vs. $\delta^{18}$O\textsubscript{NO\textsubscript{3}} space, as well as from other water column indicators. For example, using a steady state model, Casciotti and McIlvin (2007) showed that the NO\textsubscript{3} isotope anomaly at the top of the SMN could not be generated by N\textsubscript{2} fixation alone and was consistent with oxidation of NO\textsubscript{2} leaking out of the top of the SNM. However, they suggested that a combination of N\textsubscript{2} fixation and nitrite reoxidation may best fit the observations. This conclusion is echoed here where it is difficult to generate large $\Delta$(15, 18) signals at these $\delta^{15}$N\textsubscript{NO\textsubscript{3}} and $\delta^{18}$O\textsubscript{NO\textsubscript{3}} values through either N\textsubscript{2} fixation or nitrite reoxidation alone (Figure 7).

In addition to oxygen deficient zone and near-surface processes, NO\textsubscript{3} isotope enrichment during microbial nitrification, and applying this to the model from Wankel et al. (2007), they assumed that $\delta^{18}$O\textsubscript{NO\textsubscript{3}} for the same amount of nitrification contributing to nitrate uptake by phytoplankton in Monterey Bay, consistent with intensive isotope tracer incubation studies (Ward, 2005). Because $\delta^{18}$O\textsubscript{str} was uncertain at that time, they performed sensitivity studies to address the impact of different $\delta^{18}$O\textsubscript{str} values on their interpretation. We now believe that $\delta^{18}$O\textsubscript{str} 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 $\delta^{18}$O\textsubscript{NO\textsubscript{3}} for the same amount of nitrification. Thus, to achieve the same $\delta^{18}$O\textsubscript{NO\textsubscript{3}} 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 $18F\textsubscript{NKR}=18E\textsubscript{NKR}$ for nitrate processes (N\textsubscript{2} 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 $\delta^{15}$N\textsubscript{NO\textsubscript{3}} and $\delta^{18}$O\textsubscript{NO\textsubscript{3}} data from a variety of oceanographic profiles representing the major ocean basins, the impacts of partial NO\textsubscript{3} assimilation in polar regions on the N and O isotopes of NO\textsubscript{3} in the ocean interior, and of low latitude productivity on the $^{18}$O enrichment in preformed NO\textsubscript{3} was diagnosed. N budget processes (N\textsubscript{2} fixation and denitrification) led to variations in subsurface $\delta^{15}$N\textsubscript{NO\textsubscript{3}} and $\delta^{18}$O\textsubscript{NO\textsubscript{3}}, but in their absence, the large scale steady state $\delta^{18}$O value of subsurface NO\textsubscript{3} was set by nitrate assimilation in polar regions. Nitrate uptake in the southern ocean leads to heavy isotope enrichment in preformed NO\textsubscript{3}, while nitrate assimilation in low latitudes removes the $\delta^{18}$O signal of the preformed NO\textsubscript{3} and replaces it with the nitrification signal (Sigman et al., 2009). Overall, when only internal processes were active in the model, the mean ocean $\delta^{18}$O\textsubscript{NO\textsubscript{3}} value was $1.1\%$ higher than the nitrification source. When the N budget was added to the model, the mean ocean $\delta^{18}$O\textsubscript{NO\textsubscript{3}} value was $2.4\%$ higher than the nitrification source value. This analysis provides additional constraints on the $\delta^{18}$O value of newly produced NO\textsubscript{3} 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 NO\textsubscript{3} 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 $\delta^{15}$N\textsubscript{NO\textsubscript{3}} and $\delta^{18}$O\textsubscript{NO\textsubscript{3}} variations. Assuming that nitrate assimilation leads to equivalent fractionation of N and O isotopes (Granger et al., 2004), and that $\delta^{18}$O\textsubscript{str} = $2.9\%$, they estimated that nitrification could supply up to $30\%$ of NO\textsubscript{3} assimilated by phytoplankton in Monterey Bay, consistent with intensive isotope tracer incubation studies (Ward, 2005). Because $\delta^{18}$O\textsubscript{str} was uncertain at that time, they performed sensitivity studies to address the impact of different $\delta^{18}$O\textsubscript{str} values on their interpretation. We now believe that $\delta^{18}$O\textsubscript{str} 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 $\delta^{18}$O\textsubscript{NO\textsubscript{3}} for the same amount of nitrification. Thus, to achieve the same $\delta^{18}$O\textsubscript{NO\textsubscript{3}} 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 $18F\textsubscript{NKR}=18E\textsubscript{NKR}$ for nitrate
uptake and allowed branching of NH$_4^+$ (and NO$_2^-$) between nitrification and assimilation to partition isotopes between the NO$_3^-$ and particulate N pools. One important difference from the Wankel et al. (2007) model is that they assumed $\delta^{18}$O$_{\text{NO}_3}$ = +1.1% based on more recent constraints on this value (Sigman et al., 2009). They inferred that $\delta^{15}$N$_{\text{NO}_3}$ should be lowered slightly due to nitrification (offsetting the isotopic fractionation during uptake) and $\delta^{18}$O$_{\text{NO}_3}$ should be raised (because the $\delta^{18}$O of newly produced NO$_3^-$ was higher than that removed). Both of these factors should lead to negative $\Delta^{15}$ (15, 18) values, as discussed above, but they found that nitrification had a relatively small impact on $\delta^{15}$N$_{\text{NO}_3}$ and $\delta^{18}$O$_{\text{NO}_3}$ values in the Polar Antarctic Zone. They concluded that in the Polar Antarctic Zone less than 1% of 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 provides an excellent example of how NO$_3^-$ isotopes can be used to constrain N cycle processes in an appropriate model framework.

NO$_3^-$ and NO$_2^-$ isotopes have also been used to understand the sources and cycling of NO$_3^-$ in the PNM at the base of the euphotic zone. Mackey et al. (2011) used natural abundance NO$_3^-$ + NO$_2^-$ isotope data and isotope tracer experiments to determine the sources of 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, NO$_3^-$ was generated by incomplete NO$_3^-$ reduction by light-limited phytoplankton creating a broad band of NO$_3^-$. After stratification was established, NO$_3^-$ generation by ammonia oxidation contributed to maintenance of the PNM. In both cases, NO$_3^-$ was consumed by nitrite oxidation below the PNM. Once again, nitrification was interpreted to play an important role in NO$_3^-$ isotope dynamics in the upper water column where increases in $\delta^{18}$O$_{\text{NO}_3}$ were much higher than increases in $\delta^{15}$N$_{\text{NO}_3}$. In another recent study of PNM dynamics, natural abundance $\delta^{18}$O$_{\text{NO}_3}$ and $\delta^{15}$N$_{\text{NO}_3}$ values were used to infer the sources and average age of NO$_3^-$ in the PNM of the Arabian Sea (Buchwald and Casciotti, unpublished). Because the $\delta^{15}$N$_{\text{NO}_3}$ and $\delta^{18}$O$_{\text{NO}_3}$ values produced from ammonia oxidation and nitrate reduction are distinct, the sources can be readily distinguished. Based on natural abundance $\delta^{15}$N$_{\text{NO}_3}$ and $\delta^{18}$O$_{\text{NO}_3}$ data, ammonia oxidation was inferred to be the main source of NO$_3^-$ to the PNM in the Arabian Sea.

**IMPLICATIONS FOR INTERPRETING N$_2$O SOURCES**

Uncertainty in the isotopic composition of N$_2$O produced during ammonia oxidation has hampered the interpretation of near-surface N$_2$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 systematics of nitrification can provide further insight into outstanding questions in N$_2$O oxygen isotope variations, such as why $\delta^{18}$O$_{\text{N}_2\text{O}}$ in seawater is so high (Ostrom et al., 2000; Popp et al., 2002), what mechanisms of N$_2$O production operate in oxic and anoxic environments. These mechanisms and controls on N$_2$O production are in the near-surface ocean (Dore et al., 1998; Popp et al., 2002; Santoro et al., 2011).

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

**CONCLUDING REMARKS**

Understanding the nitrogen and oxygen isotopic systematics of nitrification can contribute greatly to our understanding of nitrogen cycling in the ocean, as nitrification is involved with transformations between the major pools of DIN (NH$_4^+$, NO$_2^-$, NO$_3^-$, and N$_2$O). Both ammonia and nitrite oxidation are involved with large and distinctive isotope effects, leading to predictable patterns in the isotope ratios of compounds that they transform. The discovery of AOA and their importance in ocean biogeochemistry necessitates renewed study of the isotopic systematics of nitrification. In preliminary studies, the isotopic systematics of AOA appear similar to AOB for N isotope fractionation and O atom incorporation into NO$_3^-$ (Santoro and Casciotti, 2011; Santoro et al., 2011). However, the production of N$_2$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


Paasche, K. C. (2007). The role of nitrogen fixa-

activation and metabolism of an amam-


Putnam, M. L., Venkiteswaran, J. J., Schiff, S. L., and Spoelstra, J. (2012). Deciphering the oxygen iso-


Santoro, A. E., Casciotti, K. L., and Francis, C. A. (2010). Activity, abundance, and diversity of nitri-


Santoro, A. E., and Casciotti, K. L. (2011). Enrichment and charac-


gin from nitrification or denitri-

fication? A theoretical approach from referred data and microbi-


This article was submitted to Frontiers in Aquatic Microbiology, a specialty of Frontiers in Microbiology. Copyright © 2012 Casciotti and Buchwald. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.