



# Global Biogeochemical Cycles

## RESEARCH ARTICLE

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### Key Points:

- $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only suggest expression of  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibrium isotope effect, likely due to enzyme reversibility
- Interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  is correlated with seasonal mixed layer deepening and suggests a role for entrained  $\text{NO}_2^-$  oxidizers
- Interconversion may occur where  $\text{NO}_2^-$  oxidizers are transported into regions that inhibit  $\text{NO}_2^-$  oxidation, including oxygen-deficient zones

### Supporting Information:

- Supporting Information S1

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## Enzyme-level interconversion of nitrate and nitrite in the fall mixed layer of the Antarctic Ocean

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**Abstract** In the Southern Ocean, the nitrogen (N) isotopes of organic matter and the N and oxygen (O) isotopes of nitrate ( $\text{NO}_3^-$ ) have been used to investigate  $\text{NO}_3^-$  assimilation and N cycling in the summertime period of phytoplankton growth, both today and in the past. However, recent studies indicate the significance of processes in other seasons for producing the annual cycle of N isotope changes. This study explores the impact of fall conditions on the  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) and  $^{18}\text{O}/^{16}\text{O}$  ( $\delta^{18}\text{O}$ ) of  $\text{NO}_3^-$  and nitrite ( $\text{NO}_2^-$ ) in the Pacific Antarctic Zone using depth profiles from late summer/fall of 2014. In the mixed layer, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  increase roughly equally, as expected for  $\text{NO}_3^-$  assimilation; however, the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ -only (measured after  $\text{NO}_2^-$  removal) increases more than does  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$ . Differencing indicates that  $\text{NO}_2^-$  has an extremely low  $\delta^{15}\text{N}$ , often  $< -70\text{‰}$  versus air. These observations are consistent with the expression of an equilibrium N isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , likely due to enzymatic  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion. Specifically, we propose reversibility of the nitrite oxidoreductase (NXR) enzyme of nitrite oxidizers that, having been entrained from the subsurface during late summer mixed layer deepening, are inhibited by light. Our interpretation suggests a role for  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion where nitrifiers are transported into environments that discourage  $\text{NO}_2^-$  oxidation. This may apply to surface regions with upwelling, such as the summertime Antarctic. It may also apply to oxygen-deficient zones, where NXR-catalyzed interconversion may explain previously reported evidence of  $\text{NO}_2^-$  oxidation.

### 1. Introduction

Production and export of organic carbon from the high-latitude surface ocean lowers the atmospheric concentration of carbon dioxide ( $\text{CO}_2$ ) directly through the removal of dissolved inorganic carbon to the deep ocean and indirectly by driving an increase in whole-ocean alkalinity [Sigman and Boyle, 2000]. In the modern Southern Ocean, nutrient consumption in surface waters is incomplete due to limitation of phytoplankton by iron and light [Martin, 1990; Sunda and Huntsman, 1997]. The upper ocean surrounding Antarctica is thus enriched in nitrogen (N) and phosphorus (P), the production and export of organic carbon is much less than potential export [Reuer et al., 2007], and much of the upwelled  $\text{CO}_2$  degasses to the atmosphere [Sigman et al., 2010]. Increasing nutrient consumption in the Southern Ocean could stem this  $\text{CO}_2$  leakage and has been proposed as an explanation for the lower atmospheric  $\text{CO}_2$  concentrations of past ice ages [Knox and McElroy, 1984; Sarmiento and Toggweiler, 1984; Siegenthaler and Wenk, 1984].

In the Antarctic Zone (AZ), the southernmost region of the Southern Ocean, nutrients are supplied to the surface ocean through seasonal mixing and wind-driven upwelling. In winter, the Antarctic surface cools and increases in salinity due to sea ice formation, deepening the mixed layer and importing nutrients from the deep ocean into surface waters. Furthermore,  $\text{NO}_3^-$ -rich deep water upwells into the surface ocean due to Ekman divergence induced by circumpolar westerly winds. During summer, surface warming melts the ice and shoals the mixed layer, stratifying the water column. No longer light-limited, phytoplankton in the summertime mixed layer draw down  $\text{NO}_3^-$  and other nutrients. The water below the summertime mixed layer, which was the base of the winter mixed layer, retains the low temperatures of the Antarctic winter and is called the temperature minimum ( $T_{\text{min}}$ ) layer. The  $T_{\text{min}}$  layer is thought to be a summertime record of winter

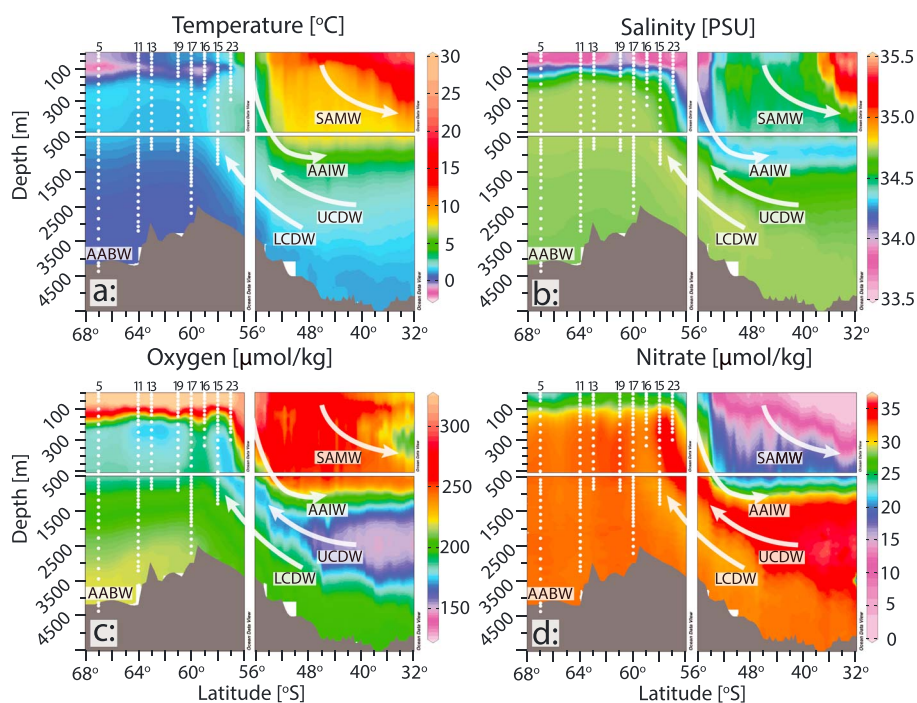
conditions as well as a reflection of the initial state from which the surface ocean evolves throughout the summer [Altabet and Francois, 2001].

The  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$  ratios of seawater  $\text{NO}_3^-$  can be used to study nutrient consumption in the Southern Ocean. Phytoplankton consuming surface  $\text{NO}_3^-$  preferentially assimilate the lighter isotopes of nitrogen and oxygen,  $^{14}\text{N}$  and  $^{16}\text{O}$ , relative to the heavier isotopes,  $^{15}\text{N}$  and  $^{18}\text{O}$  [Wada and Hattori, 1978; Pennock et al., 1996; Waser et al., 1998; Needoba et al., 2004], such that consumption increases the  $\delta^{15}\text{N}$  ( $([^{15}\text{N}/^{14}\text{N}]_{\text{sample}}/[^{15}\text{N}/^{14}\text{N}]_{\text{air}} - 1) \times 1000$ ) and  $\delta^{18}\text{O}$  ( $([^{18}\text{O}/^{16}\text{O}]_{\text{sample}}/[^{18}\text{O}/^{16}\text{O}]_{\text{VSMOW}} - 1) \times 1000$ ) of the  $\text{NO}_3^-$  remaining in seawater. This isotopic change in the  $\text{NO}_3^-$  pool is commonly simulated using the Rayleigh model, which describes an approximately linear relationship between the  $\delta^{15}\text{N}$  of the  $\text{NO}_3^-$  substrate and the natural logarithm of the fraction of  $\text{NO}_3^-$  remaining [Mariotti et al., 1981]. The slope of the line approximates  $^{15}\epsilon$ , the N isotope effect for nitrate assimilation, which is defined as  $^{15}\epsilon = ({}^{14}k/{}^{15}k - 1) \times 1000$ , where  ${}^{14}k$  and  ${}^{15}k$  are the rate coefficients for the assimilation of  $^{14}\text{N}$  and  $^{15}\text{N}$ -bearing  $\text{NO}_3^-$ , respectively. Below, we refer to plots of  $\text{NO}_3^-$   $\delta^{15}\text{N}$  or  $\delta^{18}\text{O}$  against the natural logarithm of  $\text{NO}_3^-$  as “Rayleigh space.” The Rayleigh model assumes a closed substrate pool, which is largely the case for  $\text{NO}_3^-$  in the AZ during the summer, when  $\text{NO}_3^-$  supply is weak relative to  $\text{NO}_3^-$  assimilation. Moreover, because the fractional consumption of  $\text{NO}_3^-$  in the summertime surface is relatively low, violations of the Rayleigh model assumptions due to Ekman-driven  $\text{NO}_3^-$  supply or in situ remineralization have minimal effect [Sigman et al., 1999].

The Rayleigh model, however, fails to capture seasonal patterns in the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ , which recent studies of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  isotopes from the Southern Ocean have begun to document [DiFiore et al., 2010; Smart et al., 2015]. In particular, the Rayleigh model fails to explain the chemistry of the temperature minimum layer, due at least in part to wintertime nitrification of the low- $\delta^{15}\text{N}$   $\text{NH}_4^+$  resulting from intensive biological N recycling in the late summer mixed layer [Lourey et al., 2003; Smart et al., 2015].

This study provides additional insights into the seasonal origin of non-Rayleigh  $\text{NO}_3^-$  isotope dynamics in the Southern Ocean. We report  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only in samples from eight profiles collected in the Pacific Antarctic Zone during the 2014 U.S. Repeat Hydrography cruise along the P16S line. In the combined  $\text{NO}_3^- + \text{NO}_2^-$  data, we recover the previously observed non-Rayleigh behavior of the  $T_{\text{min}}$  layer and derive values for the N and O isotope effects of  $\text{NO}_3^-$  assimilation that are consistent with prior estimates from the region. The coupled N and O isotope measurements of the combined  $\text{NO}_3^- + \text{NO}_2^-$  pool indicate close to equivalent increases in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  into the surface mixed layer, consistent with expectations for  $\text{NO}_3^-$  assimilation from culture studies [Granger et al., 2004, 2008, 2010; Karsh et al., 2012, 2014].

However, we find that the  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ -only increases more than does the  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ -only, which is not expected given known Southern Ocean processes. By differencing the  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only measurements, we calculate very low values for  $\text{NO}_2^-$   $\delta^{15}\text{N}$  in the upper ~90 m. These observations suggest the expression of an equilibrium N isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in the late summer surface ocean, which preferentially partitions  $^{15}\text{N}$  into  $\text{NO}_3^-$  and  $^{14}\text{N}$  into  $\text{NO}_2^-$ . This equilibration could result from the bidirectional interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , likely catalyzed by biochemical reaction reversibility. The  $\text{NO}_2^-$  oxidoreductase (NXR) enzyme, a bidirectional enzyme in certain nitrifying microorganisms that catalyzes both the oxidation of  $\text{NO}_2^-$  and the reduction of  $\text{NO}_3^-$  [Sundermeyer-Klinger et al., 1984], has previously been implicated in isotope exchange between  $\text{NO}_2^-$  and  $\text{NO}_3^-$  [Friedman et al., 1986; Wunderlich et al., 2013]. We suggest that  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion across NXR results in expression of the equilibrium N isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , which would explain the very low values of  $\text{NO}_2^-$   $\delta^{15}\text{N}$  that we calculate for the Southern Ocean surface. Moreover, differences in estimates of the assimilation isotope effects between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only data suggest that the intensity of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion is linked to the seasonal deepening of the mixed layer, suggesting a role for nitrifiers entrained from the dark  $T_{\text{min}}$  layer into the sunlit surface mixed layer. Enzyme-level interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  is a new complication in our understanding of the marine N cycle. This process has the potential to be important in the many oceanographic environments where  $\text{NO}_2^-$  oxidizers can be transported across light or chemical gradients into waters with conditions unfavorable for  $\text{NO}_2^-$  oxidation.



**Figure 1.** (a) Temperature ( $^{\circ}\text{C}$ ), (b) salinity (psu, practical salinity unit), (c) oxygen concentration ( $\mu\text{mol}/\text{kg}$ ), and (d)  $\text{NO}_3^-$  concentration ( $\mu\text{mol}/\text{kg}$ ) along  $150^{\circ}\text{W}$ . White dots show the locations of samples analyzed in this study, with station number given at the top of each profile. UCDW: Upper Circumpolar Deep Water; LCDW: Lower Circumpolar Deep Water; AABW: Antarctic Bottom Water; AAIW: Antarctic Intermediate Water; SAMW: Subantarctic Mode Water.

## 2. Methods

### 2.1. Cruise Track and Sample Collection

Seawater samples were collected during the 2014 U.S. Repeat Hydrography P16S cruise onboard the R/V *Nathaniel B. Palmer*. From 20 March to 5 May, the cruise occupied a total of 90 stations from  $67^{\circ}\text{S}$  to  $15^{\circ}\text{S}$  along  $150^{\circ}\text{W}$ , and hydrographic information for each cast was collected from a Sea-Bird Electronics CTD. Water samples were collected every 25 to 50 m in the surface ocean and every 100 to 400 m at depth. The onboard science crew analyzed the samples for the concentrations of major nutrients, and measurements are presented here in their reported units.

The samples analyzed in this study were collected from eight stations between  $57^{\circ}\text{S}$  and  $67^{\circ}\text{S}$  (Figure 1, white circles). While some sampled profiles extended to the ocean floor, others were sampled only through the upper water column. Seawater was collected in 50 mL bottles that were rinsed three times with sample prior to filling and were frozen at  $-20^{\circ}\text{C}$  within 2 h of collection. Inserts were added to the frozen sample bottles within 1 week of freezing. Initial isotopic analysis revealed that some of the collected samples were depleted in  $\text{NO}_3^-$  by as much as 50% of their shipboard-measured concentrations. Refractometer testing indicated that these samples also had reduced salinity, with measured-to-reported salinity ratios of 50–100%. The  $\text{NO}_3^-$  and salinity ratios fall close to a 1:1 line and suggest a linear relationship between salt loss and  $\text{NO}_3^-$  loss. These observations are consistent with the loss of brine, which likely formed and escaped when the sample froze in storage. To adjust for this brine loss, we measured the salinity of each sample and used regression lines to generate an estimate of actual  $\text{NO}_3^-$  concentration, which was then used to calculate the desired injection volume for our mass spectrometer measurements (see below). While our data set may be compromised modestly by this brine loss and associated variations in the volume of sample injections, inspection of the data for correlations between brine loss and isotopic composition yielded no compelling trends (supporting information Text S1 and Figure S1). Here we report  $\text{NO}_3^-$  concentrations as measured at sea, not the compromised concentrations in our sample bottles.

## 2.2. Isotopic Analysis of $\text{NO}_3^- + \text{NO}_2^-$ and $\text{NO}_3^-$ -Only

$\text{NO}_3^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were measured using the denitrifier method, in which bacteria lacking an active nitrous oxide ( $\text{N}_2\text{O}$ )-reductase quantitatively convert seawater  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  gas [Sigman *et al.*, 2001; Casciotti *et al.*, 2002]. Additionally, the denitrifier method converts any  $\text{NO}_2^-$  in the sample to  $\text{N}_2\text{O}$ , which can confound interpretations of measured  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ .  $\text{NO}_2^-$  in seawater affects measurements of  $\delta^{18}\text{O}$  because, during bacterial conversion,  $\text{NO}_2^-$  loses a smaller fraction of O atoms than  $\text{NO}_3^-$  as the two N species are converted to  $\text{N}_2\text{O}$  (3/4 in  $\text{NO}_2^-$  versus 5/6 in  $\text{NO}_3^-$ ). The isotopic impact of such differential O loss is that  $\text{N}_2\text{O}$  generated from  $\text{NO}_2^-$  has a  $\delta^{18}\text{O}$  that is  $\sim 25\text{‰}$  lower than  $\text{N}_2\text{O}$  produced from  $\text{NO}_3^-$  with the same initial  $\delta^{18}\text{O}$  [Casciotti *et al.*, 2007], implying that our measured values systemically underestimate the true  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$ . To correct for this methodological bias, we increased the measured  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  by the product of 25‰ and the fraction of  $\text{NO}_2^-$  in each sample ( $[\text{NO}_2^-]/[\text{NO}_3^- + \text{NO}_2^-]$ ); the magnitude of this correction ranges from 0.00 to 0.31‰. It is important to note that this correction only applies to  $\delta^{18}\text{O}$ , not to  $\delta^{15}\text{N}$ , and that the magnitude of the correction is much smaller than the  $\text{NO}_3^-$   $\delta^{18}\text{O}$  difference between the surface ocean and deep ocean. Lastly, uncertainty in the exact correction factor, here taken as 25‰, is insufficient to explain the signals identified and discussed in the interpretation below. All further references to  $\text{NO}_2^-$  effects refer to the isotopic signal of  $\text{NO}_2^-$  in the water column and not to this methodological correction.

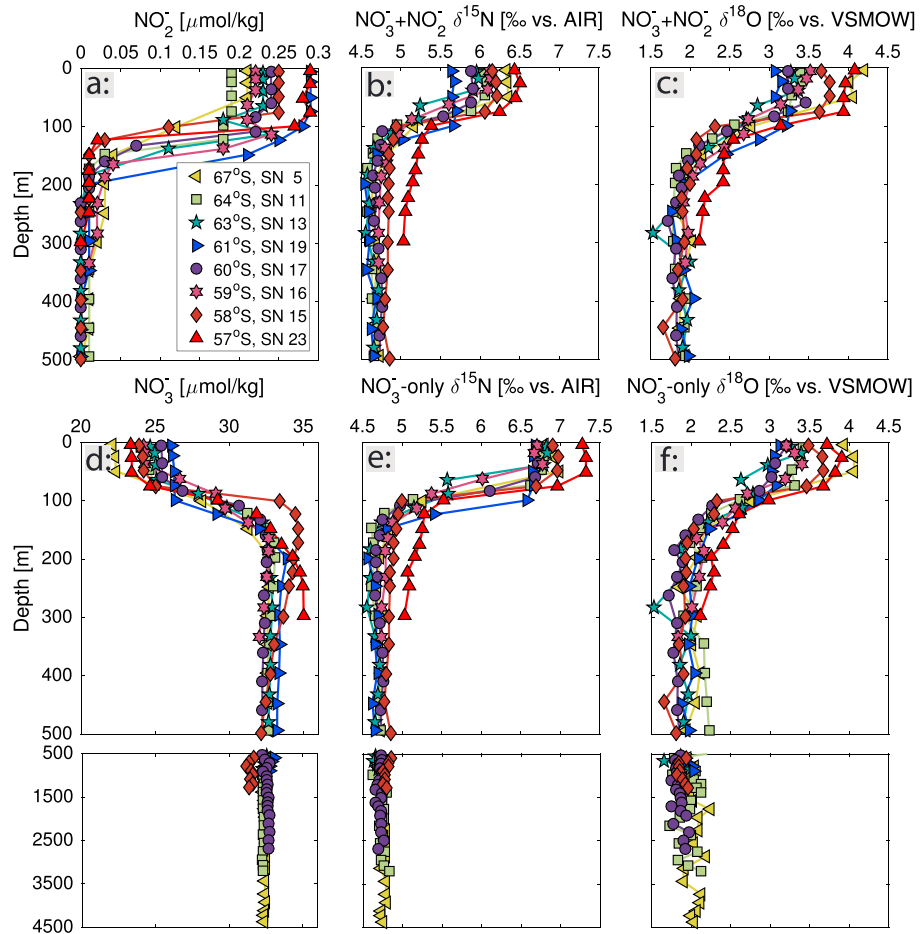
In order to isolate the  $\text{NO}_3^-$ -only isotopic signal, all samples with detectable  $\text{NO}_2^-$  (concentrations  $\geq 0.01$   $\mu\text{mol/kg}$ ) were treated with sulfanilamide prior to analysis with the denitrifier method. During treatment, 200  $\mu\text{L}$  of 1% sulfanilamide in 10% HCl were added per 10 mL of sample or standard and left for 7–10 min. 90–100  $\mu\text{L}$  of 2 M NaOH was then added to increase sample pH to 6–8, as measured using pH indicator strips. This sulfanilamide-based protocol was undertaken as part of a methodological comparison with the sulfamic acid-based protocol of Granger and Sigman [2009]. The method comparison showed extremely similar results for the two protocols, for samples both with and without  $\text{NO}_2^-$ , consistent with prior testing of nitrite-spiked nitrate reference solutions [Weigand *et al.*, 2016]. Hereafter, the results from our samples and their  $\text{NO}_2^-$ -removed counterparts are always distinguished because the presence or absence of  $\text{NO}_2^-$  is central to the discussion. We use “ $\text{NO}_3^-$ -only” to refer to samples treated for  $\text{NO}_2^-$  removal or samples without any reported  $\text{NO}_2^-$ , while “ $\text{NO}_3^- + \text{NO}_2^-$ ” refers to the original (untreated) sample.  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^-$  plotted for deep samples without any reported  $\text{NO}_2^-$  represent the same measurements of untreated samples.

The isotopic composition of  $\text{N}_2\text{O}$  was measured by gas chromatography-isotope ratio mass spectrometry using a purpose-built online  $\text{N}_2\text{O}$  extraction and purification system and Thermo MAT 253 mass spectrometer [Weigand *et al.*, 2016]. Seawater solutions of the international  $\text{NO}_3^-$  reference materials IAEA-NO3 and USGS34 as well as gaseous injections from an in-house  $\text{N}_2\text{O}$  tank were run in parallel throughout the run, interspersed among the samples. Vials containing bacteria but no injected sample were also included in every batch to constrain bacterial blanks, and we conducted both within-batch and between-batch replications. On average, every sample was measured four times, and we report the simple average of the replicate measurements. The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  was measured in 184 samples, and the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ -only was measured in 88 samples. The pooled standard deviation of replicate sample measurements was 0.05‰ for  $\delta^{15}\text{N}$  and 0.14‰ for  $\delta^{18}\text{O}$  ( $n = 272$ ). There was no significant difference in reproducibility between samples treated with sulfanilamide and their untreated counterparts. For an in-house seawater sample from the deep North Pacific that was measured two to three times in each run, the standard deviation was 0.04‰ for  $\delta^{15}\text{N}$  and 0.12‰ for  $\delta^{18}\text{O}$  ( $n = 94$ ).

## 3. Results

### 3.1. Hydrography

Major water masses are identified from temperature, salinity, oxygen, and  $\text{NO}_3^-$  concentration (Figure 1). Upper Circumpolar Deep Water (UCDW) is characterized by high  $\text{NO}_3^-$  and low oxygen concentrations, properties typically associated with the remineralization of sinking organic matter in the middepths of the low-latitude Indo-Pacific. While UCDW upwells in the Open Antarctic Zone (OAZ) north of the Southern Antarctic Circumpolar Current Front (SACCF), Lower Circumpolar Deep Water (LCDW) upwells farther south



**Figure 2.** Depth profiles of (a)  $\text{NO}_2^-$  concentration, (b)  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$ , (c)  $\text{NO}_3^- + \text{NO}_2^- \delta^{18}\text{O}$ , (d)  $\text{NO}_3^-$  concentration, (e)  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$ , and (f)  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$ . Note the different vertical scales between the first two rows of panels and the third row of panels.

within the Polar Antarctic Zone (PAZ). LCDW is relatively saline due to its incorporation of North Atlantic Deep Water (NADW) and is depleted in  $\text{NO}_3^-$  and enriched in oxygen relative to UCDW. Three stations in this analysis are in the OAZ between the Antarctic Polar Front (APF) and the SACCF, and five stations are in the PAZ south of the SACCF. Antarctic Bottom Water (AABW) is characterized by cold temperatures and high oxygen concentrations, which result from its recent contact with the surface and the high solubility of oxygen at low temperatures. Finally, Antarctic Intermediate Water (AAIW) and Subantarctic Mode Water (SAMW) are nutrient-rich, low-salinity water masses that form near the APF and the Subantarctic Front (SAF), respectively [Orsi *et al.*, 1995], and provide nutrients to the low-latitude upper ocean [Sarmiento *et al.*, 2004].

Several features of the Ross Sea Gyre between 57°S and 67°S are evident in the hydrographic data. The isolated remnant base of the winter mixed layer is identified as the  $T_{\text{min}}$  layer near 100 m, with the temperature minimum reaching between  $-2^\circ\text{C}$  and  $-0.5^\circ\text{C}$  (Figure 1a). The surface is significantly fresher than the underlying water, with the highest salinity for a given depth occurring between 62°S and 63°S (Figure 1b). The deeper isothermals and isohalines are domed, reflecting regional upwelling associated with the core of the cyclonic Ross Sea Gyre. There is also a region of greater oxygen depletion from 200 m to 350 m between 60°S and 64°S where oxygen concentrations are less than  $180 \mu\text{mol/kg}$  (Figure 1c); the same region is characterized by a maximum in  $\text{NO}_3^-$  concentration (Figure 1d). Mixed layer depths for each station were determined by visually inspecting profiles of potential density for rapid increases in density relative to the average of the surface ocean. Mixed layer depths range from 58 m to 81 m, with the deepest mixed layer occurring at the station in the core of the gyre.



### 3.2. Isotopic Impact of $\text{NO}_2^-$ Removal

Onboard nutrient analysis revealed  $\text{NO}_2^-$  concentrations less than  $0.01 \mu\text{mol/kg}$  in samples collected below 300 m and concentrations ranging from  $0.19$ – $0.29 \mu\text{mol/kg}$  in the mixed layer, up to 1.3% of the combined  $\text{NO}_3^- + \text{NO}_2^-$  pool (Figure 2a). Despite the small fractional contribution of  $\text{NO}_2^-$  to the  $\text{NO}_3^- + \text{NO}_2^-$  pool, removing  $\text{NO}_2^-$  altered  $\delta^{15}\text{N}$  by  $-0.1\text{‰}$  to  $1.0\text{‰}$ ; that is, it tended to raise  $\delta^{15}\text{N}$ , with larger changes observed in samples with higher  $\text{NO}_2^-$  concentrations (Figures 2b and 2e). Removing  $\text{NO}_2^-$  altered  $\delta^{18}\text{O}$  by  $-0.4\text{‰}$  to  $0.3\text{‰}$  but without a clear tendency (Figures 2c and 2f) (supporting information Text S2 and Figure S2).

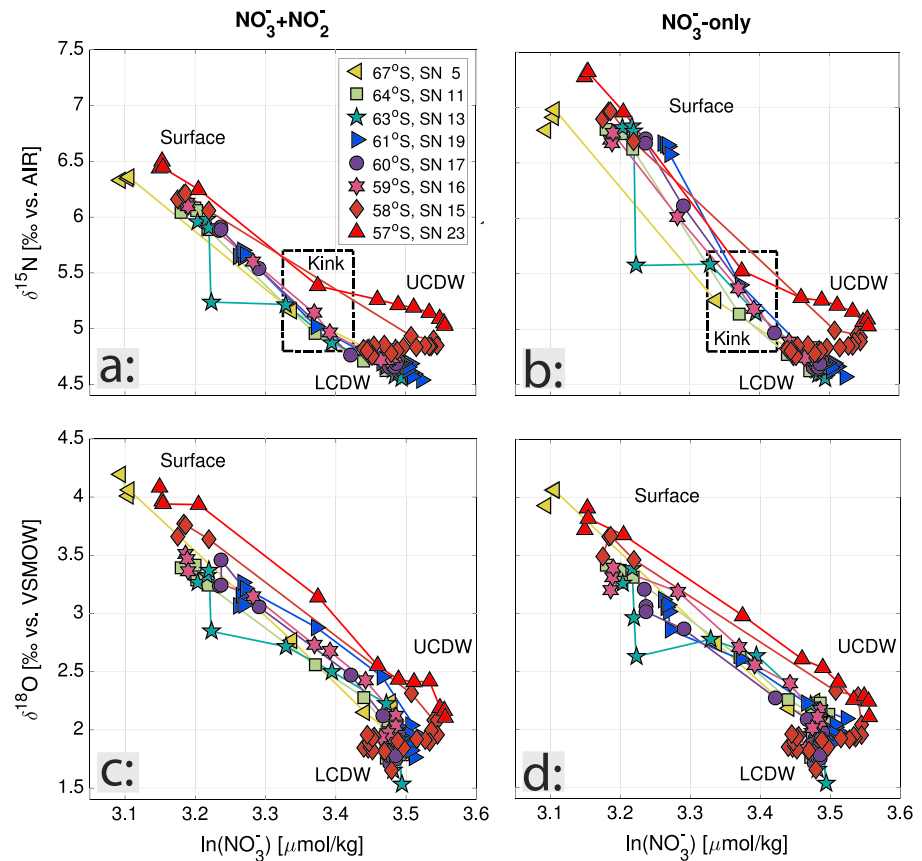
### 3.3. Major Isotopic Signals in the Water Column

At all stations, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  are quite uniform in the deeper water column (Figure 2, third row of panels). Below 1000 m, where there is no detectable  $\text{NO}_2^-$ ,  $\text{NO}_3^- \delta^{15}\text{N}$  is  $4.7 \pm 0.0\text{‰}$  and  $\text{NO}_3^- \delta^{18}\text{O}$  is  $2.0 \pm 0.1\text{‰}$  ( $1\sigma$ ,  $n = 46$ ). In the upper 200 m, the dominant signal is a rise in both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  (Figures 2b, 2c, 2e, and 2f) coincident with the decrease in  $\text{NO}_3^-$  concentration (Figure 2d). The largest changes in all three parameters occur from below 200 m to above 50 m, where the mean  $\text{NO}_3^-$  concentration decreases from  $32.6 \pm 0.6 \mu\text{mol/kg}$  to  $24.3 \pm 1.2 \mu\text{mol/kg}$ . For  $\text{NO}_3^- + \text{NO}_2^-$ , the mean  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  increase from  $4.7 \pm 0.1\text{‰}$  to  $6.1 \pm 0.2\text{‰}$  and from  $1.9 \pm 0.1\text{‰}$  to  $3.5 \pm 0.3\text{‰}$ , respectively; for  $\text{NO}_3^-$ -only, the mean  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  increase from  $4.7 \pm 0.1\text{‰}$  to  $6.8 \pm 0.2\text{‰}$  and from  $1.9 \pm 0.1\text{‰}$  to  $3.4 \pm 0.3\text{‰}$ , respectively ( $1\sigma$ ,  $n = 118$  below 200 m,  $n = 22$  above 50 m). In the upper 50 m,  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$  are relatively constant, while  $\delta^{18}\text{O}$  varies slightly but without a clear trend. The large changes in  $\text{NO}_3^-$  concentration and isotope ratios from the subsurface into the surface ocean are attributable to  $\text{NO}_3^-$  assimilation by phytoplankton, which removes nutrients and causes the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of the remaining  $\text{NO}_3^-$  to rise [Wada and Hattori, 1978; Pennock et al., 1996; Waser et al., 1998; Needoba et al., 2004]. We do not observe strong meridional trends in  $\text{NO}_3^-$  concentration or isotopic composition, but the stations nearest to  $61^\circ\text{S}$  are consistently characterized by higher  $\text{NO}_3^-$  concentrations and lower  $\text{NO}_3^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ . This is likely due to the proximity of these stations to the core of the Ross Sea Gyre, where upwelling is expected to be strongest.

### 3.4. Non-Rayleigh Dynamics and Estimation of Isotope Effects

In most profiles,  $\text{NO}_3^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  resemble LCDW at depth, consistent with their position south of the SACCF, while the northernmost stations 15 ( $58^\circ\text{S}$ ) and 23 ( $57^\circ\text{S}$ ) transition upward from LCDW to UCDW before the  $T_{\text{min}}$  is reached (Figure 3). According to the Rayleigh model, the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  versus  $\ln(\text{NO}_3^-)$  should fall along linear trends connecting the subsurface water  $\text{NO}_3^-$  source, either UCDW or LCDW, with the surface samples. However, in all profiles the  $\delta^{15}\text{N}$  of the  $T_{\text{min}}$  layer falls well below the linear trend defined by deeper and shallower  $\text{NO}_3^-$  (Figure 3, dashed black box indicating the concave-up “kink”), except for at station 15 ( $58^\circ\text{S}$ ) where samples from the  $T_{\text{min}}$  layer resemble underlying deep water. No comparable nonlinearity is observed for  $\delta^{18}\text{O}$ . Plots of the depth profiles in Rayleigh space are thus consistent with previous observations of non-Rayleigh behavior for  $\text{NO}_3^-$  samples in the remnant of the winter mixed layer [Sigman et al., 1999; DiFiore et al., 2010; Smart et al., 2015].

As a result of this non-Rayleigh behavior, we estimate larger N and O isotope effects for  $\text{NO}_3^-$  assimilation,  $^{15}\epsilon$  and  $^{18}\epsilon$ , respectively, when taking the linear trend connecting the mixed layer samples with samples in the  $T_{\text{min}}$  than when regressing against full depth profiles. Because the  $T_{\text{min}}$  layer is thought to represent the initial concentration and isotopic composition of  $\text{NO}_3^-$  in the summertime surface [Altabet and Francois, 2001], regressions through the  $T_{\text{min}}$  likely yield the best approximation for the isotope effect of  $\text{NO}_3^-$  assimilation over a single season [DiFiore et al., 2010]. For  $\text{NO}_3^- + \text{NO}_2^-$  samples, estimates of  $^{15}\epsilon$  calculated from regressions on samples from the surface to the core of the  $T_{\text{min}}$  layer range from  $4.8 \pm 0.3\text{‰}$  to  $6.1 \pm 0.4\text{‰}$ , and estimates of  $^{18}\epsilon$  range from  $2.5 \pm 1.5\text{‰}$  to  $5.6 \pm 0.7\text{‰}$ . For  $\text{NO}_3^-$ -only samples, estimates of  $^{15}\epsilon$  range from  $6.9 \pm 0.4\text{‰}$  to  $11.7 \pm 1.0\text{‰}$ , and estimates of  $^{18}\epsilon$  range from  $2.5 \pm 0.8\text{‰}$  to  $5.4 \pm 0.7\text{‰}$  (supporting information Text S3 and Figure S3). These cited ranges exclude station 15 ( $58^\circ\text{S}$ ), where sampled  $T_{\text{min}}$  water was nearly identical to underlying deep water in nitrate  $\delta^{15}\text{N}$  and concentration and thus did not capture the characteristic  $\delta^{15}\text{N}/\ln(\text{NO}_3^-)$  relationship of the temperature minimum layer.



**Figure 3.** The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only data in Rayleigh space. Except for station 15, all stations show a concave-up  $\delta^{15}\text{N}$  “kink” at the  $T_{\min}$  layer (dashed black box), where  $\text{NO}_3^- \delta^{15}\text{N}$  is lower for its concentration than the Rayleigh model predicts [Sigman *et al.*, 1999; DiFiore *et al.*, 2010]. No corresponding feature is seen in  $\delta^{18}\text{O}$ . Values do not change substantially when plotted against  $\ln(\text{NO}_3^- + \text{NO}_2^-)$  instead of  $\ln(\text{NO}_3^-)$ .

## 4. Discussion

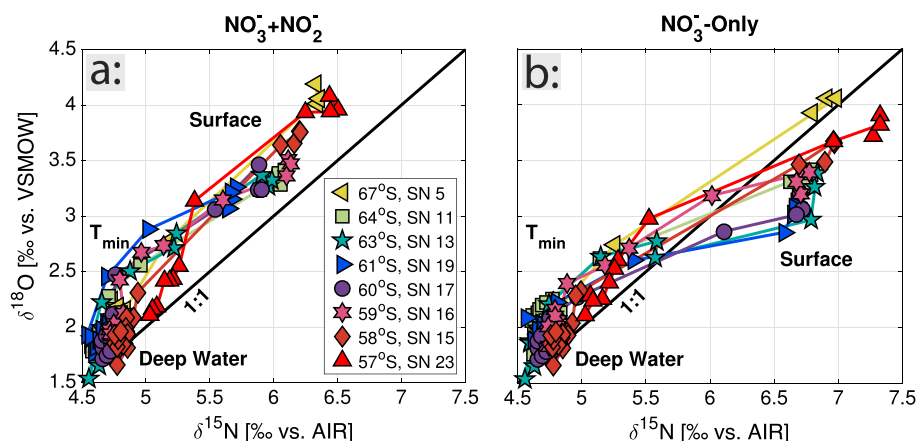
### 4.1. Observed $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in $\text{NO}_3^- + \text{NO}_2^-$ and $\text{NO}_3^-$ -Only Samples

#### 4.1.1. $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$ and $\delta^{18}\text{O}$

In the absence of other processes, the nearly equal N and O isotope effects of  $\text{NO}_3^-$  assimilation would cause surface  $\text{NO}_3^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  to increase in parallel [Granger *et al.*, 2004, 2008, 2010; Karsh *et al.*, 2012, 2014]. Graphically,  $\text{NO}_3^-$  assimilation causes the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  to rise along a 1:1 slope in  $\delta^{18}\text{O}$  versus  $\delta^{15}\text{N}$  space. Below the  $T_{\min}$ ,  $\text{NO}_3^- + \text{NO}_2^-$  samples show a uniform difference between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\sim 3\text{‰}$  (Figure 4a), consistent with previous Southern Ocean measurements [Raftar *et al.*, 2013; Smart *et al.*, 2015]. In the  $T_{\min}$ ,  $\text{NO}_3^- + \text{NO}_2^-$  samples fall slightly above the 1:1 line in  $\delta^{18}\text{O}$  versus  $\delta^{15}\text{N}$  space, reflecting the decrease in  $\text{NO}_3^- \delta^{15}\text{N}$  relative to  $\ln(\text{NO}_3^-)$  within the  $T_{\min}$  layer (i.e., the kink). This feature has been observed previously in winter data from the Atlantic sector of the AZ and is hypothesized to result from the remineralization of low- $\delta^{15}\text{N}$  N remaining in the mixed layer at the end of the summer [Smart *et al.*, 2015]. Moving into the surface ocean, the difference between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  remains constant as the N and O isotope systems evolve in approximately the 1:1 fashion expected from  $\text{NO}_3^-$  assimilation acting alone. This 1:1 evolution continues until, in the surface samples,  $\delta^{18}\text{O}$  changes slightly in some profiles.

#### 4.1.2. $\text{NO}_3^-$ -Only $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$

For the  $\text{NO}_3^-$ -only samples,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  show the same uniform difference of  $\sim 3\text{‰}$  below the  $T_{\min}$  layer that is observed in the  $\text{NO}_3^- + \text{NO}_2^-$  data (Figure 4b). Likewise, in  $\text{NO}_3^-$ -only as for  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\delta^{15}\text{N}$  rises less than  $\delta^{18}\text{O}$  from depth into the  $T_{\min}$  layer. However, into the surface mixed layer,  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  diverge. For the  $\text{NO}_3^-$ -only data,  $\delta^{15}\text{N}$  increases relative to  $\delta^{18}\text{O}$  such that the shallowest samples fall below the 1:1 line extending from deep  $\text{NO}_3^-$ . Such a large increase in  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$



**Figure 4.** Cross plots of (a)  $\text{NO}_3^- + \text{NO}_2^-$  and (b)  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$  against  $\delta^{15}\text{N}$ .

has not been observed previously. Moreover, the observations of both the 1:1 evolution of  $\text{NO}_3^- + \text{NO}_2^-$  from the  $T_{\min}$  into the surface and the larger increase in  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$  for  $\text{NO}_3^-$ -only are inconsistent with the expected results from previously described Southern Ocean dynamics.

#### 4.2. Estimated and Predicted $\text{NO}_2^-$ $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$

##### 4.2.1. Anomalously Low $\delta^{15}\text{N}$ of $\text{NO}_2^-$ Derived Through Mass Balance

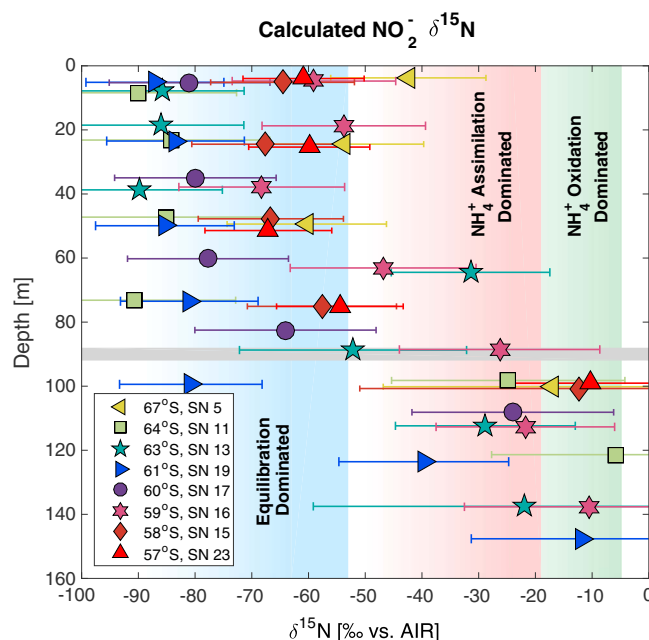
The  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$ -only samples is higher than that of the  $\text{NO}_3^- + \text{NO}_2^-$  samples, indicating a low  $\delta^{15}\text{N}$  for  $\text{NO}_2^-$  relative to  $\text{NO}_3^-$ . The  $\delta^{15}\text{N}$  of  $\text{NO}_2^-$  was calculated using an isotope mass balance, with error propagation derived from a Monte Carlo simulation. For each sample, we adjusted the reported concentration of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , as well as the measured values of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , by values drawn randomly from normal distributions with characteristic standard deviations. In accordance with estimates of accuracy and precision derived from shipboard measurements of the Reference Materials for Nutrients in Seawater, the standard deviation was  $0.21 \mu\text{mol/kg}$  for the  $\text{NO}_3^-$  distribution and  $0.006 \mu\text{mol/kg}$  for the  $\text{NO}_2^-$  distribution. The standard deviation was  $0.05\text{‰}$  for  $\delta^{15}\text{N}$  in untreated samples and  $0.04\text{‰}$  for  $\delta^{15}\text{N}$  in treated samples, reflecting the pooled standard deviations of replicate measurements across our entire data set. After shifting each term in the mass balance equation, we recalculated  $\text{NO}_2^-$   $\delta^{15}\text{N}$  100,000 times, and here we report the standard deviation of the resulting distribution as the uncertainty in  $\text{NO}_2^-$   $\delta^{15}\text{N}$  for each sample. The uncertainties in average  $\text{NO}_2^-$   $\delta^{15}\text{N}$  are given as the standard deviation of the constituent  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values. Excluding samples with  $\text{NO}_2^-$  concentrations less than  $0.10 \mu\text{mol/kg}$ , for which uncertainty in the calculation is highest,  $\text{NO}_2^-$   $\delta^{15}\text{N}$  ranges from  $-91 \pm 18\text{‰}$  to  $-6 \pm 22\text{‰}$ , with an average value of  $-58 \pm 52\text{‰}$  ( $2\sigma$ ,  $n = 55$ ; Figure 5).  $\text{NO}_2^-$   $\delta^{15}\text{N}$  is depth dependent, with an average value above 90 m of  $-69 \pm 33\text{‰}$  ( $2\sigma$ ,  $n = 41$ ) that is lower than the value below 90 m of  $-24 \pm 38\text{‰}$  ( $2\sigma$ ,  $n = 14$ ; Figure 5, grey bar). In addition, the surface  $\text{NO}_2^-$   $\delta^{15}\text{N}$  is significantly lower than that calculated by *Smart et al.* [2015] for the Atlantic sector of the Antarctic Zone; using a similar approach, they estimated  $\text{NO}_2^-$   $\delta^{15}\text{N}$  to range from  $-40\text{‰}$  to  $-20\text{‰}$  in the winter mixed layer.

##### 4.2.2. $\text{NO}_2^-$ $\delta^{15}\text{N}$ Predicted From $\text{NH}_4^+$ and $\text{NO}_2^-$ Oxidation and Assimilation

We first consider a mechanism suggested previously to explain observations of low- $\delta^{15}\text{N}$   $\text{NO}_2^-$  in samples from the wintertime Atlantic AZ. *Lourey et al.* [2003] report that the  $\delta^{15}\text{N}$  of surface suspended particulate nitrogen (PN) in the Pacific AZ decreases from  $\sim 0\text{‰}$  to  $\sim -5\text{‰}$  from early to late summer, an observation best explained as reflecting an increase in the assimilation of regenerated N, predominantly  $\text{NH}_4^+$  [*Fawcett et al.*, 2011, 2014]. Relative to  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  is low in  $\delta^{15}\text{N}$  because of the net isotopic fractionation imparted by zooplankton metabolism and excretion [*Checkley and Miller*, 1989] and by the bacterial degradation of PN [*Lehmann et al.*, 2002]. At the end of the summer, the combined  $\text{NH}_4^+$  and organic N pool in the AZ mixed layer will thus be low in  $\delta^{15}\text{N}$  [*Lourey et al.*, 2003].

Simplistically, there are two possible fates for  $\text{NH}_4^+$  in surface waters: assimilation by phytoplankton or oxidation to  $\text{NO}_2^-$  by nitrifying bacteria and archaea (i.e., the first step of nitrification). Nitrifier activity has been found to be inhibited by light [*Vanzella et al.*, 1989; *Guerrero and Jones*, 1996], although it may not be





**Figure 5.**  $\text{NO}_2^- \delta^{15}\text{N}$  with  $2\sigma$  error for samples with  $\text{NO}_2^-$  concentrations greater than  $0.10 \mu\text{mol/kg}$ . Predicted values for a system dominated by  $\text{NH}_4^+$  oxidation are shown in green, a system dominated by  $\text{NH}_4^+$  assimilation in red, and accounting for  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibration in blue. The fading of the shaded regions at lower  $\delta^{15}\text{N}$  indicates the influence of net  $\text{NO}_2^-$  oxidation. The grey bar at 90 m separates surface and deep values of  $\text{NO}_2^- \delta^{15}\text{N}$ .

ammonium concentrations [Casciotti *et al.*, 2003]. Assuming a starting  $\text{NH}_4^+ \delta^{15}\text{N}$  of  $-5\text{‰}$  in the late summer AZ surface [Lourey *et al.*, 2003], if most of the  $\text{NH}_4^+$  is oxidized and only a small fraction is assimilated, despite the substantial isotope effect of  $\text{NH}_4^+$  oxidation, the  $\delta^{15}\text{N}$  of the  $\text{NO}_2^-$  produced by oxidation will approximate the  $\delta^{15}\text{N}$  of the  $\text{NH}_4^+$  source,  $-5\text{‰}$ . Conversely, if assimilation is the dominant process and only a small fraction of the available  $\text{NH}_4^+$  is oxidized, the  $\text{NH}_4^+$  oxidation isotope effect will lower the  $\delta^{15}\text{N}$  of  $\text{NO}_2^-$  produced by oxidation to roughly  $-24\text{‰}$  to  $-19\text{‰}$ .

The  $\text{NO}_2^-$  produced from  $\text{NH}_4^+$  oxidation can either be assimilated with an isotope effect of  $\sim 0\text{‰}$  [Waser *et al.*, 1998] or be oxidized to  $\text{NO}_3^-$  with an inverse isotope effect of  $-12.8\text{‰}$  [Casciotti, 2009]. If most of the  $\text{NO}_2^-$  is assimilated, the  $\delta^{15}\text{N}$  of the unassimilated  $\text{NO}_2^-$  will remain effectively unchanged, at either  $-5\text{‰}$  or in the range of  $-24\text{‰}$  to  $-19\text{‰}$ . If the regime is dominated by  $\text{NO}_2^-$  oxidation, however, the inverse isotope effect will have a significant impact. If  $\sim 63\%$  of the available  $\text{NO}_2^-$  is oxidized, the unoxidized  $\text{NO}_2^-$  will be left with a  $\delta^{15}\text{N}$  of  $-17.8\text{‰}$  for the system dominated by  $\text{NH}_4^+$  oxidation (Figure 5, green shading) or  $-36.8\text{‰}$  for the system dominated by  $\text{NH}_4^+$  assimilation (Figure 5, red shading). Although the minimum  $\text{NO}_2^- \delta^{15}\text{N}$  suggested by the scenarios outlined above is very low, it is still significantly higher than many of the  $\text{NO}_2^- \delta^{15}\text{N}$  values calculated here for the AZ surface.

Furthermore,  $\text{NH}_4^+$  oxidation and  $\text{NH}_4^+$  assimilation cannot explain our observation that  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  evolve along an approximately 1:1 trend while  $\text{NO}_3^-$ -only samples exhibit elevated  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$ . The generation of a low- $\delta^{15}\text{N}$   $\text{NO}_2^-$  pool would cause a larger discrepancy from expectations in the  $\delta^{15}\text{N}$  to  $\delta^{18}\text{O}$  relationship of  $\text{NO}_3^- + \text{NO}_2^-$  than of  $\text{NO}_3^-$ -only, with the latter only impacted if there is some oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$ . Instead, we observe the opposite situation, in which the expected 1:1 relationship between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  is seen in the  $\text{NO}_3^- + \text{NO}_2^-$  data but we observe a substantial deviation from expectations in the  $\text{NO}_3^-$ -only data.

#### 4.2.3. $\text{NO}_2^- \delta^{18}\text{O}$ Altered During Storage

Abiotic oxygen exchange between  $\text{NO}_2^-$  and  $\text{H}_2\text{O}$  during sample storage very likely removed any relevant oceanographic information from the  $\text{NO}_2^- \delta^{18}\text{O}$  of our samples [Casciotti *et al.*, 2007; Casciotti and McIlvin,

completely halted [Ward, 2005]. Coupled with the significant competitive advantage that phytoplankton have over nitrifiers for  $\text{NH}_4^+$  [Ward, 1985; Smith *et al.*, 2014], this typically leads to  $\text{NH}_4^+$  oxidation being restricted to the dark waters below the photic zone and  $\text{NH}_4^+$  assimilation dominating in surface waters. However, in the late summer AZ, the deepening mixed layer results in a decrease in the mean light level experienced by mixed layer organisms, potentially leading to the co-occurrence of  $\text{NH}_4^+$  assimilation and nitrification. In this case, the  $\delta^{15}\text{N}$  of the produced upper ocean  $\text{NO}_2^-$  depends on the relative rates of  $\text{NH}_4^+$  assimilation and  $\text{NH}_4^+$  oxidation. At the low  $\text{NH}_4^+$  concentrations typical of AZ surface waters [Gordon *et al.*, 2000], the isotope effect of  $\text{NH}_4^+$  assimilation is expected to be near  $0\text{‰}$  [Hoch *et al.*, 1992; Liu *et al.*, 2013]. By contrast, the  $\text{NH}_4^+$  oxidation isotope effect is thought to be  $14$ – $19\text{‰}$  across a broader range of

2007] but does not appear to have significantly altered the  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$ . The incorporation of O from  $\text{H}_2\text{O}$  likely had only minor impacts on the  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  because the  $\delta^{18}\text{O}$  of  $\text{NO}_2^-$  equilibrated with  $\text{H}_2\text{O}$  is roughly comparable to the  $\delta^{18}\text{O}$  of partially consumed  $\text{NO}_3^-$  [Buchwald and Casciotti, 2013], an interpretation that is supported by the observation that  $\delta^{18}\text{O}$  is very similar between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only analyses (Figure 2). While the  $\delta^{18}\text{O}$  of  $\text{NO}_2^-$  in our samples does not contain relevant information, the  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  may record previous interactions with the  $\text{NO}_2^-$  pool, as described below.

### 4.3. Evidence for Enzyme-Level $\text{NO}_3^-$ - $\text{NO}_2^-$ Interconversion

#### 4.3.1. Nitrogen Isotopes in $\text{NO}_3^-$ and $\text{NO}_2^-$

Interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  at an intracellular/periplasmic, enzyme scale can explain both the positive deviation of mixed layer  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$  (Figure 4b) and the extremely low  $\delta^{15}\text{N}$  of surface  $\text{NO}_2^-$  (Figure 5). Such interconversion would lead to expression of the N equilibrium isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , which would enrich  $\text{NO}_3^-$  in  $^{15}\text{N}$  and deplete  $\text{NO}_2^-$  in  $^{15}\text{N}$  [Casciotti, 2009] while not altering the combined  $\delta^{15}\text{N}$  of the  $\text{NO}_3^- + \text{NO}_2^-$  present in the summer mixed layer. Thus, the  $\text{NO}_3^- + \text{NO}_2^-$  data would evolve according to expectations for  $\text{NO}_3^-$  assimilation, but because  $\text{NO}_2^-$ -removed samples contain only the  $\text{NO}_3^-$  enriched in  $^{15}\text{N}$  by interconversion,  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  would increase more rapidly than does  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$ . Likewise, because  $\text{NO}_2^-$  is depleted in  $^{15}\text{N}$  during interconversion, we would calculate low values for  $\text{NO}_2^-$   $\delta^{15}\text{N}$ . The impact of interconversion on the  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  is complex, involving multiple processes and considerations, including the incorporation of oxygen from ambient  $\text{H}_2\text{O}$  during nitrification and abiotic exchange as well as the isotope effects of oxygen incorporation and loss [Buchwald and Casciotti, 2013]. As discussed below, the  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$  and  $\text{NO}_2^-$  does appear to be impacted by  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion, with data indicating a  $\delta^{18}\text{O}$  decline in both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only measurements.

Previous evidence for N isotopic equilibration between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  includes a study by Brunner *et al.* [2013] wherein a large increase in  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and a corresponding decrease in  $\text{NO}_2^-$   $\delta^{15}\text{N}$  was measured in cultures of anaerobic ammonia oxidizing (anammox) bacteria. These observations were interpreted to reflect N exchange and the expression of an equilibrium isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , proposed to result from the reversibility of a biochemical reaction. One mechanism that could drive interconversion and N exchange involves the reversibility of the nitrite oxidoreductase (NXR) enzyme, which is used by some nitrifying organisms to oxidize  $\text{NO}_2^-$  to  $\text{NO}_3^-$  and which can also catalyze the reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  [Sundermeyer-Klinger *et al.*, 1984]. NXR is a membrane-associated enzyme with both cytoplasmic and periplasmic types, which occur in distinct groups of  $\text{NO}_2^-$ -oxidizing bacteria [Pester *et al.*, 2014]. Friedman *et al.* [1986] undertook some of the initial work on the NXR enzyme, using  $^{15}\text{N}$ - and  $^{18}\text{O}$ -labeled  $\text{NO}_3^-$  and  $\text{NO}_2^-$  to demonstrate O transfer between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in cultures of nitrifying bacteria. Wunderlich *et al.* [2013] later used  $^{18}\text{O}$  labeling to provide further evidence for O atom exchange among  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{H}_2\text{O}$  in both natural populations of  $\text{NO}_3^-$ -reducing microorganisms and pure cultures of  $\text{NO}_2^-$ -oxidizing bacteria under anoxic conditions, which they attributed to the reversibility of NXR. It thus appears that the bidirectional NXR enzyme can catalyze the intracellular coupled oxidation of  $\text{NO}_2^-$  and reduction of  $\text{NO}_3^-$  to result in the expression of a  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibrium N isotope effect, without requiring net oxidation at either the scale of the enzyme or the organism [Friedman *et al.*, 1986; Brunner *et al.*, 2013; Wunderlich *et al.*, 2013].

When  $\text{NO}_2^-$  oxidation and  $\text{NO}_3^-$  reduction occur at the organismal scale, the enzymatic isotope effects of the two processes can be underexpressed due to substantial consumption of the substrate within the cells of nitrifiers and denitrifiers [e.g., Kritee *et al.*, 2012]. Thus, compensating  $\text{NO}_3^-$  reduction and  $\text{NO}_2^-$  oxidation by a consortium of nitrate reducers and nitrite oxidizers in an ocean water parcel are unlikely to approximate the equilibrium N isotope effect between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . Conversely, the full magnitude of the equilibrium N isotope effect can be captured when the coupled oxidation and reduction occurs intracellularly, where the  $\text{NO}_2^-$  oxidoreductase enzyme operates on an intracellular (or periplasmic) N pool composed of both  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The proposed intracellular coupling of  $\text{NO}_2^-$  oxidation and  $\text{NO}_3^-$  reduction thus would have a different isotopic impact from the counteraction of  $\text{NO}_2^-$  oxidation and  $\text{NO}_3^-$  reduction by distinct  $\text{NO}_2^-$ -oxidizing and  $\text{NO}_3^-$ -reducing organisms in an ocean water parcel.

Casciotti [2009] used vibrational partition functions to calculate an N equilibrium fractionation factor of 0.9454 at 24.85°C for the reaction  $^{14}\text{NO}_2^- + ^{15}\text{NO}_3^- \rightleftharpoons ^{15}\text{NO}_2^- + ^{14}\text{NO}_3^-$ , corresponding to an inverse isotope

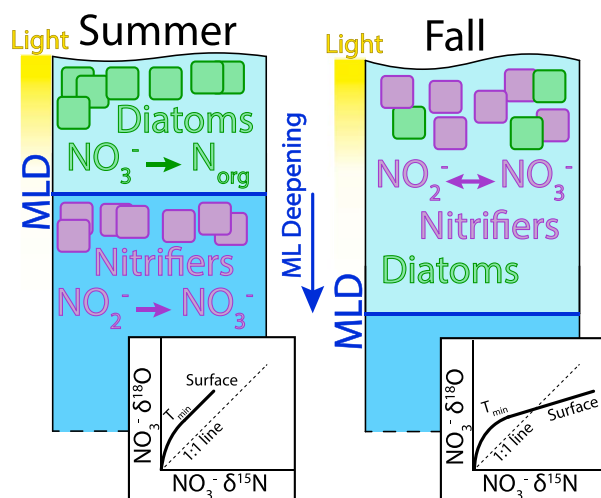
**Table 1.** Estimates of the Equilibrium N Isotope Effect Between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  Using Four Different Sets of Molecular Vibrational Frequencies in  $^{15}\text{N}$  and  $^{14}\text{N}$ -bearing  $\text{NO}_3^-$  and  $\text{NO}_2^-$ <sup>a</sup>

	$T = 0^\circ\text{C}$	$T = 24.85^\circ\text{C}$	$T = 31^\circ\text{C}$
	Southern Ocean	Standard Temperature	Brunner <i>et al.</i> [2013]
Walters and Michalski [2015]	-59.9‰	-52.9‰	-51.4‰
Casciotti [2009]	-61.7‰	-54.6‰	-53.0‰
Begun and Fletcher [1960]	-69.2‰	-61.2‰	-59.4‰
Spindel [1954]	-93.3‰	-83.0‰	-80.7‰

<sup>a</sup>Vibrational frequencies are for the aqueous phase in Walters and Michalski [2015] (averaging results from the B3LYP and EDF2 levels of theory), Casciotti [2009], and Begun and Fletcher [1960] but are unknown for Spindel [1954].

effect of  $-54.6\text{‰}$ . We extended this calculation to a range of temperatures for four different sets of reported molecular vibrational frequencies in  $^{15}\text{N}$  and  $^{14}\text{N}$ -bearing  $\text{NO}_3^-$  and  $\text{NO}_2^-$  (Table 1). The frequencies are derived from a combination of computational chemistry simulations [Casciotti, 2009; Walters and Michalski, 2015], empirical observations [Spindel, 1954; Begun and Fletcher, 1960], and calculations [Spindel, 1954]. The resulting estimates of the  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibrium isotope effect range from  $-83.0\text{‰}$  to  $-52.9\text{‰}$  at  $24.85^\circ\text{C}$  and from  $-93.3\text{‰}$  to  $-59.9\text{‰}$  at  $0^\circ\text{C}$ .

In their experiments incubating cultures of anammox bacteria at  $31^\circ\text{C}$ , Brunner *et al.* [2013] empirically derived a value for the  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibrium isotope effect of  $-60.5 \pm 1\text{‰}$ . This value is very close to the theoretical value of  $-59.4\text{‰}$  derived at this temperature from the vibrational frequencies of Begun and Fletcher [1960] but is significantly lower than the values of  $-51.4\text{‰}$  and  $-53.0\text{‰}$  predicted using the frequencies reported in Walters and Michalski [2015] and Casciotti [2009], respectively. The data from



**Figure 6.** Proposed dynamics of interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . (left) During the summer, the surface ocean is thermally stratified. Diatoms, the dominant phytoplankton group in the Ross Sea region during the growing season [Peloquin and Smith, 2007], consume  $\text{NO}_3^-$  in the photic zone, while nitrifiers are largely restricted to the waters below the mixed layer.  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  increase in a 1:1 relationship into the mixed layer as a result of nitrate assimilation. This relationship is preserved in the isotopic composition of the combined  $\text{NO}_3^- + \text{NO}_2^-$ . (right) As the mixed layer deepens into the fall and nitrifying organisms are entrained into the surface ocean, the reversible nitrite oxidoreductase enzyme catalyzes the oxidation of  $\text{NO}_2^-$  and the reduction of  $\text{NO}_3^-$ . Such a bidirectional reaction may allow for the expression of a large equilibrium N isotope effect, resulting in the production of extremely low- $\delta^{15}\text{N}$   $\text{NO}_2^-$  and increasing  $\text{NO}_3^-$   $\delta^{15}\text{N}$  relative to  $\text{NO}_3^-$   $\delta^{18}\text{O}$ , causing  $\text{NO}_3^-$ -only depth profiles to deviate from the 1:1 line in  $\delta^{18}\text{O}$  versus  $\delta^{15}\text{N}$  space in the manner illustrated by the black curves.

Spindel [1954] differ considerably from the other studies and are based on early calculations, so they are excluded from the remainder of this analysis. Details aside, there is still uncertainty in the magnitude of the  $\text{NO}_3^-$ - $\text{NO}_2^-$  equilibrium isotope effect, deriving largely from uncertainty in the different molecular vibrational frequencies of  $^{15}\text{N}$  and  $^{14}\text{N}$ -bearing  $\text{NO}_3^-$  and  $\text{NO}_2^-$ .

In the Southern Ocean surface, the expression of an equilibrium isotope effect of  $-69.2\text{‰}$  to  $-59.9\text{‰}$  would generate  $\text{NO}_2^-$   $\delta^{15}\text{N}$  ranging from roughly  $-63\text{‰}$  to  $-53\text{‰}$ ; this range of  $\text{NO}_2^-$   $\delta^{15}\text{N}$  can be expected for a large range of initial (pre-equilibrated)  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values, assuming a  $\text{NO}_3^-$  concentration of  $\sim 25 \mu\text{mol/kg}$ , a  $\text{NO}_2^-$  concentration of  $\sim 0.25 \mu\text{mol/kg}$ , and an initial  $\text{NO}_3^-$   $\delta^{15}\text{N}$  of  $\sim 6\text{‰}$ . The  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values expected to result from equilibration with  $\text{NO}_3^-$  (Figure 5, blue shading) are in much better agreement with the observed values than are the predictions resulting from  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation and assimilation. Even accounting for this equilibrium, however, some

samples have a lower  $\text{NO}_2^- \delta^{15}\text{N}$  than expected. This observation is potentially explained by  $\text{NO}_2^-$  oxidation subsequent to the interconversion, which would further decrease  $\text{NO}_2^- \delta^{15}\text{N}$  [Casciotti, 2009].

The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only from the wintertime AZ do not show signs of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion [Smart *et al.*, 2015]. Rather, Smart *et al.* [2015] observed deviations in both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only toward lower  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$ , which is a pattern that calls for the nitrification of low- $\delta^{15}\text{N}$  N. An intensive comparison of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only isotope data from the Sargasso Sea near Bermuda also showed no sign of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion in that subtropical gyre environment [Fawcett *et al.*, 2015]. Thus, while there are few comparisons of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only isotopic data across the global ocean, it appears that some aspect of the late summer/fall AZ mixed layer is important in leading to  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion.

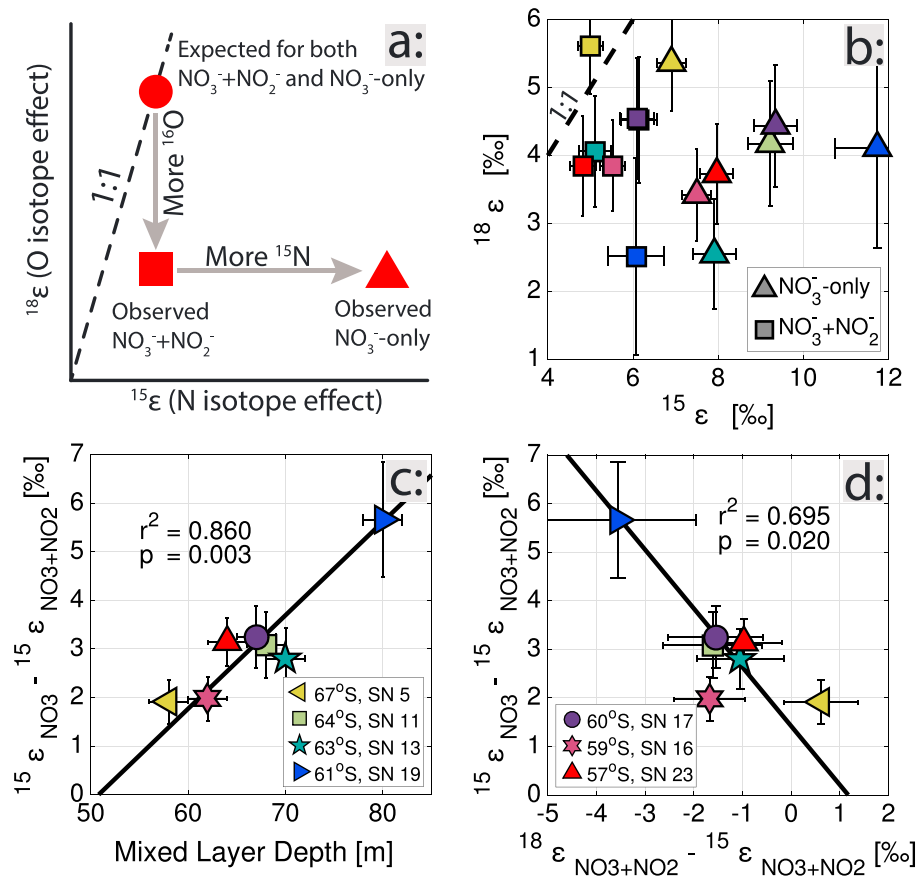
We suggest that the  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion is encouraged by the deepening of the mixed layer in late March and early April. As the mixed layer deepens into the fall and erodes into the underlying  $T_{\text{min}}$  layer, where nitrifiers have presumably been active during the summer, some of these nitrifiers may be entrained from the  $T_{\text{min}}$  layer into the fall surface ocean. Upon exposure to elevated levels of light or other new conditions, the  $\text{NO}_2^-$  oxidizers may slow their activity, such that the unidirectional oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  is reduced [Vanzella *et al.*, 1989]. Under these conditions, the bifunctional NXR enzyme may catalyze both the forward and back reactions, yielding the isotopic distribution expected from  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion (Figure 6). Our hypothesis of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion in the Pacific AZ surface is supported by the rapid increase in  $\text{NO}_2^- \delta^{15}\text{N}$  near the base of the mixed layer in each profile (Figure 5, grey bar), which suggests that the production of low- $\delta^{15}\text{N}$   $\text{NO}_2^-$  is confined to the mixed layer, where light levels are highest.

Additional evidence for the scenario proposed in Figure 6 is the difference between the apparent isotope effects of  $\text{NO}_3^-$  assimilation derived from  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only samples. If high- $\delta^{15}\text{N}$  N were shuttled into the  $\text{NO}_3^-$  pool, the apparent value of  $^{15}\epsilon$  in  $\text{NO}_3^-$ -only data would increase and result in  $^{15}\epsilon_{\text{NO}_3\text{-only}} > ^{15}\epsilon_{\text{NO}_3+\text{NO}_2}$  (Figure 7a, triangle). By this logic, the difference in  $^{15}\epsilon$  between  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^-$  can be taken as a measure of the degree of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion. In our data, the  $\text{NO}_3^-$ -only samples at all stations yield estimates of  $^{15}\epsilon$  that are higher than those derived for  $\text{NO}_3^- + \text{NO}_2^-$  samples (Figure 7b). This is best explained as resulting from the  $^{15}\text{N}$  enrichment of  $\text{NO}_3^-$  relative to  $\text{NO}_2^-$  during interconversion. The difference in  $^{15}\epsilon$  between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only data, which we propose as a reflection of the intensity of interconversion, is strongly positively correlated with the depth of the mixed layer (Figure 7c). This may be because greater mixed layer deepening entrains more nitrifying organisms into the surface ocean, leading to greater  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion. The highest intensity of interconversion occurs directly above the core of Ross Sea Gyre upwelling at station 19 (61°S), which we attribute to increased excavation of  $\text{NO}_2^-$  oxidizers from the  $T_{\text{min}}$  layer into surface waters due to strong Ekman upwelling and its tendency to yield faster mixed layer deepening in the late summer/fall.

In this framework, the lack of evidence for  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion in the wintertime AZ [Smart *et al.*, 2015] can be explained by the low light of the ~175 m deep winter AZ mixed layer, which reduces inhibition of  $\text{NO}_2^-$  oxidation. While the Sargasso Sea has well-lit surface waters, the strength of density stratification and the net convergence of surface water in the subtropics work against the upward transport of nitrite oxidizers into those well-lit waters, explaining the lack of evidence for  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion in the region [Fawcett *et al.*, 2015]. Given a general lack of complementary  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only isotope data, it is unclear whether  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion occurs in the midsummer AZ. While the deepening of the mixed layer would not apply to the early summer or midsummer, wind-driven upwelling (of ~3 m/month) may be adequate to drive some transport of nitrite oxidizers into the sunlit summer mixed layer and potentially lead to  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion.

#### 4.3.2. Oxygen Isotope Impacts of the Proposed Interconversion

Above, we noted that we observe an approximately 1:1 relationship between  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in  $\text{NO}_3^- + \text{NO}_2^-$  but an increase in  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$  in  $\text{NO}_3^-$ -only (Figure 4). When we look in greater detail, however, the  $\text{NO}_3^- + \text{NO}_2^-$  data also show a discrepancy from the 1:1  $\delta^{15}\text{N}$ -to- $\delta^{18}\text{O}$  relationship expected from nitrate assimilation acting alone (Figure 7a, circle) [Granger *et al.*, 2004, 2008, 2010; Karsh *et al.*, 2012, 2014]. Our



**Figure 7.** (a) Schematic showing how interconversion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  and the associated incorporation of O atoms from  $\text{H}_2\text{O}$  into either  $\text{NO}_3^-$  or  $\text{NO}_2^-$  may affect the apparent values of  $^{15}\epsilon$  and  $^{18}\epsilon$ . (b) The  $^{18}\epsilon$  versus  $^{15}\epsilon$  for  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only profiles. Note the different scales for  $^{18}\epsilon$  and  $^{15}\epsilon$ . (c) The difference between  $^{15}\epsilon_{\text{NO}_3^-}$  and  $^{15}\epsilon_{\text{NO}_3^- + \text{NO}_2^-}$ , a proposed measure for the intensity of  $\text{NO}_3^- - \text{NO}_2^-$  interconversion, is strongly correlated with mixed layer depth, consistent with mixed layer deepening entraining  $\text{NO}_2^-$  oxidizers into the surface ocean. (d) The apparent intensity of  $\text{NO}_3^- - \text{NO}_2^-$  interconversion is also strongly inversely correlated with the offset in  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  data from 1:1 expectations, consistent with the incorporation of water oxygen during the interconversion process. Plots exclude station 15 (58°S), where sampled  $T_{\text{min}}$  water was nearly identical to underlying deep water in nitrate  $\delta^{15}\text{N}$  and concentration.

estimates of  $^{18}\epsilon$  from  $\text{NO}_3^- + \text{NO}_2^-$  samples are up to 3.6‰ lower than the estimates of  $^{15}\epsilon$  at the same stations (Figure 7b), The implication of this observation is that low- $\delta^{18}\text{O}$  oxygen is introduced into either mixed layer  $\text{NO}_3^-$  or  $\text{NO}_2^-$  by  $\text{NO}_3^- - \text{NO}_2^-$  interconversion, lowering the apparent value of  $^{18}\epsilon$  in  $\text{NO}_3^- + \text{NO}_2^-$  such that  $^{15}\epsilon_{\text{NO}_3^- + \text{NO}_2^-} > ^{18}\epsilon_{\text{NO}_3^- + \text{NO}_2^-}$  (Figure 7a, square). Consistent with this view, the offset of  $^{18}\epsilon$  from  $^{15}\epsilon$  in the  $\text{NO}_3^- + \text{NO}_2^-$  samples is inversely correlated with the difference in  $^{15}\epsilon$  between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only (Figure 7d), the latter having been proposed above as a reflection of the intensity of  $\text{NO}_3^- - \text{NO}_2^-$  interconversion. The decrease in the  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  may result from the incorporation of O atoms from ambient  $\text{H}_2\text{O}$  into either  $\text{NO}_2^-$  or  $\text{NO}_3^-$ , as  $\text{NO}_3^- - \text{NO}_2^-$  interconversion will ensure that the  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ -only is lowered in either case. Indeed, estimates of  $^{18}\epsilon$  from  $\text{NO}_3^-$ -only measurements are also lower than  $^{15}\epsilon$  from  $\text{NO}_3^- + \text{NO}_2^-$  measurements (Figure 7b). While the O isotope systematics expected from the  $\text{NO}_3^- - \text{NO}_2^-$  interconversion cannot easily be extracted from existing information on other processes (see supporting information Text S4), it appears that  $\text{NO}_3^- - \text{NO}_2^-$  interconversion causes an underestimation of the O isotope effect of  $\text{NO}_3^-$  assimilation. Despite these observations, we emphasize that incorporation of low- $\delta^{18}\text{O}$  oxygen during  $\text{NO}_3^- - \text{NO}_2^-$  interconversion can explain only a small portion of the offset of the  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  data from 1:1 expectations (Figure 4b); most of this offset results from the increase in  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  relative to  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$ .



#### 4.4. Implications

##### 4.4.1. Southern Ocean

The observed  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion represents a previously unrecognized influence on nitrate N and O isotopic dynamics in the Southern Ocean. Our data indicate that this process can significantly alter the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of both  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , whereas the two pools were previously thought to be isotopically related only through the kinetic isotope effects associated with  $\text{NO}_2^-$  oxidation and  $\text{NO}_3^-$  reduction. Moreover, in the sunlit upper ocean, vigorous interaction between  $\text{NO}_2^-$  and  $\text{NO}_3^-$  has generally not been expected, with the dominant relevant processes formerly thought to be assimilatory  $\text{NO}_3^-$  reduction followed quickly by  $\text{NO}_2^-$  reduction.

As described above, the existing data suggest that  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion is important only in specific upper ocean environments. This study captured AZ conditions in the late summer and early fall, when the summertime nutrient uptake has essentially ended and the isotopic signal from interconversion is likely only minimally incorporated into sinking phytoplankton biomass. However, if the imprint of isotopic equilibration is preserved through the winter or redevelops early in the summer, the main pulse of phytoplankton biomass production, including that of diatoms, will incorporate it. This is relevant to paleoceanographic reconstructions of  $\text{NO}_3^-$  consumption using  $\delta^{15}\text{N}$  data. The wintertime AZ shows no sign of the  $\delta^{15}\text{N}$ -to- $\delta^{18}\text{O}$  elevation in  $\text{NO}_3^-$ -only that we observe in the late summer [Smart et al., 2015]. This implies that wintertime processes destroy the signal, most likely by dilution of the fall mixed layer  $\text{NO}_3^-$  with  $\text{NO}_3^-$  from the underlying  $T_{\text{min}}$ . However, as discussed above,  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion may occur during summer high-growth periods; this will be critical to evaluate with new data.

##### 4.4.2. Oxygen-Deficient Zones

A counterintuitive finding in the ocean's subsurface oxygen-deficient zones (ODZs), both from  $^{15}\text{N}$  tracer studies [Lipschultz et al., 1990; Füssel et al., 2012; Beman et al., 2013; Peng et al., 2015] and natural abundance isotope observations [Casciotti and McIlvin, 2007; Casciotti et al., 2013; Gaye et al., 2013; Buchwald et al., 2015], is that  $\text{NO}_2^-$  appears to undergo oxidation to  $\text{NO}_3^-$  despite the lack of appropriate oxidants in the environment. In tracer studies, this has been shown through the addition of  $^{15}\text{N}$ -labeled  $\text{NO}_2^-$  to seawater samples collected from suboxic zones and the subsequent detection of  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$  after incubation under anoxic conditions [Füssel et al., 2012; Beman et al., 2013; Peng et al., 2015]. In natural abundance studies, the same conclusion of  $\text{NO}_2^-$  oxidation is reached through the observation of a decoupling of  $\text{NO}_3^-$   $\delta^{15}\text{N}$  from  $\text{NO}_3^-$   $\delta^{18}\text{O}$  and of very low values of  $\text{NO}_2^-$   $\delta^{15}\text{N}$  relative to  $\text{NO}_3^-$   $\delta^{15}\text{N}$  in suboxic waters; these observations have been attributed to forcings from the combined isotope effects of  $\text{NO}_3^-$  reduction,  $\text{NO}_2^-$  reduction and oxidation, and anammox [Casciotti and McIlvin, 2007; Casciotti, 2009; Casciotti et al., 2013; Gaye et al., 2013; Buchwald et al., 2015; Martin and Casciotti, 2016]. Again, the perplexing aspect of interpretations suggesting  $\text{NO}_2^-$  oxidation in ODZ waters is the lack of appropriate oxidized species in the water column to serve as electron acceptors [Buchwald et al., 2015; Peng et al., 2015].

The subsurface ODZs are another candidate environment for  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion, due to possible transport of  $\text{NO}_2^-$  oxidizers into these zones. Indeed, enzyme-level interconversion between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  provides a straightforward explanation for the evidence from tracer studies of  $\text{NO}_2^-$  oxidation in ODZs. If  $\text{NO}_3^-$  and  $\text{NO}_2^-$  are able to interconvert without net oxidation due to biochemical reversibility, the high  $^{15}\text{N}/^{14}\text{N}$  ratio of  $^{15}\text{N}$ -labeled  $\text{NO}_2^-$  will appear in  $\text{NO}_3^-$  even when incubated under anoxic conditions. Thus, tracer study data can be explained without invoking net  $\text{NO}_2^-$  oxidation at either the enzyme or organism scale. We suggest that the results of such studies be reevaluated in the context of possible enzyme-level  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion.

Enzyme-level  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion also provides an alternative explanation for natural abundance isotopic patterns in ODZs that have been interpreted as evidence of organism-scale  $\text{NO}_2^-$  oxidation. The decoupling of  $\text{NO}_3^-$   $\delta^{15}\text{N}$  from  $\text{NO}_3^-$   $\delta^{18}\text{O}$  and the increase in  $\text{NO}_3^-$   $\delta^{15}\text{N}$  relative to  $\text{NO}_2^-$   $\delta^{15}\text{N}$  may both reflect the equilibrium isotope effect-driven partitioning of  $^{15}\text{N}$  into  $\text{NO}_3^-$  and  $^{14}\text{N}$  into  $\text{NO}_2^-$ . As  $^{15}\text{N}$  is concentrated in  $\text{NO}_3^-$ ,  $\text{NO}_3^-$   $\delta^{15}\text{N}$  increases,  $\text{NO}_2^-$   $\delta^{15}\text{N}$  decreases, and  $\text{NO}_3^-$   $\delta^{18}\text{O}$  changes. While the calculated values of  $\text{NO}_2^-$   $\delta^{15}\text{N}$  in our surface samples are lower than those observed in ODZs, this can be explained by net  $\text{NO}_2^-$  reduction in ODZs, which would act to increase  $\text{NO}_2^-$   $\delta^{15}\text{N}$  relative to our observations. We do not intend to suggest that the observed  $\text{NO}_3^-/\text{NO}_2^-$   $\delta^{15}\text{N}$  differences and the decoupling of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in and near ODZs are explained entirely by enzyme-level  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion;  $\text{NO}_3^-$  and  $\text{NO}_2^-$

reduction, organism-level net  $\text{NO}_2^-$  oxidation, and the various processes involved in anammox are all probably involved. Even regional N fixation may play a role in the decoupling of  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  in the ODZs [Sigman *et al.*, 2005]. Nevertheless,  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion may represent an important component process, especially at the ODZ boundaries.

## 5. Conclusions

We report  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only in samples from eight depth profiles in the Pacific sector of the Southern Ocean's Antarctic Zone, collected in late March and early April. We find that the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of the combined  $\text{NO}_3^- + \text{NO}_2^-$  pool increase roughly in parallel in accordance with expectations for  $\text{NO}_3^-$  assimilation, but  $\text{NO}_3^-$ -only data show a strong elevation in  $\delta^{15}\text{N}$  relative to  $\delta^{18}\text{O}$  in the surface mixed layer. Differencing the measurements reveals that  $\text{NO}_2^-$  in the surface ocean is extremely low in  $\delta^{15}\text{N}$ , on average about  $-70\text{‰}$  versus air. These observations are interpreted to reflect intracellular (cytoplasmic or periplasmic), enzyme-level interconversion between  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , possibly mediated by biochemical reversibility of the nitrite oxidoreductase enzyme of  $\text{NO}_2^-$  oxidizing organisms entrained from the  $T_{\min}$  layer into the sunlit mixed layer during the early phases of mixed layer deepening in the late summer. According to this interpretation,  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion can be important where  $\text{NO}_2^-$  oxidizing organisms are transported into conditions that discourage  $\text{NO}_2^-$  oxidation; in the case of the late summer Antarctic,  $\text{NO}_2^-$  oxidizers are mixed into sunlit surface waters and experience light inhibition.

$\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion represents a previously unrecognized influence on  $\text{NO}_3^-$  N and O isotopic distributions in the Southern Ocean, and its consequences for our understanding of upper ocean  $\text{NO}_3^-$  isotope dynamics are not yet clear. If the isotopic signal of interconversion is preserved in the Antarctic mixed layer through the winter or develops early in the summer, then the  $\delta^{15}\text{N}$  of phytoplankton (including diatoms) will be affected by it, with possible implications for the paleoceanographic record. In addition, intracellular, enzyme-level interconversion between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  may help to explain previous evidence from tracer and natural abundance studies of  $\text{NO}_2^-$  oxidation in oxygen-deficient zones, a possibility that warrants further investigation.

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