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

P. C. Kemeny1,2, M. A. Weigand1, R. Zhang3, B. R. Carter4,5, K. L. Karsh6, S. E. Fawcett7, and D. M. Sigman1

1Department of Geosciences, Princeton University, Princeton, New Jersey, USA, 2Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, USA, 3College of Ocean and Earth Sciences, Xiamen University, Xiamen, China, 4Joint Institute for the Study of the Atmosphere and Ocean, University of Washington, Seattle, Washington, USA, 5NOAA Pacific Marine Environmental Laboratory, Seattle, Washington, USA, 6Antarctic Climate & Ecosystems Cooperative Research Centre, University of Tasmania, Hobart, Tasmania, Australia, 7Department of Oceanography, University of Cape Town, Rondebosch, South Africa

Abstract In the Southern Ocean, the nitrogen (N) isotopes of organic matter and the N and oxygen (O) isotopes of nitrate (NO3−) have been used to investigate NO3− 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 15N/14N(δ15N(N03–)) and 18O/16O(δ18O(N03–)) in the Pacific Antarctic Zone using depth profiles from late summer/fall of 2014. In the mixed layer, the δ15N and δ18O of NO3− increase roughly equally, as expected for NO3− assimilation; however, the δ15N of NO3− -only (measured after NO3− removal) increases more than does NO3− -only δ18O. Differentiation indicates that NO3− has an extremely low δ15N, often < −70‰ versus air. These observations are consistent with the expression of an equilibrium N isotope effect between NO3− and NO2−, likely due to enzymatic NO3− -NO2− 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 NO3− -NO2− interconversion where nitrifiers are transported into environments that discourage NO2− 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 NO2− oxidation.

1. Introduction

Production and export of organic carbon from the high-latitude surface ocean lowers the atmospheric concentration of carbon dioxide (CO2) 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 CO2 degasses to the atmosphere [Sigman et al., 2010]. Increasing nutrient consumption in the Southern Ocean could stem this CO2 leakage and has been explained as a potential mechanism for the lower atmospheric CO2 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 shoals the mixed layer, stratifying the water column. No longer light-limited, phytoplankton in the summertime mixed layer draw down NO3− 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 (Tmin) layer. The Tmin 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, $^{15}\text{N}$ and $^{16}\text{O}$, relative to the heavier isotopes, $^{14}\text{N}$ 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 $\text{NO}_2^-$ and $\text{NO}_3^-$, 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 $\text{NO}_3^-$ $\delta^{15}\text{N}$ 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 $\text{NO}_3^-$ oxidizers can be transported across light or chemical gradients into waters with conditions unfavorable for $\text{NO}_2^-$ oxidation.
2. Methods

2.1. Cruise Track and Sample Collection

Seawater samples were collected during the 2014 U.S. Repeat Hydrography P16S cruise onboard the RVIB Nathaniel B. Palmer. From 20 March to 5 May, the cruise occupied a total of 90 stations from 67°S to 15°S along 150°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°S and 67°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°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 NO₃⁻ 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 NO₃⁻ and salinity ratios fall close to a 1:1 line and suggest a linear relationship between salt loss and NO₃⁻ 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 estimate the actual NO₃⁻ 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 NO₃⁻ concentrations as measured at sea, not the compromised concentrations in our sample bottles.
2.2. Isotopic Analysis of NO$_3^-$ + NO$_2^-$ and NO$_3^-$-Only

NO$_3^-$ δ$^{15}$N and δ$^{18}$O were measured using the denitrifier method, in which bacteria lacking an active nitrous oxide (N$_2$O)-reductase quantitatively convert seawater NO$_3^-$ to N$_2$O gas [Sigman et al., 2001; Casciotti et al., 2002]. Additionally, the denitrifier method converts any NO$_2^-$ in the sample to N$_2$O, which can confound interpretations of measured δ$^{15}$N and δ$^{18}$O. NO$_2^-$ in seawater affects measurements of δ$^{18}$O because, during bacterial conversion, NO$_3^-$ loses a smaller fraction of O atoms than NO$_3^-$ as the two N species are converted to N$_2$O (3/4 in NO$_3^-$ versus 5/6 in NO$_3^-$). The isotopic impact of such differential O loss is that N$_2$O generated from NO$_2^-$ has a δ$^{18}$O that is ~25‰ lower than N$_2$O produced from NO$_3^-$ with the same initial δ$^{18}$O [Casciotti et al., 2007], implying that our measured values systemically underestimate the true δ$^{18}$O of NO$_3^-$ + NO$_2^-$.

To correct for this methodological bias, we increased the measured δ$^{18}$O of NO$_3^-$ + NO$_2^-$ by the product of 25% and the fraction of NO$_2^-$ in each sample ([NO$_2^-$]/[NO$_3^-$ + 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 δ$^{18}$O, not to δ$^{15}$N, and that the magnitude of the correction is much smaller than the NO$_3^-$ δ$^{18}$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 NO$_2^-$ effects refer to the isotopic signal of NO$_2^-$ in the water column and not to this methodological correction.

In order to isolate the NO$_3^-$-only isotopic signal, all samples with detectable NO$_2^-$ (concentrations ≥0.01 μmol/kg) were treated with sulfanilamide prior to analysis with the denitrifier method. During treatment, 200 μL of 1% sulfanilamide in 10% HCl were added per 10 mL of sample or standard and left for 7–10 min. 90–100 μL 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 NO$_2^-$, consistent with prior testing of nitrite-spiked nitrate reference solutions [Weigand et al., 2016]. Hereafter, the results from our samples and their NO$_2^-$-removed counterparts are always distinguished because the presence or absence of NO$_2^-$ is central to the discussion. We use “NO$_3^-$-only” to refer to samples treated for NO$_2^-$ removal or samples without any reported NO$_2^-$, while “NO$_3^-$ + NO$_2^-$” refers to the original (untreated) sample. NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ plotted for deep samples without any reported NO$_2^-$ represent the same measurements of untreated samples.

The isotopic composition of N$_2$O was measured by gas chromatography-isotope ratio mass spectrometry using a purpose-built online N$_2$O extraction and purification system and Thermo MAT 253 mass spectrometer [Weigand et al., 2016]. Seawater solutions of the international NO$_3^-$ reference materials IAEA-NO3 and USGS34 as well as gaseous injections from an in-house N$_2$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 δ$^{15}$N and δ$^{18}$O of NO$_3^-$ + NO$_2^-$ was measured in 184 samples, and the δ$^{15}$N and δ$^{18}$O of NO$_3^-$-only was measured in 88 samples. The pooled standard deviation of replicate sample measurements was 0.05‰ for δ$^{15}$N and 0.14‰ for δ$^{18}$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 δ$^{15}$N and 0.12‰ for δ$^{18}$O (n = 94).

3. Results

3.1. Hydrography

Major water masses are identified from temperature, salinity, oxygen, and NO$_3^-$ concentration (Figure 1). Upper Circumpolar Deep Water (UCDW) is characterized by high 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 (OA2) north of the Southern Antarctic Circumpolar Current Front (SACCF), Lower Circumpolar Deep Water (LCDW) upwells farther south.
within the Polar Antarctic Zone (PAZ). LCDW is relatively saline due to its incorporation of North Atlantic Deep Water (NADW) and is depleted in 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 $T_{min}$ layer near 100 m, with the temperature minimum reaching between $-2^\circ$C and $-0.5^\circ$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 $\mu$mol/kg (Figure 1c); the same region is characterized by a maximum in 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.

Figure 2. Depth profiles of (a) NO$_2^-$ concentration, (b) NO$_3^-$ + NO$_2^-$ $\delta^{15}$N, (c) NO$_3^-$ + NO$_2^-$ $\delta^{18}$O, (d) NO$_3^-$ concentration, (e) NO$_3^-$ only $\delta^{15}$N, and (f) NO$_3^-$ only $\delta^{18}$O. Note the different vertical scales between the first two rows of panels and the third row of panels.
3.2. Isotopic Impact of NO₂⁻ Removal

Onboard nutrient analysis revealed NO₃⁻ concentrations less than 0.01 μmol/kg in samples collected below 300 m and concentrations ranging from 0.19–0.29 μmol/kg in the mixed layer, up to 1.3% of the combined NO₃⁻ + NO₂⁻ pool (Figure 2a). Despite the small fractional contribution of NO₃⁻ to the NO₃⁻ + NO₂⁻ pool, removing NO₂⁻ altered δ¹⁵N by −0.1‰ to 1.0‰; that is, it tended to raise δ¹⁵N, with larger changes observed in samples with higher NO₂⁻ concentrations (Figures 2b and 2e). Removing NO₂⁻ altered δ¹⁸O by −0.4‰ to 0.3‰ 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 δ¹⁵N and δ¹⁸O of NO₃⁻ are quite uniform in the deeper water column (Figure 2, third row of panels). Below 1000 m, where there is no detectable NO₂⁻, NO₃⁻ δ¹⁵N is 4.7 ± 0.0‰ and NO₃⁻ δ¹⁸O is 2.0 ± 0.1‰ (1σ, n = 46). In the upper 200 m, the dominant signal is a rise in both δ¹⁵N and δ¹⁸O (Figures 2b, 2c, 2e, and 2f; coincident with the decrease in NO₃⁻ concentration (Figure 2d). The largest changes in all three parameters occur from below 200 m to above 50 m, where the mean NO₃⁻ concentration decreases from 32.6 ± 0.6 μmol/kg to 24.3 ± 1.2 μmol/kg. For NO₃⁻ + NO₂⁻, the mean δ¹⁵N and δ¹⁸O increase from 4.7 ± 0.1‰ to 6.1 ± 0.2‰ and from 1.9 ± 0.1‰ to 3.5 ± 0.3‰, respectively; for NO₃⁻ only, the mean δ¹⁵N and δ¹⁸O increase from 4.7 ± 0.1‰ to 6.8 ± 0.2‰ and from 1.9 ± 0.1‰ to 3.4 ± 0.3‰, respectively (1σ, n = 118 below 200 m, n = 22 above 50 m). In the upper 50 m, NO₃⁻ concentration and δ¹⁵N are relatively constant, while δ¹⁸O varies slightly but without a clear trend. The large changes in NO₃⁻ concentration and isotope ratios from the subsurface into the surface ocean are attributable to NO₃⁻ assimilation by phytoplankton, which removes nutrients and causes the δ¹⁵N and δ¹⁸O of the remaining NO₃⁻ 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 NO₃⁻ concentration or isotopic composition, but the stations nearest to 61°S are consistently characterized by higher NO₃⁻ concentrations and lower NO₃⁻ δ¹⁵N and δ¹⁸O. 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, NO₃⁻ δ¹⁵N and δ¹⁸O resemble LCDW at depth, consistent with their position south of the SACCF, while the northernmost stations 15 (58°S) and 23 (57°S) transition upward from LCDW to UCDW before the Tₘᵢₙ is reached (Figure 3). According to the Rayleigh model, the δ¹⁵N and δ¹⁸O of NO₃⁻ versus ln(NO₃⁻) should fall along linear trends connecting the subsurface water NO₃⁻ source, either UCDW or LCDW, with the surface samples. However, in all profiles the δ¹⁵N of the Tₘᵢₙ layer falls well below the linear trend defined by deeper and shallower NO₃⁻ (Figure 3, dashed black box indicating the concave-up “kink”), except for at station 15 (58°S) where samples from the Tₘᵢₙ layer resemble underlying deep water. No comparable nonlinearity is observed for δ¹⁸O. Plots of the depth profiles in Rayleigh space are thus consistent with previous observations of non-Rayleigh behavior for NO₃⁻ 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 NO₃⁻ assimilation, ¹⁵ε and ¹⁸ε, respectively, when taking the linear trend connecting the mixed layer samples with samples in the Tₘᵢₙ than when regressing against full depth profiles. Because the Tₘᵢₙ layer is thought to represent the initial concentration and isotopic composition of NO₃⁻ in the summertime surface [Altabet and Francois, 2001], regressions through the Tₘᵢₙ likely yield the best approximation for the isotope effect of NO₃⁻ assimilation over a single season [DiFiore et al., 2010]. For NO₃⁻ + NO₂⁻ samples, estimates of ¹⁵ε calculated from regressions on samples from the surface to the core of the Tₘᵢₙ layer range from 4.8 ± 0.3‰ to 6.1 ± 0.4‰, and estimates of ¹⁸ε range from 2.5 ± 1.5‰ to 5.6 ± 0.7‰. For NO₃⁻ only samples, estimates of ¹⁵ε range from 6.9 ± 0.4‰ to 11.7 ± 1.0‰, and estimates of ¹⁸ε range from 2.5 ± 0.8‰ to 5.4 ± 0.7‰ (supporting information Text S3 and Figure S3). These cited ranges exclude station 15 (58°S), where sampled Tₘᵢₙ water was nearly identical to underlying deep water in nitrate δ¹⁵N and concentration and thus did not capture the characteristic δ¹⁵N/ln(NO₃⁻) relationship of the temperature minimum layer.
4. Discussion

4.1. Observed δ15N and δ18O in NO3−/C0 + NO2−/C0 and NO3−-Only Samples

4.1.1. NO3−/C0 + NO2−/C0 δ15N and δ18O

In the absence of other processes, the nearly equal N and O isotope effects of NO3−/C0 assimilation would cause surface NO3−/C0 δ15N and δ18O to increase in parallel [Granger et al., 2004, 2008, 2010; Karsh et al., 2012, 2014]. Graphically, NO3−/C0 assimilation causes the δ15N and δ18O of NO3−/C0 to rise along a 1:1 slope in δ18O versus δ15N space. Below the Tmin, NO3−/C0 + NO2−/C0 samples show a uniform difference between δ15N and δ18O of ~3‰ (Figure 4a), consistent with previous Southern Ocean measurements [Rafter et al., 2013; Smart et al., 2015]. In the Tmin, NO3−/C0 + NO2−/C0 δ15N rises less than δ18O from depth into the Tmin 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-δ15N N remaining in the mixed layer at the end of the summer [Smart et al., 2015]. Moving into the surface mixed layer, the difference between δ15N and δ18O remains constant as the N and O isotope systems evolve in approximately the 1:1 fashion expected from NO3−/C0 assimilation acting alone. This 1:1 evolution continues until, in the surface samples, δ18O changes slightly in some profiles.

4.1.2. NO3−-Only δ15N and δ18O

For the NO3−-only samples, δ15N and δ18O show the same uniform difference of ~3‰ below the Tmin layer that is observed in the NO3− + NO2− data (Figure 4b). Likewise, in NO3−-only as for NO3− + NO2−, δ15N rises less than δ18O from depth into the Tmin layer. However, into the surface mixed layer, NO3− + NO2− δ15N and NO3−-only δ15N diverge. For the NO3−-only data, δ15N increases relative to δ18O such that the shallowest samples fall below the 1:1 line extending from deep NO3−. Such a large increase in δ15N relative to δ18O
has not been observed previously. Moreover, the observations of both the 1:1 evolution of NO$_3^-$ + NO$_2^-$ from the $T_{\text{min}}$ into the surface and the larger increase in $\delta^{15}$N relative to $\delta^{18}$O for NO$_3^-$ only are inconsistent with the expected results from previously described Southern Ocean dynamics.

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

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

The $\delta^{15}$N of NO$_3^-$ only samples is higher than that of the NO$_3^-$ + NO$_2^-$ samples, indicating a low $\delta^{15}$N for NO$_2^-$ relative to NO$_3^-$. The $\delta^{15}$N of 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 NO$_3^-$ and NO$_2^-$, as well as the measured values of $\delta^{15}$N and $\delta^{18}$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 $\mu$mol/kg for the NO$_3^-$ distribution and 0.006 $\mu$mol/kg for the NO$_2^-$ distribution. The standard deviation was 0.05‰ for $\delta^{15}$N in untreated samples and 0.04‰ for $\delta^{15}$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 NO$_2^-$ $\delta^{15}$N 100,000 times, and here we report the standard deviation of the resulting distribution as the uncertainty in NO$_2^-$ $\delta^{15}$N for each sample. The uncertainties in average NO$_2^-$ $\delta^{15}$N are given as the standard deviation of the constituent NO$_2^-$ $\delta^{15}$N values. Excluding samples with NO$_2^-$ concentrations less than 0.10 $\mu$mol/kg, for which uncertainty in the calculation is highest, NO$_2^-$ $\delta^{15}$N ranges from $-91 \pm 18$‰ to $-6 \pm 22$‰, with an average value of $-58 \pm 52$‰ ($2\sigma$, $n = 55$; Figure 5). NO$_2^-$ $\delta^{15}$N is depth dependent, with an average value above 90 m of $-69 \pm 33$‰ ($2\sigma$, $n = 41$) that is lower than the value below 90 m of $-24 \pm 38$‰ ($2\sigma$, $n = 14$; Figure 5, grey bar). In addition, the surface NO$_2^-$ $\delta^{15}$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 NO$_2^-$ $\delta^{15}$N to range from $-40$‰ to $-20$‰ in the winter mixed layer.

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

We first consider a mechanism suggested previously to explain observations of low-$\delta^{15}$N NO$_2^-$ in samples from the wintertime Atlantic AZ. Lourey et al. [2003] report that the $\delta^{15}$N of surface suspended particulate nitrogen (PN) in the Pacific AZ decreases from ~0‰ to ~−5‰ from early to late summer, an observation best explained as reflecting an increase in the assimilation of regenerated N, predominantly NH$_4^+$ [Fawcett et al., 2011, 2014]. Relative to NO$_3^-$, NH$_4^+$ is low in $\delta^{15}$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 NH$_4^+$ and organic N pool in the AZ mixed layer will thus be low in $\delta^{15}$N [Lourey et al., 2003].

Simplistically, there are two possible fates for NH$_4^+$ in surface waters: assimilation by phytoplankton or oxidation to 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
ammonium concentrations [Casciotti et al., 2003]. Assuming a starting NH$_4^+$ $\delta^{15}$N of $-0$‰ in the late summer AZ surface [Lourey et al., 2002], if most of the NH$_4^+$ is oxidized and only a small fraction is assimilated, despite the substantial isotope effect of NH$_4^+$ oxidation, the $\delta^{15}$N of the NO$_2^-$ produced by oxidation will approximate the $\delta^{15}$N of the NH$_4^+$ source, $-5$‰. Conversely, if assimilation is the dominant process and only a small fraction of the available NH$_4^+$ is oxidized, the NH$_4^+$ oxidation isotope effect will lower the $\delta^{15}$N of NO$_2^-$ produced by oxidation to roughly $-24$‰ to $-19$‰.

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

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

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

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

### 4.3. Evidence for Enzyme-Level NO$_3^-$-NO$_2^-$ Interconversion

#### 4.3.1. Nitrogen Isotopes in NO$_3^-$ and NO$_2^-$

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

Previous evidence for N isotope equilibration between NO$_3^-$ and NO$_2^-$ includes a study by Brunner et al. [2013] wherein a large increase in NO$_3^-$ $\delta^{15}N$ and a corresponding decrease in NO$_2^-$ $\delta^{15}N$ 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 NO$_3^-$ and 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 NO$_2^-$ to NO$_3^-$ and which can also catalyze the reduction of NO$_3^-$ to 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 NO$_2^-$-oxidizing bacteria [Pester et al., 2014]. Friedman et al. [1986] undertook some of the initial work on the NXR enzyme, using $^{15}N$- and $^{18}O$-labeled NO$_3^-$ and NO$_2^-$ to demonstrate O transfer between NO$_3^-$ and NO$_2^-$ in cultures of nitrifying bacteria. Wunderlich et al. [2013] later used $^{18}O$ labeling to provide further evidence for O atom exchange among NO$_3^-$, NO$_2^-$, and H$_2$O in both natural populations of NO$_3^-$-reducing microorganisms and pure cultures of 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 NO$_2^-$ and reduction of NO$_3^-$ to result in the expression of a NO$_3^-$-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 NO$_2^-$ oxidation and 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 NO$_3^-$ reduction and 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 NO$_3^-$ and 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 NO$_2^-$ oxidoreductase enzyme operates on an intracellular (or periplasmic) N pool composed of both NO$_3^-$ and NO$_2^-$. The proposed intracellular coupling of NO$_2^-$ oxidation and NO$_3^-$ reduction thus would have a different isotopic impact from the counteraction of NO$_2^-$ oxidation and NO$_3^-$ reduction by distinct NO$_2^-$-oxidizing and 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 $^{14}NO_2^- + ^{15}NO_3^- \rightleftharpoons ^{15}NO_2^- + ^{14}NO_3^-$, corresponding to an inverse isotope
The resulting estimates of the NO$_3^-$ frequencies reported in Walters and Michalski [1960] but is significant lower than the values of $\delta^{15}$N predicted using the frequencies reported in Walters and Michalski [1960] and Casciotti [2009], respectively. The data from 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 NO$_3^-$-NO$_2^-$ equilibrium isotope effect, deriving largely from uncertainty in the different molecular vibrational frequencies of $^{15}$N and $^{14}$N-bearing NO$_3^-$ and NO$_2^-$.

In their experiments incubating cultures of anammox bacteria at 31°C, Brunner et al. [2013] empirically derived a value for the NO$_3^-$-NO$_2^-$ equilibrium isotope effect of $-60.5 \pm 1\%$. This value is very close to the theoretical value of $-59.4\%$ derived at this temperature from the vibrational frequencies of Begun and Fletcher [1960] but is significantly lower than the values of $-51.4\%$ and $-53.0\%$ predicted using the frequencies reported in Walters and Michalski [2015] and Casciotti [2009], respectively. The data from 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 NO$_3^-$-NO$_2^-$ equilibrium isotope effect, deriving largely from uncertainty in the different molecular vibrational frequencies of $^{15}$N and $^{14}$N-bearing NO$_3^-$ and NO$_2^-$.

In the Southern Ocean surface, the expression of an equilibrium isotope effect of $-69.2\%$ to $-59.9\%$ would generate NO$_2^-$ $\delta^{15}$N ranging from roughly $-63\%$ to $-53\%$; this range of NO$_2^-$ $\delta^{15}$N values expected to result from equilibration with NO$_3^-$ (Figure 5, blue shading) are in much better agreement with the observed values than are the predictions resulting from NH$_4^+$ and NO$_2^-$ oxidation and assimilation. Even accounting for this equilibrium, however, some
samples have a lower NO$_2^-$-$\delta^{15}$N than expected. This observation is potentially explained by NO$_2^-$ oxidation subsequent to the interconversion, which would further decrease NO$_2^-$-$\delta^{15}$N [Casciotti, 2009].

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

We suggest that the NO$_3^-$-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 $T_{min}$ layer, where nitrifiers have presumably been active during the summer, some of these nitrifiers may be entrained from the $T_{min}$ layer into the fall surface ocean. Upon exposure to elevated levels of light or other new conditions, the NO$_2^-$ oxidizers may slow their activity, such that the unidirectional oxidation of NO$_2^-$ to 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 of NO$_2^-$ assimilation acting alone (Figure 7a, circle). 

$\delta^{15}$N and $\delta^{18}$O enrichment of NO$_3^-$ relative to NO$_2^-$ during interconversion can be explained by the low light of the ~175 m deep winter AZ mixed layer, which reduces inhibition of 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 NO$_3^-$-NO$_2^-$ interconversion in the region [Fawcett et al., 2015]. Given a general lack of complementary NO$_3^-$ + NO$_2^-$ and NO$_3^-$-only isotope data, it is unclear whether NO$_3^-$-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 NO$_3^-$-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 $\delta^{15}$N and $\delta^{18}$O in NO$_3^-$ + NO$_2^-$ but an increase in $\delta^{15}$N relative to $\delta^{18}$O in NO$_3^-$-only (Figure 4). When we look in greater detail, however, the NO$_3^-$ + NO$_2^-$ data also show a discrepancy from the 1:1 $\delta^{15}$N-to-$\delta^{18}$O relationship expected from nitrate assimilation acting alone (Figure 7a, circle) [Granger et al., 2004, 2008, 2010; Karsh et al., 2012, 2014]. Our
estimates of $^{18}\varepsilon$ from NO$_3^-$ and NO$_2^-$ samples are up to 3.6‰ lower than the estimates of $^{15}\varepsilon$ at the same stations (Figure 7b). The implication of this observation is that low-$^{18}\delta$O oxygen is introduced into either mixed layer NO$_3^-$ or NO$_2^-$ by NO$_3^-$-NO$_2^-$ interconversion, lowering the apparent value of $^{18}\varepsilon$ in NO$_3^-$+NO$_2^-$ such that $^{15}\varepsilon$NO$_3^-$+NO$_2^->^{18}\varepsilon$NO$_3^-$+NO$_2^-$ (Figure 7a, square). Consistent with this view, the offset of $^{18}\varepsilon$ from $^{15}\varepsilon$ in the NO$_3^-$+NO$_2^-$ profiles is inversely correlated with the difference in $^{15}\varepsilon$ between NO$_3^-$+NO$_2^-$ and NO$_3^-$-only (Figure 7d), the latter having been proposed above as a reflection of the intensity of NO$_3^-$-NO$_2^-$ interconversion. The apparent intensity of NO$_3^-$-NO$_2^-$ interconversion is also strongly inversely correlated with the offset in $^{18}\delta$O of NO$_3^-$+NO$_2^-$ data from 1:1 expectations, consistent with the incorporation of water oxygen during the interconversion process. Plots exclude station 1S (58°S), where sampled $T_{\text{min}}$ water was nearly identical to underlying deep water in nitrate $^{15}\delta$N and concentration.

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

4.4.1. Southern Ocean

The observed NO$_3^-$-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 $\delta^{15}$N and $\delta^{18}$O of both NO$_3^-$ and NO$_2^-$, whereas the two pools were previously thought to be isotopically related only through the kinetic isotope effects associated with NO$_2^-$ oxidation and NO$_3^-$ reduction. Moreover, in the sunlit upper ocean, vigorous interaction between NO$_2^-$ and NO$_3^-$ has generally not been expected, with the dominant relevant processes formerly thought to be assimilatory NO$_3^-$ reduction followed quickly by NO$_2^-$ reduction.

As described above, the existing data suggest that NO$_3^-$-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. If NO$_3^-$ 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. In tracer studies, this has been shown through the addition of $^{15}$N-labeled NO$_2^-$ to seawater samples collected from suboxic zones and the subsequent detection of $^{15}$N-labeled NO$_3^-$ after incubation under anoxic conditions. In natural abundance studies, the same conclusion of NO$_2^-$ oxidation is reached through the observation of a decoupling of NO$_3^-$ $\delta^{15}$N from NO$_2^-$ $\delta^{16}$O and of very low values of NO$_2^-$ $\delta^{15}$N relative to NO$_3^-$ $\delta^{15}$N in suboxic waters; these observations have been attributed to forcings from the combined isotope effects of NO$_3^-$ reduction, NO$_2^-$ reduction and oxidation, and anammox [Casciotti and McIlvain, 2007; Casciotti et al., 2013; Gyllensten et al., 2013; Buchwald et al., 2015]. In natural abundance studies, the perplexing aspect of interpretations suggesting 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 NO$_3^-$-NO$_2^-$ interconversion, due to possible transport of NO$_2^-$ oxidizers into these zones. Indeed, enzyme-level interconversion between NO$_3^-$ and NO$_2^-$ provides a straightforward explanation for the evidence from tracer studies of NO$_2^-$ oxidation in ODZs. If NO$_3^-$ and NO$_2^-$ are able to interconvert without net oxidation due to biochemical reversibility, the high $^{15}$N/$^{14}$N ratio of $^{15}$N-labeled NO$_2^-$ will appear in NO$_3^-$ even when incubated under anoxic conditions. Tracer study data can be explained without invoking net 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 NO$_3^-$-NO$_2^-$ interconversion.

Enzyme-level NO$_3^-$-NO$_2^-$ interconversion also provides an alternative explanation for natural abundance isotopic patterns in ODZs that have been interpreted as evidence of organism-scale NO$_2^-$ oxidation. The decoupling of NO$_3^-$ $\delta^{15}$N from NO$_2^-$ $\delta^{18}$O and the increase in NO$_2^-$ $\delta^{15}$N relative to NO$_3^-$ $\delta^{15}$N may both reflect the equilibrium isotope effect-driven partitioning of $^{15}$N into NO$_3^-$ and $^{14}$N into NO$_2^-$ As $^{15}$N is concentrated in NO$_3^-$, NO$_3^-$ $\delta^{15}$N increases, NO$_2^-$ $\delta^{15}$N decreases, and NO$_3^-$ $\delta^{18}$O changes. While the calculated values of NO$_2^-$ $\delta^{15}$N in our surface samples are lower than those observed in ODZs, this can be explained by net NO$_2^-$ reduction in ODZs, which would act to increase NO$_2^-$ $\delta^{15}$N relative to our observations. We do not intend to suggest that the observed NO$_3^-$/NO$_2^-$ $\delta^{15}$N differences and the decoupling of $\delta^{15}$N and $\delta^{18}$O in and near ODZs are explained entirely by enzyme-level NO$_3^-$-NO$_2^-$ interconversion; NO$_3^-$ and NO$_2^-$...
reduction, organism-level net 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 NO$_3^-\delta^{15}N$ and $\delta^{18}O$ in the ODZs [Sigman et al., 2005]. Nevertheless, NO$_3^-\cdot$NO$_2^-$ interconversion may represent an important component process, especially at the ODZ boundaries.

5. Conclusions

We report $\delta^{15}N$ and $\delta^{18}O$ for NO$_3^-\cdot$NO$_2^-$ and 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 $\delta^{15}N$ and $\delta^{18}O$ of the combined NO$_3^-\cdot$NO$_2^-$ pool increase roughly in parallel in accordance with expectations for NO$_3^-\cdot$assimilation, but NO$_3^-\cdot$-only data show a strong elevation in $\delta^{15}N$ relative to $\delta^{18}O$ in the surface mixed layer. Differencing the measurements reveals that NO$_2^+$ in the surface ocean is extremely low in $\delta^{15}N$, on average about $\sim70\%$ versus air. These observations are interpreted to reflect intracellular (cytoplasmic or periplasmic), enzyme-level interconversion between NO$_3^-$ and NO$_2^-$, possibly mediated by biochemical reversibility of the nitrite oxidoreductase enzyme of NO$_2^-$-oxidizing organisms entrained from the $T_{\text{min}}$ layer into the sunlit mixed layer during the early phases of mixed layer deepening in the late summer. According to this interpretation, NO$_3^-\cdot$NO$_2^-$ interconversion can be important where NO$_2^-$-oxidizing organisms are transported into conditions that discourage NO$_2^-$ oxidation; in the case of the late summer Antarctic, NO$_2^-$ oxidizers are mixed into sunlit surface waters and experience light inhibition.

NO$_3^-\cdot$NO$_2^-$ interconversion represents a previously unrecognized influence on NO$_3^-\cdot$N and O isotopic distributions in the Southern Ocean, and its consequences for our understanding of upper ocean 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 $\delta^{15}N$ of phytoplankton (including diatoms) will be affected by it, with possible implications for the paleoceanographic record. In addition, intracellular, enzyme-level interconversion between NO$_3^-$ and NO$_2^-$ may help to explain previous evidence from tracer and natural abundance studies of NO$_2^-$ oxidation in oxygen-deficient zones, a possibility that warrants further investigation.

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