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The isotope effect of nitrate assimilation in the Antarctic Zone: Improved estimates and paleoceanographic implications

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Abstract

Both the nitrogen (N) isotopic composition (δ15N) of the nitrate source and the magnitude of isotope discrimination associated with nitrate assimilation are required to estimate the degree of past nitrate consumption from the δ15N of organic matter in Southern Ocean sediments (e.g., preserved within diatom microfossils). It has been suggested that the amplitude of isotope discrimination (i.e. the isotope effect) correlates with mixed layer depth, driven by a physiological response of phytoplankton to light availability, which introduces complexity to the interpretation of sedimentary records. However, most of the isotope effect estimates that underpin this hypothesis derive from acid-preserved water samples, from which nitrite would have been volatilized and lost during storage. Nitrite δ15N in Antarctic Zone surface waters is extremely low (-61 ± 20‰), consistent with the expression of an equilibrium isotope effect associated with nitrate–nitrite interconversion. Its loss from the combined nitrate+nitrite pool would act to raise the δ15N of nitrate, potentially yielding overestimation of the isotope effect. Here, we revisit the nitrate assimilation isotope effect in the Antarctic Zone with measurements of the δ15N and concentration of nitrate with and without nitrite, using frozen sea water samples from 5 different cruises that collectively cover all sectors of the Southern Ocean. The N isotope effect estimated using nitrate+nitrite δ15N is relatively constant (5.5 ± 0.6‰) across the Antarctic Zone, shows no relationship with mixed layer depth, and is in agreement with sediment trap δ15N measurements. Estimates of the N isotope effect derived from nitrate-only δ15N are higher and more variable (7.9 ± 1.5‰), consistent with an artifact from nitrate-nitrite isotope exchange. In the case of the Southern Ocean, we conclude that the δ15N of nitrate + nitrite better reflects the isotope effect of nitrate assimilation. The stability of this isotope effect across the Antarctic Zone simplifies the effort to reconstruct past degree of nitrate consumption.

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1. INTRODUCTION

In the Southern Ocean and especially south of the Polar Front (i.e. the Antarctic Zone), deep nutrient-rich waters rise to the surface and are returned to the subsurface before nitrate and phosphate are fully used by phytoplankton. The

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Nitrates (NO₃⁻) are significant nutrient sources in Southern Ocean surface waters (Granger and Sigman, 2009; Weigand et al., 2016). In seawater samples stored frozen since collection, nitrite (NO₂⁻) ably removed prior to isotope analysis through the addition of iron and light (Martin et al., 1990; Mitchell et al., 1991) and allows CO₂ sequestered in the deep ocean to be released to the atmosphere (Sigman et al., 2010) and (ii) the unused nutrient pool in the surface waters of the Southern Ocean to be exported to the low latitudes (Sarmiento et al., 2004; Palter et al., 2010).

Nitrate (NO₃⁻) assimilation by phytoplankton preferentially incorporates ¹⁵N into new biomass, leaving the residual NO₃⁻ pool enriched in ¹⁵N (Wada and Hattori, 1978; Montoya and McCarthy, 1995; Granger et al., 2004; 2010). The kinetic isotope effect (ε) expresses the degree of isotope discrimination, and is commonly defined as the ratio of reaction rates at which the two isotopes are converted from reactant to product (i.e. ε (‰) = ((1 - 15/k/14k) × 1000); where k is the rate constant for the ¹⁴N-containing reactant). The isotopic fractionation of NO₃⁻ assimilation links the degree of NO₃⁻ consumption to the δ¹⁵N (=(¹⁵N/¹⁴N)sample/(¹⁵N/¹⁴N)reference - 1) × 1000, with atmospheric N₂ as the reference) of both the NO₃⁻ and the newly produced organic matter (Altabet and Francois, 1994a,b; Sigman et al., 1999a). Thus, the δ¹⁵N of fossil-bound organic matter recovered from Southern Ocean sediment cores provides a measure of the degree of nitrate consumption in the past and has been used to investigate possible mechanisms for driving changes in atmospheric CO₂ observed over glacial cycles (Robinson and Sigman, 2008; Martinez-Garcia et al., 2014; Studer et al., 2015; Wang et al., 2017).

During at least the last two ice ages, the δ¹⁵N of diatom- and deep-sea coral-bound organic N in Antarctic sediments was ~4‰ higher than it is today (Studer et al., 2015; Wang et al., 2017), indicative of an enhanced degree of nitrate consumption during the ice ages. Together with the observed glacial decrease in Antarctic productivity (Kohfeld et al., 2005; Jaccard et al., 2013), this implies that the supply of NO₃⁻ to Antarctic Zone surface waters was significantly reduced during the ice ages, leading to the hypothesis of Antarctic “stratification” as one of the dominant drivers of glacial-interglacial variation in atmospheric pCO₂ (Francois et al., 1997; Sigman et al., 2010).

The δ¹⁵N of the NO₃⁻ supply and the isotope effect of NO₃⁻ assimilation are two key parameters required for estimating the degree of past NO₃⁻ consumption. Previous studies in the Southern Ocean have suggested systematic variations in the isotope effect with mixed layer depth that would have implications for the δ¹⁵N of sinking particulate N (PN) and fossil-bound N under changing environmental conditions (DiFiore et al., 2010). However, most of these measurements were made on acidified water samples, from which nitrite (NO₂⁻) would have been largely lost during storage, due to the volatility of HNO₂ followed by its rapid conversion into gaseous nitrogen oxide (NO and NO₂) (Park and Lee, 1988; Rayson et al., 2012). NO₂ can be reliably removed prior to isotope analysis through the addition of sulfamic acid or sulfamidate, allowing the measurement of both NO₃⁻ + NO₂ and NO₃⁻ only δ¹⁵N and δ¹⁸O for seawater samples stored frozen since collection (Granger and Sigman, 2009; Weigand et al., 2016). In late-summer water column profiles, Kemeny et al. (2016) reported a systematic difference between NO₃⁻ + NO₂⁻ δ¹⁵N and NO₃⁻ δ¹⁵N in Antarctic Zone surface waters, with NO₃⁻ δ¹⁵N typically being 0.5–1.0‰ higher than NO₃⁻ + NO₂⁻ δ¹⁵N. They suggested that NO₃⁻ – NO₂ interconversion occurs in the euphotic zone, leading to the expression of an N equilibrium isotope effect between NO₃⁻ and NO₂, enriching NO₃⁻ and depleting NO₂ in ¹⁵N. Seasonal mixed layer deepening was suggested to entrain nitrite oxidizers from the subsurface into the late-summer mixed layer, discouraging NO₂ oxidation due to light inhibition and favoring the reversibility of the nitrite oxidoreductase (NXR) enzyme.

In this study, we revisit the mean value and variability of the isotope effect of NO₃⁻ assimilation in the Antarctic Zone using isotopic data from seawater samples that were stored frozen and not acidified, thus avoiding potential artifacts from the coupling of putative NO₃⁻–NO₂ interconversion with subsequent NO₂ loss during storage.

2. MATERIALS AND METHODS

2.1. Background: hydrography

We draw a distinction between the Polar Antarctic Zone (PAZ), the area south of the Southern Antarctic Circumpolar Current Front (SACCF), and the Open Antarctic Zone (OAZ), the area between the SACCF and the Polar Front (PF).

Fig. 1. Location of the stations (symbols) overlaid on mixed layer depth (MLD) climatology for February (Pellichero et al., 2017). The Polar Front (PF) and the Southern Antarctic Circumpolar Current Front (SACCF) according to Orsi et al. (1993) are indicated by the dashed and solid black lines, respectively. The solid and dashed white lines represent the mean winter maximal (WSI) and summer minimal (SSI) sea ice extent (1979-2008, ≥ 15% ice cover) (Raymond, 2014). The cyan inverted triangles indicate the stations for SANAES4, the orange triangles for P18S, the purple squares for P16S (Kemeny et al., 2016), the blue-green circles for IO8S, and the red stars for the NBP cruises (NBP01-1 and NBP06-8) (DiFiore et al., 2009).
160 rejection-driven increase in salinity, a prerequisite for the
159 shelf water mass. The latter has undergone a brine
158 usually caps a relatively homogeneous, dense, near-freezing
157 2017). Near and above the continental shelves (hereafter
156 ice covered areas to the south (Fig. 1) (Pellichero et al.,
155 with generally shallower mixed layers in the seasonally sea-
154 stress and thus deeper vertical mixing near the Polar Front,
153 AASW tends to be thicker in the OAZ due to higher wind
152 summer warming and freshening of the overlying water.
151 become isolated in the shallow subsurface by spring-to-
150 the remnant of the previous winter mixed layer that has
149 the remnant of the previous winter mixed layer and the
148 The latter is also known as “Winter Water
147 referred to as the ‘T min’ stations) (e.g., Park et al., 1998).
146 minimum (T min) layer below (Fig. 2c and A1c; hereafter
145 well-mixed surface layer, and a subsurface temperature
144 Water, AASW) is characterized by both a relatively fresh,
143 Antarctic Zone (hereafter referred as Antarctic Surface
142 In summer, the upper halocline layer in most of the
141 by mesoscale eddies (Abernathey and Ferreira, 2015).
140 Zone away from the coasts, for example, due to mixing
139 (Fig. 2). Deep ventilation may also occur in the Antarctic
138 northernmost extent of winter sea-ice or the southernmost
137 these two zones is also roughly delineated by either the
136 nocline. The remainder loses buoyancy near Antarctica,
135 sinking north of the Polar Front into the main oceanic pyc-
134 causing it to flow northward in the Ekman layer, ultimately
133 CDW gains buoyancy due to warming and freshening,
132 (CDW) upwells in the Antarctic Zone. Part of the upwelled
131 mixed layer. Nutrient-rich Circumpolar Deep Water
130 pumping and vertical mixing across the base of the winter
129 Across the Antarctic Zone, NO 3
128 et al., 1995).
126 northernmost extent of winter sea-ice or the southernmost
125 these two zones is also roughly delineated by either the
124 (Fig. 1) (e.g., Sigman et al., 2009a). The boundary between
123 northernmost extent of winter sea-ice or the southernmost
122 extent of Upper Circumpolar Deep Water (UCDW) (Orsi
121 et al., 1995).

2.2. Sample collection

New and previously published NO 3 + NO 2 and NO 3 - only δ 15N and δ 18O measurements are reported from the Atlantic, Indian, and Pacific sectors of the Antarctic Zone (Fig. 1 and Table 1). New hydrographic sections are GOSHIP IO8S (February 2016) in the eastern Indian sector, P18S (January 2017) in the eastern Pacific sector, and SANAE54 (December-January 2014–2015) in the Atlantic sector along the Greenwich Meridian. These new datasets are compiled in combination with previous measurements located near the Antarctic continent (DiFiore et al., 2009; NBP cruises) and in the western Pacific sector (Kemeny et al., 2016; GOSHIP P16S transect).

2.3. Nitrate isotope analysis

NO 3 + NO 2 δ 15N and δ 18O were determined using the denitrifier method (Sigman et al., 2001; Casciotti et al., 1999).
Table 1
Averages (±sd) for the isotope effects estimated by pooling different datasets (Eq. (4)). The “AASW average” isotope effects are calculated using the entire AASW dataset. “Regional average” isotope effects are calculated using data from individual cruises (IO8S, P16S, P18S, and SANAE54) or from the distinct regional continental shelf settings of the NBP cruises (Dumont D’Urville Sea, Davis Sea, Prydz Bay, and Ross Sea). “Station average” isotope effects are estimated by first calculating the isotope effect for each station and then the average for each cruise or regional setting.

<table>
<thead>
<tr>
<th>Cruise Name (Sector)</th>
<th>Location</th>
<th>Date</th>
<th>PAZ</th>
<th>OAZ</th>
<th>Regional ε average (%)</th>
<th>NO3 + NO2</th>
<th>NO3-only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
<td></td>
<td>( ^{15}E )</td>
<td>( ^{18}E )</td>
</tr>
<tr>
<td>IO8 (Indian)</td>
<td>83°E</td>
<td>Feb. 2016</td>
<td>6</td>
<td>3</td>
<td>5.9 ± 0.4</td>
<td>4.4 ± 0.3</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>P16 (Pacific)*</td>
<td>150°W</td>
<td>Apr. 2014</td>
<td>6</td>
<td>2</td>
<td>4.9 ± 0.3</td>
<td>4.6 ± 0.3</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>P18 (Pacific)</td>
<td>103°W</td>
<td>Jan. 2017</td>
<td>2</td>
<td>5</td>
<td>5.8 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>8.4 ± 0.7</td>
</tr>
<tr>
<td>SANAE54 (Atlantic)</td>
<td>0°W</td>
<td>Dec-Feb 2014-2015</td>
<td>7</td>
<td>1</td>
<td>4.8 ± 0.3</td>
<td>4.3 ± 0.2</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>NBP01-01 (DDU)b</td>
<td>146°E</td>
<td>Feb. 2001</td>
<td>6</td>
<td>0</td>
<td>3.2 ± 0.4</td>
<td>1.9 ± 0.9</td>
<td>5.7 ± 2.7</td>
</tr>
<tr>
<td>NBP01-01 (Davis Sea)c</td>
<td>93°E</td>
<td>Feb. 2001</td>
<td>3</td>
<td>0</td>
<td>4.9 ± 0.4</td>
<td>4.8 ± 0.8</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>NBP01-01 (Prydz Bay)c</td>
<td>76°E</td>
<td>Mar. 2001</td>
<td>5</td>
<td>0</td>
<td>3.8 ± 0.6</td>
<td>4.2 ± 0.7</td>
<td>6.7 ± 2.8</td>
</tr>
<tr>
<td>NBP06-08 (Ross Sea)c</td>
<td>174°E</td>
<td>Nov. 2006</td>
<td>4</td>
<td>0</td>
<td>5.8 ± 0.5</td>
<td>6.3 ± 0.8</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>AASW average</td>
<td></td>
<td></td>
<td>39</td>
<td>11</td>
<td>5.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>7.3 ± 0.3</td>
</tr>
<tr>
<td>AASW average (without DDU and Ross Sea)d</td>
<td></td>
<td></td>
<td>29</td>
<td>11</td>
<td>5.4 ± 0.2</td>
<td>4.6 ± 0.1</td>
<td>7.6 ± 1.0</td>
</tr>
</tbody>
</table>

a Kemeny et al. (2016).
b Dumont D’Urville Sea.
c DiFiore et al. (2009).
d The variability in the AASW for these areas is close to the analytical precision.
e Only the stations with a vertical isotopic gradient greater than 0.2‰ are used.
During the reduction of NO$_3$ and NO$_2$ to N$_2$O by the denitrifier method, O atoms are either transferred to the product of NO$_2$ and if the reactant N pool (NO$_3$) is not fully assimilated, only Eq. (1) is valid for the O isotopes. Because phytoplankton discriminate against $^{15}$N and $^{18}$O, both isotopes are lost during NO$_3$ reduction (3/4 vs. 5/6), the N$_2$O generated from NO$_3$ by the denitrifier method is $\sim$25% lower in $^{18}$O than N$_2$O generated from NO$_3$ with the same initial $^{15}$O (Casciotti et al., 2007). This methodological bias is corrected for each sample by adding to NO$_3$ + NO$_2$ $^{15}$O the product of 25% and the relative contribution of NO$_2$ to NO$_3$ + NO$_2$ (Kemeny et al., 2016). In order to isolate and measure the NO$_3$-only $^{15}$N and $^{18}$O samples with detectable NO$_2$ concentrations were treated with sulfamic acid prior to NO$_3$ isotope analysis (Granger and Sigman, 2009).

Replicate analyses (100% of the samples) at MPIC indicate median 1σ reproducibility of $<0.07^\circ$ and $<0.13^\circ$ for $^{15}$N and $^{18}$O, respectively (i.e., similar to $<0.05^\circ$ and $<0.14^\circ$ for P165 and SANAES4 analyzed at Princeton). There was no significant difference in reproducibility between samples treated with sulfamic acid and their untreated counterparts. Seawater samples from the deep North Atlantic (MPIC) and Pacific (Princeton University) were used as in-house standards and measured two to three times in each run, the long-term reproducibility was $<0.08^\circ$ and $<0.12^\circ$ for $^{15}$N and $^{18}$O, respectively. In the case of the NBP samples, which were analyzed a decade earlier using the protocol and extraction system of Casciotti et al. (2002), the replicate analyses indicated a median 1σ reproducibility of 0.17 for $^{15}$N and 0.26 for $^{18}$O (DiFiore et al., 2009).

### 2.4. Estimating the isotope effect of nitrate assimilation

If NO$_3$ assimilation proceeds with a constant isotope effect and if the reactant N pool (NO$_3$) is neither significantly replenished nor subject to any loss, then the isotopic evolution of the residual NO$_3$, instantaneous PN (hereafter indicated with the superscript "inst."), and accumulated PN (hereafter indicated with the superscript "acc") are described by Rayleigh fractionation kinetics, with the following equations (Mariotti et al., 1981):

\[
R_{NO_3} = R_{NO_3}^0 \cdot f^{(-10^{-3}f)}
\]  
\[
R_{PN} = (1 - 10^{-3}f) \cdot R_{NO_3}
\]  
\[
R_{PN} = R_{NO_3}^0 \cdot \frac{1 - f^{(-10^{-3}f)}}{1 - f}
\]

where \( f \) is the fraction of NO$_3$ remaining (i.e. \( f = [NO_3]/[NO_3]_{initial} \)), the superscript 0 is the initial condition, R is the $^{15}$N/$^{14}$N or $^{18}$O/$^{16}$O ratio, and e is the isotope effect for either N or O isotopes. Since O atoms are not incorporated into biomass, only Eq. (1) is valid for the O isotopes. Because phytoplankton discriminate against $^{15}$N and $^{18}$O to the same extent during NO$_3$ assimilation ($^{15}$e $\approx$ $^{18}$e; Granger et al., 2004, 2010; Karsh et al., 2012, 2014), the residual NO$_3$ $^{15}$N and $^{18}$O rise equally if only assimilation is taking place in a water parcel. Eqs. (1)-(3) include the reasonable assumption that the abundances of both the $^{15}$N and $^{18}$O isotopes are low, implying that $^{15}$N $\sim$ $^{14}$N + $^{15}$N and $^{18}$O $\sim$ $^{16}$O + $^{17}$O + $^{18}$O (Mariotti et al., 1981). Rearranging Eq. (1) and using delta notation instead of the ratio allows us to formulate a linear relationship that has negative c as its slope and initial conditions (NO$_3$ concentration and $^{15}$N) as intercept (Mariotti et al., 1981), as shown here for the N isotopes:

\[
10^c \cdot \ln \left( \frac{[NO_3]}{[NO_3]^0} + 1 \right) = -c \cdot \ln \left( \frac{[NO_3]}{[NO_3]^0} + 1 \right)
\]

In logarithmic equations of the form \( \ln(1 + u)/(1 + v) \), where \( u \) and \( v \) are real numbers that are small relative to 1, which is the case for most $10^{-3} \cdot \delta$ values, \( \ln(1 + u)/(1 + v) \) can be approximated by \( u - v \). Consequently, Eq. (4) can be simplified to give the following widely applied approximate equation (Mariotti et al., 1981):

\[
^{15}N_{NO_3} = -e \cdot \ln \left( \frac{[NO_3]_0}{[NO_3]} \right) + \left( ^{15}N_{NO_3}^0 + e \cdot \ln \left( \frac{[NO_3]_0}{[NO_3]} \right) \right)
\]

We calculate a difference of less than $\sim 0.1^\circ$ between Eqs. (4) and (5) for estimates of the NO$_3$ assimilation isotope effect in the Antarctic Zone. While Eq. (5) is useful for illustrating Rayleigh fractionation trends in the plotting spaces of NO$_3$ $^{15}$N vs. $\ln([NO_3]_0$) and NO$_3$ $^{18}$O vs. $\ln([NO_3]_0$), we use the more accurate Eq. (4) to estimate isotope effects.

### 3. RESULTS

#### 3.1. Nitrate concentration and impact on isotope distribution

In all the Southern Ocean stations analyzed, there is a small but significant accumulation of NO$_2$ near the surface, increasing from near-zero concentrations in deep water to $\sim$0.25 $\mu$mol l$^{-1}$ (Fig. 3). This is in contrast to low-latitude areas, where a primary NO$_2$ maximum is typically found at the base of the euphotic layer, with $<0.02$ $\mu$mol l$^{-1}$ in the surface mixed layer (Lomas and Lipschultz, 2006;
Ekman transport of surface water from the Antarctic Zone result from progressive NO$_3^-$ increases in NO$_3^-$ the same extent, from 2.0‰ to 4.8‰ NO$_2$ 3.7‰ to 3.0‰ throughout this depth interval, from 1.9‰ to 5.0‰ on average, and from 5.0‰ to 3.0‰ face on average, and from 5.0‰ to 3.0‰. In the OAZ, NO$_3^-$ assimilation of 14N- and 16O-bearing NO$_3^-$ increase toward the surface in concert with the upward return flow of the ocean’s “conveyor belt” circulation (Sarmiento et al., 2007; Talley, 2013). The 15N enrichment by phytoplankton (Figs. 2 and 4). In the OAZ, NO$_3^-$ + NO$_2^-$ δ$^{15}$N is higher than NO$_3^-$ + NO$_2^-$ δ$^{15}$N by ~0.7 ± 0.2‰ in the summer surface mixed layer, but it is not significantly different deeper in the water column. In contrast, there is no clear difference (0.0 ± 0.2‰) between NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ δ$^{18}$O throughout the upper water column (Figs. 4 and 5b).

3.2. Nitrate isotope distribution in the Antarctic Zone

The δ$^{15}$N and δ$^{18}$O of both NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ increase toward the surface in concert with the upward decline in NO$_3^-$ concentration, reflecting the preferential assimilation of 15N- and 16O-bearing NO$_3^-$ by phytoplankton (Figs. 2 and 4). In the OAZ, NO$_3^-$ + NO$_2^-$ δ$^{15}$N increases from 5.0‰ in the deeper layers to 6.6‰ at the surface on average, and from 5.0‰ to 7.4‰ for NO$_3^-$-only δ$^{15}$N. Both NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ δ$^{18}$O increase to the same extent across this depth interval, from 1.9‰ to 3.7‰ on average. At the T$_{\min}$ stations in the PAZ, NO$_3^-$ + NO$_2^-$ δ$^{15}$N increases from 4.8‰ to 6.2‰ on average, and from 4.8‰ to 6.9‰ for NO$_3^-$-only δ$^{15}$N. Similar to the OAZ, both NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ δ$^{18}$O increase to the same extent, from 2.0‰ to 3.4‰. The northward increases in NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ δ$^{15}$N and δ$^{18}$O result from progressive NO$_3^-$ consumption during the Ekman transport of surface water from the Antarctic Zone to the Polar Frontal and Subantarctic Zones (Figs. 2 and A1) (Sigman et al., 1999a; DiFiore et al., 2006).

4. DISCUSSION

4.1. Nitrate supply to Antarctic surface waters

Below AAWS lie two distinct water masses and, therefore, two different sources of NO$_3^-$ to Antarctic Zone surface waters: Lower Circumpolar Deep Water (LCDW) and Upper Circumpolar Deep Water (UCDW), which supply NO$_3^-$ to the PAZ and OAZ, respectively. UCDW is characterized on average by higher NO$_3^-$ concentration and δ$^{15}$N than LCDW (by 1.5 μmol l$^{-1}$ and 0.2–0.3‰, respectively; Sigman et al., 2000; DiFiore et al., 2010) but is similar in NO$_3^-$ δ$^{18}$O (Figs. 2, 4, and A1). The NO$_3^-$ concentration maximum in UCDW results from exchange with the Indian and Pacific Oceans, where regenerated nutrients accumulate along the mid-depth return flow of the ocean’s “conveyor belt” circulation (Sarmiento et al., 2007; Talley, 2013). The 15N enrichment in UCDW ultimately derives from the transfer (via Ekman
transport) of $^{15}$N- and $^{18}$O-enriched residual NO$_3$ from the polar ocean to lower latitude intermediate, thermocline, and surface waters (Fig. 2; Sigman et al., 1999a; Rafter et al., 2013). In low-latitude areas where NO$_3$ consumption is complete, export production and remineralization produce regenerated NO$_3$ with the same $\delta^{15}$N as the NO$_3$ originally supplied to the euphotic zone from the underlying thermocline. The NO$_3$ in the thermocline was, in turn, elevated in $\delta^{15}$N (and $\delta^{18}$O) by partial NO$_3$ consumption when the thermocline water was previously at the surface of the Antarctic zone (AZ), polar front zone (PFZ) and subantarctic zone (SAZ). Its regeneration thus elevates the $\delta^{15}$N of UCDW and its deep Pacific and Indian precursors (e.g., Pacific Deep Water). The same processes do not elevate the NO$_3$ $\delta^{18}$O of UCDW because regenerated NO$_3$ has a $\delta^{18}$O equal to that of ambient water plus $1.1$‰ (Sigman et al., 2009a; Buchwald et al., 2012), which is lower than the ambient NO$_3$ $\delta^{18}$O (>1.8‰). Water column deni-

Fig. 4. Profiles of (a,b) NO$_3$+NO$_2$ concentration, (c,d) NO$_3$+NO$_2$ $\delta^{15}$N and $\delta^{18}$O, and (e,f) NO$_3$-only $\delta^{15}$N and $\delta^{18}$O for the Polar Antarctic Zone (PAZ) (a,c,e) and Open Antarctic Zone (OAZ) (b,d,f) for all stations. In the PAZ, the stations are grouped by their upper ocean thermohaline structure into three categories: $T_{\text{min}}$ stations (white circles), ‘margin’ stations (orange triangles) or ‘$T_{\text{max}}$’ stations (green-blue squares). The mean profiles for each category are given by the corresponding solid symbols (averages for 0-20, 20-40, 40-60, 60-80, 80-100, 100-125, 125-150, 150-200, 200-300, 300-400, and 400-500 m depth intervals). All stations in the OAZ fall into the $T_{\text{min}}$ category.
trification in the tropical oxygen deficient zones also works to increase the NO$_3$ $\delta^{15}$N in this mid-depth return flow, again causing a much weaker increase in NO$_3$ $\delta^{18}$O due to the low latitude NO$_3$ assimilation/regeneration cycle described above (Sigman et al., 2009b).

4.2. Nitrate isotope variation in Antarctic surface waters

4.2.1. Nitrite interference and the interconversion of nitrate and nitrite

During the analysis of the NO$_3$ isotopes by N$_2$O-based methods, both NO$_3$ and NO$_2$ are converted to N$_2$O, but the contribution from NO$_2$ to the measured isotopic composition is often considered negligible due to its typically low concentration relative to NO$_3$ in seawater (Sigman et al., 2001; Casciotti et al., 2002; McIlvin and Altabet, 2005). However, in the Southern Ocean mixed layer, there is commonly a small but significant accumulation of NO$_2$ near the surface. Most of the previous NO$_3$ isotope measurements in the Southern Ocean have been performed on acidified samples (Sigman et al., 1999a; Altabet and Francois, 2001; Karsh et al., 2003; DiFiore et al., 2006), which would have affected the preservation of NO$_3$. Under acidic conditions, nitrous acid (HNO$_2$) forms, some of which may be lost to the gas phase. In addition, HNO$_2$ can decompose to nitrogen oxides (NO and NO$_2$), with some of the NO$_2$ being hydrolyzed to form NO$_3$ and the rest escaping from the high-density polyethylene sample bottles typically used for storage (Park and Lee, 1988; Rayson et al., 2012). NO$_3$ disappearance during storage could plausibly be argued to be beneficial for the understanding of the isotope dynamics associated with NO$_3$ assimilation, with the caveat that a portion of the HNO$_2$ breakdown can yield NO$_2$. Freezing of seawater samples is thought to better preserve both NO$_3$ and NO$_2$ concentrations as well as their $\delta^{15}$N and $\delta^{18}$O values, a view that is supported by the stability of $\delta^{15}$N and $\delta^{18}$O of NO$_3$ + -NO$_2$ measurements in frozen samples over time (e.g., Smart et al., 2015; Kemeny et al., 2016; this study).

Because of the small NO$_3$ contribution to the NO$_3$ + -NO$_2$ pool (<1%) in the Southern Ocean, prior studies assumed NO$_3$ to have a negligible effect on NO$_3$ + NO$_2$ $\delta^{15}$N and $\delta^{18}$O (DiFiore et al., 2009). However, applying NO$_3$ removal to Southern Ocean surface mixed layer samples has shown that NO$_3$ can have a significant effect on NO$_3$ + NO$_2$ $\delta^{15}$N, challenging those previous assumptions (Rafter et al., 2013; Smart et al., 2015; Kemeny et al., 2016). This is further confirmed by our compilation, in which NO$_3$-only $\delta^{15}$N is higher than NO$_3$ + NO$_2$ $\delta^{15}$N by $\sim$0.7 ± 0.2‰ in the summer surface mixed layer but is not significantly different deeper in the water column (Fig. 5a). Given the small contribution of NO$_3$ to the NO$_3$ + NO$_2$ pool (median = 0.9%), the $\delta^{15}$N difference implies that NO$_3$ $\delta^{15}$N is extremely low, ranging from $-90$‰ to $-17$‰ (averaging $-61 \pm 20$‰) in the mixed layer and increasing with depth toward $-0$‰ at the T$_{min}$ (Fig. 3). NO$_3$ is thought to be generated in the subsurface largely as a result of the low-light conditions. Here, NO$_3$ is produced during the first step of nitrification (NH$_4^+$ $\rightarrow$ NO$_2$) and as a result of efflux of NO$_3$ out of phytoplankton cells due to an imbalance between NO$_3$ and NO$_2$ reduction during assimilatory NO$_3$ uptake. NO$_3$ is consumed by both the second step of nitrification (NO$_2$ $\rightarrow$ NO$_3$) and NO$_2$ assimilation by phytoplankton (Ward, 1985; Lomas and Lipschultz, 2006). This combination of processes can yield NO$_2$ with a $\delta^{15}$N between $\sim0$‰ and $-35$‰, depending on the isotope effects of each process and their relative contributions to NH$_4^+$ and NO$_2$ removal (Fripiat et al., 2015a). We observe NO$_3$ $\delta^{15}$N values that fall roughly within this range in the subsurface (i.e., below the mixed layer), consistent with NO$_3$ being produced and consumed by the processes outlined above.

However, another mechanism is required to produce the extremely low NO$_2$ $\delta^{15}$N estimated for the surface mixed layer (Fig. 3) and thus explain the measured $\delta^{15}$N difference between NO$_3$ + NO$_2$ and NO$_2$ only (Fig. 5a). Kemeny et al. (2016; P16S, Fig. 1) suggested that NO$_3$ + NO$_2$ interconversion can occur in the Southern Ocean mixed layer. This interconversion would lead to the expression of the large equilibrium N isotope effect between NO$_3$ and NO$_2$ (60–90‰ under relevant conditions) (Casciotti, 2009; Kemeny et al., 2016), enriching NO$_3$ and depleting NO$_2$.
in $^{15}$N and yielding NO$_3^-$ and NO$_2^-$ with $^{15}$N values in the
ranges that we observe (Figs. 3 and 5a). At this time, the
expression of the equilibrium isotope effect between NO$_3^-$
and NO$_2^-$ is the only viable proposal for generating the
observed extreme depletions of NO$_2^-$ $^{15}$N.

Most of the stations in both the PAZ and OAZ are
found to have low NO$_3^-$ $^{15}$N in the mixed layer, suggesting
that NO$_3^-$–NO$_2^-$ interconversion is ubiquitous in summer in
these regions, and not only in fall as suggested by Kemeny
et al. (2016). Deep mixed layers prevail in the Southern
Ocean (down to $\sim$100 m in December), and mixed layer
depth (MLD) commonly exhibits short-term variability of
up to 40 m during the summer months (Pellichero et al.,
2017; Holte et al., 2017). Subsurface microbial communities
(i.e., nitrifiers) may end up trapped in the mixed layer fol-
lowing their entrainment into it such that they spend a sig-
nificant amount of time in the euphotic layer (Fripiat et al.,
2015b). Once in the mixed layer with elevated levels of light,
light-inhibition is likely to decrease the activity of NO$_2^-$ ox-
dizers and, therefore, the unidirectional oxidation of NO$_2^-$
to NO$_3^-$ (Ward, 1985; Vanzella et al., 1989). Under these
conditions and according to Kemeny et al. (2016), the
bifunctional nitrite oxidoreductase enzyme may catalyze
both the forward and reverse reactions, enriching NO$_3^-$
and depleting NO$_2^-$ in $^{15}$N. The effective co-occurrence of
NO$_2^-$ oxidation and NO$_3^-$ reduction in the same intracellu-
lar (or periplasmic) space could allow for the full expression
of the equilibrium isotope effect at the environmental scale.

While the biological catalysis of NO$_3^-$–NO$_2^-$ equilibrium
has previously been demonstrated in culture studies
(Sundermeyer-Klinger et al., 1984; Friedman et al., 1986;
Bruner et al., 2013; Wunderlich et al., 2013), the existence
and operation of specific microbial consortia and enzymatic
machinery that could explain our observations remains
speculative.

From the perspective of the N atoms in NO$_3^-$ + NO$_2^-$,
interconversion operates as a closed system, with N atoms
exchanged between NO$_3^-$ and NO$_2^-$ without the NO$_3^-$ -
NO$_2^-$ pool experiencing any N loss or gain. This means that
$^{15}$e associated with the assimilation of NO$_3^-$ + NO$_2^-$ should
be unaffected by NO$_3^-$–NO$_2^-$ interconversion. However, as
interconversion enriches NO$_3^-$ in $^{15}$N, the NO$_3^-$-only $^{15}$e will
be affected, and will be larger than the NO$_3^-$ + NO$_2^-$ $^{15}$e
(Table 1; Fig. 6a and b). Consistent with Kemeny et al.
(2016), we observe that the $^{15}$e derived from NO$_3^-$-only pro-
files is higher than the $^{15}$e derived from profiles of NO$_3^-$ -
NO$_2^-$ (Fig. 7a). However, in contrast to the observation of
Kemeny et al. (2016), our larger dataset suggests that there
is no relationship between MLD and the difference in $^{15}$e
estimated from the NO$_3^-$ + NO$_2^-$ and NO$_3^-$-only data
($R^2 = 0.01; p$-value $= 0.63$) (Fig. 7a). The $^{15}$e difference
has been suggested to reflect the extent of mixed layer
NO$_3^-$–NO$_2^-$ interconversion at any given location. How-
ever, we hypothesize that short-term variability in the
MLD provides an efficient mechanism for entrainment of
the subsurface microbial communities (i.e., nitrifiers) into
the euphotic layer, largely independent of the seasonal aver-
age MLD. This is likely to lead to conditions favorable for
NO$_3^-$–NO$_2^-$ interconversion. Moreover, the mean ($\pm$1sd) $^{15}$e
difference (for NO$_3^-$ + NO$_2^-$ versus NO$_3^-$) is similar for sta-
tions in the OAZ (2.9 ± 1.3‰) and the PAZ (2.8 ± 1.9‰).

In the PAZ, sea-ice melting in spring-summer seeds the
mixed layer with a sea-ice microbial community, which
grew in a low-light environment that is favorable to nitrifi-
cation (Priscu et al., 1990; Fripiat et al., 2014), and the nitri-
fiers of this community may also facilitate NO$_3^-$–NO$_2^-$
interconversion in the mixed layer.

From the perspective of the O atoms in NO$_3^-$ + NO$_2^-$,
nitrate-nitrite interconversion implies an open system in
which both the NO$_3^-$-only and NO$_3^-$ + NO$_2^-$ pools are con-
tinuously supplied with O deriving predominantly from water
(Kemeny et al., 2016). This is illustrated by comparing $^{18}$O
$^{15}$N. The estimates of $^{18}$O that we estimate from the NO$_3^-$ + NO$_3^-$-only
and NO$_3^-$-only data (Table 1; Fig. 6c and 6d). However, the very low con-
centration ratios of NO$_2^-$ to NO$_3^-$ may mask a large range
in $^{15}$N $^{18}$O$^{15}$O (~50‰) that yield no significant difference
($\pm$0.2‰) in NO$_3^-$ + NO$_2^-$ and NO$_3^-$-only $^{18}$O, precluding
an assessment of the O isotope systematics of NO$_3^-$–NO$_2^-$
interconversion. We expect kinetic isotope effects during
the incorporation and removal of O atoms, as well as equi-
librium isotope effects between both NO$_3^-$ and NO$_2^-$
and NO$_3^-$ and NO$_2^-$, and water (e.g., Buchwald et al., 2012).

We conclude that, in the AZ, the N isotopic composition
of NO$_3^-$ + NO$_2^-$ is more representative than that of NO$_3^-$
only with regard to the true isotope effect of NO$_3^-$ assimila-
tion because NO$_3^-$-only $^{15}$e is altered during the putative
NO$_3^-$–NO$_2^-$ interconversion due to $^{14}$N-enrichment. The
$^{15}$e estimated from both NO$_3^-$ + NO$_2^-$ and NO$_3^-$-only is also
vulnerable to alteration during interconversion, with O
atoms likely being exchanged with water and then redis-
tributed between NO$_3^-$ and NO$_2^-$ in the process.

4.2.2. Isotope fractionation during nitrate assimilation in
Antarctic Surface Waters

The negative correlation between [NO$_3^-$] and both NO$_3^-$
$^{15}$N and $^{18}$O$^{15}$O reflects the link between NO$_3^-$ consump-
tion and the NO$_3^-$ isotopes in the Antarctic Zone (Fig. 6),
whereby preferential assimilation of $^{14}$N and $^{16}$O by phyto-
plankton leaves the residual NO$_3^-$ pool enriched in $^{14}$N and
$^{18}$O (Sigman et al., 1999a). There is strong seasonality asso-
ciated with both the supply of NO$_3^-$ to the mixed layer and
consumption by phytoplankton. Primary production and
NO$_3^-$ assimilation are restricted to the late spring and sum-
mer when total insolation is higher and surface mixed layers
are shallower. Nitrate supply to the water column above the
base of the winter mixed layer occurs year-round, but
nitrate supply to the sunlit surface waters is dominated by
wintertime vertical mixing. A high ratio of assimilation to
supply is therefore expected during the productive period,
and the isotope fractionation associated with nutrient consumption is likely to approximate Rayleigh fractionation kinetics (Eqs. (1)-(5), Section 2.3) (DiFiore et al., 2010; Fripiat et al., 2012). Accordingly, NO$_3$ assimilation in AASW should generate a linear trend in NO$_3$ $\delta^{15}$N vs. ln[NO$_3$/C$_0$] space starting from LCDW and UCDW as initial conditions (with the dashed colored lines (blue and red, respectively). The mean profiles for stations near the continental shelf (i.e., ‘T$_{max}$’ and ‘margin’ stations) are shown as green lines in (a) and (c), and are indistinguishable from the mean NO$_3$ $\delta^{15}$N/[NO$_3$]) profiles at the more pelagic ‘T$_{min}$’ PAZ stations. In terms of NO$_3$ $\delta^{18}$O/[NO$_3$], the stations near the continental shelf have similar surface values to the pelagic PAZ stations, but with a lower NO$_3$ $\delta^{18}$O for a given NO$_3$ concentration deeper in the water column (i.e., below ~140 m).

The data generally fall on a single $\delta^{18}$O/[ln(NO$_3$)] line consistent with a Rayleigh trend connecting deep water below AASW with the summertime mixed layer (Fig. 6c and d). In both the PAZ and OAZ, there is a progressive $^{18}$O enrichment (~0.2‰) in the mean profiles from the deeper layers up to below AASW, despite the lack of a clear decline in NO$_3$ concentration. This small $^{18}$O enrichment may be caused by the co-occurrence of partial assimilation and subsurface nitrification (Fawcett et al., 2015; Peng et al., in press), which will cause the $\delta^{18}$O of NO$_3$ in the upper ocean to increase upwards because the assimilated NO$_3$ is initially lower in $\delta^{18}$O (~$-3$‰ to ~$-2$‰) than the regenerated NO$_3$ produced by nitrification (i.e., $\sim$H$_2$O $^{18}$O + 1.1‰; Sigman et al., 2009a,b).

The AASW NO$_3$ $\delta^{15}$N/[ln(NO$_3$)] relationship displays upward concavity that causes T$_{min}$ samples to fall below a Rayleigh fractionation trend with CDW as the NO$_3$ source, by 0.2‰ and 0.5‰ for the PAZ and OAZ, respectively (Fig. 6a and b) (Sigman et al., 1999a; DiFiore et al., 2010; Smart et al., 2015; Kemeny et al., 2016). Lateral exchange with waters harboring a lower NO$_3$ $\delta^{15}$N/[NO$_3$] relationship than CDW has been put forward to explain the OAZ T$_{min}$ $\delta^{15}$N anomaly (DiFiore et al., 2010). Exchange of AASW between the PAZ and OAZ was suggested to be the most likely candidate. However, a similar albeit weaker anomaly in $\delta^{15}$N is also observed in

the PAZ, arguing that lateral exchange cannot be the only process at play. Remineralization of low-$^{15}$N PN represents an alternative hypothesis (Sigman et al., 1999a; Smart et al., 2015). It has been suggested that regenerated NO$_3^-$ with a much lower $^{15}$N ($\sim$5%$\epsilon$) than expected from NO$_3^-$ assimilation is required to explain the T$_{min}$ anomaly (Smart et al., 2015). A related possibility is that sinking PN is remineralized with net isotope fractionation as it is exported through the T$_{min}$, generating low-$^{15}$N NH$_4^+$ that is subsequently oxidized to NO$_3^-$; this possibility will be addressed in a separate manuscript. Compared to the variations observed in downcore diatom-bound $^{15}$N records (up to $\sim$4%$\epsilon$) (Robinson and Sigman, 2008; Studer et al., 2015), the NO$_3^-$ $^{15}$N/ln [NO$_3^-$] anomaly is small ($\sim$0.5%$\epsilon$). Nevertheless, it may have repercussions for paleoceanographic reconstructions (Kemeny et al., submitted for publication). Within AASW from the T$_{min}$ upward, a strong negative linear correlation is observed for NO$_3^-$ isotopes vs. ln[NO$_3^-$] (p-value < 0.001; R$^2$ > 0.76), indicating that the dominant biogeochemical process at play is NO$_3^-$ assimilation, mostly occurring in the spring-summer with the shoaling of the mixed layer (Sigman et al., 1999a; DiFiore et al., 2010; Rafter et al., 2013). Thus, our AASW-based estimates of the isotope effect (described below) are not affected by the T$_{min}$ $^{15}$N anomaly.

### 4.2.3. Estimating the isotope effect of nitrate assimilation in the Antarctic Zone

The “AASW average” isotope effects are calculated using the entire AASW dataset (Table 1). “Regional average” isotope effects are calculated using data from individual cruises (IO8S, P16S, P18S, and SANAE54) or from the distinct regional continental shelf settings of the NBP cruises (Dumont D’Urville’s Sea, Davis Sea, Prydz Bay, and Ross Sea). “Station average” isotope effects are estimated by first calculating the isotope effect for each station and then averaging these estimates for each cruise or regional setting. All estimates were calculated using Eq. (4). The ammonium (Altabet, 1988; Fawcett et al., 2011) but requires further investigation. When this low-$^{15}$N PN is regenertated to NO$_3^-$ upon wintertime deep mixing, it lowers NO$_3^-$ $^{15}$N throughout the (winter) mixed layer (Smart et al., 2015). A related possibility is that sinking PN is remineralized with net isotope fractionation as it is exported through the T$_{min}$, generating low-$^{15}$N NH$_4^+$ that is subsequently oxidized to NO$_3^-$; this possibility will be addressed in a separate manuscript.

Compared to the variations observed in downcore diatom-bound $^{15}$N records (up to $\sim$4%$\epsilon$) (Robinson and Sigman, 2008; Studer et al., 2015), the NO$_3^-$ $^{15}$N/ln [NO$_3^-$] anomaly is small ($\sim$0.5%$\epsilon$). Nevertheless, it may have repercussions for paleoceanographic reconstructions (Kemeny et al., submitted for publication). Within AASW from the T$_{min}$ upward, a strong negative linear correlation is observed for NO$_3^-$ isotopes vs. ln[NO$_3^-$] (p-value < 0.001; R$^2$ > 0.76), indicating that the dominant biogeochemical process at play is NO$_3^-$ assimilation, mostly occurring in the spring-summer with the shoaling of the mixed layer (Sigman et al., 1999a; DiFiore et al., 2010; Rafter et al., 2013). Thus, our AASW-based estimates of the isotope effect (described below) are not affected by the T$_{min}$ $^{15}$N anomaly.

### Fig. 7. $^{15}$e (a) and $^{18}$e (b) vs. mixed layer depth (MLD) for NO$_3^+$-NO$_2^-$ (black circles) and NO$_3^-$-only (black open circles). The solid line is for the average value of NO$_3^+$-NO$_2^-$ $^{15}$e (5.7%$\epsilon$) and $^{18}$e (4.4%$\epsilon$), and the dashed lines are for the average values of NO$_3^-$-only $^{15}$e (8.4%$\epsilon$) and $^{18}$e (4.2%$\epsilon$) (station averages in Table 1). MLD was computed based on the threshold method with a finite-density difference criterion (0.03 kg m$^{-3}$) from near-surface reference values (Dong et al., 2008).

### Fig. 8. $^{15}$e vs. $^{18}$e for NO$_3^+$-NO$_2^-$ (gray circles) and NO$_3^-$-only (gray open circles) (station averages in Table 1). NBP stations near and on the continental shelf are shown with the gray triangles (i.e., no NO$_3^-$-only measurements). The averages are shown with larger corresponding black symbols and error bars. The solid line is for $^{15}$e = $^{18}$e.

Fig. 7. $^{15}$e (a) and $^{18}$e (b) vs. mixed layer depth (MLD) for NO$_3^+$-NO$_2^-$ (black circles) and NO$_3^-$-only (black open circles). The solid line is for the average value of NO$_3^+$-NO$_2^-$ $^{15}$e (5.7%$\epsilon$) and $^{18}$e (4.4%$\epsilon$), and the dashed lines are for the average values of NO$_3^-$-only $^{15}$e (8.4%$\epsilon$) and $^{18}$e (4.2%$\epsilon$) (station averages in Table 1). MLD was computed based on the threshold method with a finite-density difference criterion (0.03 kg m$^{-3}$) from near-surface reference values (Dong et al., 2008).

Fig. 8. $^{15}$e vs. $^{18}$e for NO$_3^+$-NO$_2^-$ (gray circles) and NO$_3^-$-only (gray open circles) (station averages in Table 1). NBP stations near and on the continental shelf are shown with the gray triangles (i.e., no NO$_3^-$-only measurements). The averages are shown with larger corresponding black symbols and error bars. The solid line is for $^{15}$e = $^{18}$e.
AASW $^{15}$e average (±1sd) is estimated to be 5.2 ± 0.1%, with the regional $^{15}$e averages varying from 3.2% to 5.9% (Table 1). A low $^{15}$e is reported for Dumont D’Ursville Sea and Prydz Bay, both of which are characterized by small amplitudes of NO$_3^-$ depletion (~1.7 µmol l$^{-1}$) and $^{15}$N-enrichment (~0.3‰). These amplitudes are relatively close to the analytical precision (±2sd) at the time when the samples from the NBP cruises were measured. Excluding these two areas, the AASW $^{15}$e average (±1sd) is 5.4 ± 0.2‰, with the regional $^{15}$e averages varying between 4.8‰ and 5.9‰. Consistent with the expected effect of NO$_3^-$–NO$_2^-$ interconversion, the $^{15}$e estimate is higher and more variable for NO$_3$-only, with an AASW $^{15}$e average of 7.3 ± 0.3‰, which varies regionally from 6.2‰ to 8.4‰, similar to what has been reported previously for acidified samples from the OAZ and SAZ (Altabet and Francois, 2001; Karsh et al., 2003; DiFiore et al., 2006).

We calculate values of $^{15}$e that are lower than 15‰, counter to expectations for NO$_3$ assimilation (Granger et al., 2004, 2010; Rohde et al., 2015). No significant difference is observed between NO$_3^+$ + NO$_2^-$ (4.3 ± 0.1‰) and NO$_3$-only (4.5 ± 0.2‰) $^{15}$e estimates. We interpret both estimates to be artificially low, likely as a result of the NO$_3^-$–NO$_2^-$ interconversion process.

Compared to the PAZ, the OAZ NO$_3^-$ $^{15}$N vs. ln ([NO$_3^-$]) relationship is indistinguishable in slope but is shifted upward by ~0.3‰ (Fig. 6), reflecting the $^{15}$N difference in the sources of NO$_3^-$ to the AASW in the PAZ (LCDW) and OAZ (UCDW). Despite this offset, similar isotope effects are reported for the two zones (Table 2). Over the summer months, lateral transport is likely decoupled between the surface mixed layer and the T$_{min}$ layer, which could bias the estimation of the isotope effect based on vertical profile data. For example, if PAZ surface waters are advected above the OAZ T$_{min}$, the isotope effect will be underestimated. To estimate the maximal bias associated with this decoupling, we first calculate the average NO$_3$ concentration and $^{15}$N of the T$_{min}$ and mixed layer for the PAZ and OAZ. An equal-volume mixture of PAZ and OAZ water is also considered for the T$_{min}$. Using this range of scenarios for T$_{min}$ conditions (i.e., PAZ, OAZ and a mixture), we then re-estimate the isotope effects for both the OAZ and PAZ mixed layers (Table 2). The results show that lateral exchange between the OAZ and PAZ can significantly bias our estimates, but that the resulting isotope effects are still within the range of both the full and regional datasets. Accordingly, we cannot confidently assess whether lateral transport is occurring and affecting our results.

A second approach is to estimate the isotope effect for each station individually. Due to the analytical precision of 0.2‰ (±2sd), only the stations with a vertical isotopic gradient greater than 0.2‰ in the AASW are used. Regrouped by hydrographic surveys, the averages of these station-by-station estimates are not significantly different from the “regional average” isotope effects described above (Table 1). In addition, no significant relationship with MLD is observed (p-value > 0.32; $R^2 < 0.04$; Fig. 7), nor with sampling date (p-value > 0.34; $R^2 < 0.03$, data not shown).

By breaking the problem into a series of individual stations, where each station within each region can be described with the Rayleigh model (Eq. (4)), our dataset admits one equation for each station with only two unknowns per region, which are the source NO$_3$ concentration and $^{15}$N. We use method of least-squares to solve for these two parameters given the calculated intercept of each profile in Rayleigh space. The least-square solutions for the more pelagic stations, for which both NO$_3$ + NO$_2$ and NO$_3$-only $^{15}$N are available, the least-square solutions are (P16S, P18S, IO8S, and SANAE54), are 29.6 ± 1.1 µmol l$^{-1}$, 5.2 ± 0.2‰, and 5.6 ± 0.3‰ for NO$_3$ concentration, NO$_3$ + NO$_2$ $^{15}$N, and NO$_3$-only $^{15}$N, respectively. These values are indistinguishable from the mean (±1sd) T$_{min}$ conditions inferred from the observations at these stations: 29.6 ± 1.9 µmol l$^{-1}$, 5.2 ± 0.3‰, and 5.4 ± 0.4‰. This analysis further confirms that the T$_{min}$ conditions are likely to be representative of the source conditions to the summer AZ surface waters. By including the NPB cruises, which are more representative of coastal Antarctic conditions and for which only NO$_3$ $-$ + NO$_2^-$ $^{15}$N is available, the least-square solutions are 26.8 ± 3.8 µmol l$^{-1}$ and 5.6 ± 0.4‰ for NO$_3$ concentration and NO$_3$ + NO$_2$ $^{15}$N, respectively. These values are in agreement with the lower T$_{max}$ NO$_3$ concentration (25.8 ± 3.0 µmol l$^{-1}$) and higher T$_{max}$ NO$_3$ + NO$_2$ $^{15}$N (5.9 ± 0.6‰) observed at these stations.

Table 2

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Averaging (±sd) both the regional and station $^{15}\varepsilon$ averages, we estimate a nitrate assimilation isotope effect of 5.5 ± 0.6‰ for the Antarctic Zone, based on the NO$_3$ + NO$_2$ measurements (Table 1). An isotope effect of 7.8 ± 1.5‰ is derived from the NO$_3$-only measurements; we interpret this to be artificially high due to the isotopic impacts of NO$_3$–NO$_2$ interconversion described above. The relative stability of the isotope effect calculated from NO$_3$ + NO$_2$ measurements, as well as the absence of any correlation with MLD (Fig. 7a) are in disagreement with previous studies. Using a compilation of NO$_3$ isotope data from the Australian sector of the Southern Ocean, DiFiore et al. (2010) observed a poleward decrease in $^{15}\varepsilon$ from the SAZ to the PAZ (i.e., from ~9‰ to 5‰). However, except for the PAZ dataset, the OAZ-SAZ estimates were derived from acidified samples (Sigman et al., 1999a; Altabet and Francois, 2001; Karsh et al., 2003; DiFiore et al., 2006). DiFiore et al. (2010) hypothesized that this variation was driven by the southward shoaling of the mixed layer (Fig. 1), consistent with culture evidence for a physiological response of NO$_3$ assimilation and cellular NO$_3$ efflux to light availability (Needoba et al., 2004). Given our new understanding of the role of NO$_3$, we now propose that the higher OAZ $^{15}\varepsilon$ estimates were due to NO$_3$–NO$_2$ interconversion, leading to higher apparent values of $^{15}\varepsilon$ driven by the subsequent loss of NO$_3$ during acidified storage in the OAZ samples (Table 1; Fig. 6a, b) (Park and Lee, 1988; Rayson et al., 2012). The spatial variability of the MLD in the Antarctic Zone is significant (Fig. 1), and MLD is likely to have been significantly different during ice ages. Thus, our finding of no impact of MLD on $^{15}\varepsilon$ simplifies paleoceanographic interpretation of N isotope data in terms of the degree of NO$_3$ consumption (Robinson and Sigman, 2008).

4.3. Comparison of nitrate-based $^{15}\varepsilon$ estimates with sinking PN $^{15}$N data

Based on the observed Rayleigh fractionation trend in the AASW NO$_3$ + NO$_2$ data and the N isotope effect of NO$_3$ consumption that we estimate from them (Table 1), we infer an exported PN $^{15}$N of ~0.4‰ (~0.2‰ to 1.3‰), which is in the range of the annual-weighted sinking PN $^{15}$N from sediment traps in the Antarctic Zone, ~0.1% to 1.7% (Altabet and Francois, 2001). In contrast, if NO$_2$-only data are used to estimate $^{15}\varepsilon$, then the resulting export production $^{15}$N is too low (~4.4‰ to ~0.2‰) given the constraints offered by the sediment trap data. This supports our argument that the NO$_3$ + NO$_2$ pool is more representative of the N available for consumption and is thus the appropriate substrate pool from which to estimate $^{15}\varepsilon$. This is confirmed more broadly using sediment trap data from both the OAZ and the Polar Frontal Zone (Altabet and Francois, 2001; Lourey et al., 2003). To account for the effect of varying source NO$_3$ concentration and $^{15}$N, the difference between annual-weighted sinking PN $^{15}$N and initial $^{15}$N of NO$_3$ is plotted against the degree of nitrate consumption (Fig. 9) (Altabet and Francois, 2001). Estimates of the degree of nitrate consumption are based on winter to summer seasonal NO$_3$ consumption, and initial NO$_3$ $^{15}$N on winter observations in the mixed layer at the corresponding sediment trap locations. The values for both degree of nitrate consumption and initial NO$_3$ $^{15}$N are given in Altabet and Francois (2001) and Lourey et al. (2003). Annual-weighted sinking PN $^{15}$N falls on the AASW Rayleigh fractionation trend for the accumulated product if the $^{15}\varepsilon$ estimated from the NO$_3$ + NO$_2$ pool is used (Fig. 9); in contrast, the trend underestimates sinking PN $^{15}$N if the $^{15}\varepsilon$ estimated from the NO$_3$-only data is used. This analysis confirms a tight connection between the degree of NO$_3$ consumption and the $^{15}$N of (1) NO$_3$ – NO$_2$ and (2) export production (Sigman et al., 1999a; Altabet and Francois, 2001).

4.4. Paleoceanographic implications

The strong and consistent relationship between NO$_3$ + NO$_2$ concentration and $^{15}$N, which yields a relatively constant isotope effect in the lower range of previous estimates (~5.5 ± 0.6‰ from 4.5‰ to 6.2‰, Table 1), has implications for reconstructions of the past degree of NO$_3$ consumption and thus the NO$_3$ concentration in Antarctic Zone surface waters (Table 3). The $^{15}$N of coral- and diatom-bound organic N in the Antarctic Zone during the last ice age was 4‰ higher than today (Sigman et al., 1999b; Robinson and Sigman, 2008; Studer et al., 2015; Wang et al., 2017), indicating elevated nitrate consumption during glacial periods (Francois et al., 1997). In order to apply our new isotope effect estimates to a sedimentary diatom frustule-bound $^{15}$N record from the Pacific sector covering the last two glacial cycles (Studer et al., 2015), we need to take into account an isotopic offset between diatom-bound and sinking PN $^{15}$N, recognizing...
that bulk sinking PN is not entirely composed of diatoms and that diatom-bound N has a different isotopic composition from that of bulk diatom biomass (Sigman et al., 1999b; Brunelle et al., 2007; Robinson and Sigman, 2008; Horn et al., 2011a; Morales et al., 2014). We estimate the isotopic offset from the difference (∼1.8‰) between expected sinking PN δ15N in the modern Antarctic Zone (∼0.4‰) and core-top diatom-bound δ15N (∼2.2‰; Studer et al., 2015), and apply this correction to the down-core diatom-bound δ15N record (Table 3). This isotopic offset is relatively consistent, with the mean δ15N offset reported for sinking PN and core-top diatom-bound δ15N in high-latitude regions (2.4‰) (Brunelle et al., 2007; Robinson and Sigman, 2008) and for net-collected bulk organic matter and diatom-bound N in the sea-ice covered water column of the Bering Shelf (2.6 ± 2.5‰) (Morales et al., 2014). A similar offset (∼3‰) was also recovered in a frustule cleaning study of diatoms grown for aquaculture (Morales et al., 2011), while a culture study recovered in a frustule cleaning study of diatoms grown (Morales et al., 2014). A similar offset (2.2‰) Based on the average for the Antarctic Zone Tmin and summer mixed layer. 

Based on Studer et al. (2015).

Estimated based on the AASW Rayleigh fractionation trends.

Difference between core-top diatom-bound and expected sinking PN δ15N.

LGM diatom-bound δ15N minus the isotopic offset.

Table 3

<table>
<thead>
<tr>
<th>N isotope effect</th>
<th>NO3 + NO2</th>
<th>NO3-only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source NO3 δ15N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5–6.7‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.2–10.8‰&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Source NO3 concentration&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0–6.2‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.1–6.0‰&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Summer surface NO3 concentration&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24–32 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>24–32 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Modern surface NO3 consumption&lt;sup&gt;d&lt;/sup&gt;</td>
<td>~24 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>~24 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Core-top diatom-bound δ15N</td>
<td>~20%</td>
<td>~20%</td>
</tr>
<tr>
<td>Expected sinking PN δ15N</td>
<td>0.4‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.1‰&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isotopic offset&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.8‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.3‰&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>LGM diatom-bound δ15N&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.2‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.2‰&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>LGM Expected sinking PN δ15N&lt;sup&gt;h&lt;/sup&gt;</td>
<td>4.4‰&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.9‰&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>LGM surface NO3 consumption&lt;sup&gt;i&lt;/sup&gt;</td>
<td>79–97%</td>
<td>53–87%</td>
</tr>
<tr>
<td>LGM summer surface NO3 concentration&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.7–6.7 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>3.1–15.4 µmol l&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Range given by the regional and station 15e averages (Table 1).<br><sup>b</sup> Range given by the Tmin average for each individual cruises.<br><sup>c</sup> Average for the Antarctic Zone summer mixed layer.<br><sup>d</sup> Based on the average for the Antarctic Zone Tmin and summer mixed layer.<br><sup>e</sup> Based on Studer et al. (2015).
<sup>f</sup> Estimated based on the AASW Rayleigh fractionation trends.<br><sup>g</sup> Difference between core-top diatom-bound and expected sinking PN δ15N.<br><sup>h</sup> LGM diatom-bound δ15N minus the isotopic offset.
lower NO$_3$ + NO$_2$ $^{15}$e (4.7–5.5‰) (Brunelle, 2009) than for NO$^-$-only $^{15}$e (5.8–9.1‰) (Altabet and Francois, 1994a,b; Wu et al., 1997).

In lower latitude regions, NO$_3$-only $^{15}$e appears to be more representative than NO$_3$ + NO$_2$ $^{15}$e of the ‘true’ isotope effect for NO$_3$ assimilation (Table 3) (Fawcett et al., 2015; Rafter and Sigman, 2016), in which case the former could be compared with $^{15}$e estimates from acidified samples in similar environments (Altabet et al., 1999). The evidence in favor of this view is that (1) NO$_3$-only (rather than NO$_3$ + NO$_2$) $^{15}$N and $^{18}$O increase in unison from below the euphotic zone toward the surface, in agreement with NO$_3$ assimilation, and (2) NO$_2$ $^{15}$N is higher than in AASW and thus less clearly affected by NO$_3$–NO$_2$ interconversion (Fawcett et al., 2015; Rafter and Sigman, 2016; Peng et al., in press). The implication is that, rather than being strongly influenced by NO$_3$–NO$_2$ interconversion, the $^{15}$N of NO$_3$ at the lower latitude regions is dominantly altered by other NO$_2$-oxidizing and -reducing processes. In nutrient-depleted regions, the $^{15}$e estimates tend to be lower and more variable (Table 3). We cannot rule out underestimation of the isotope effect due to artifacts from mixing in these settings. However, culture studies report systematically lower isotope effects (<5‰) for haptophytes, chlorophytes and cyanobacteria, which are the phytoplankton groups that are known to prevail in these environments (Montoya and McCarthy, 1995; Granger et al., 2010). Alternatively, at adequately low [NO$_3$], there may be a reduction in the $^{15}$e of a given phytoplankton strain (Granger et al., 2004).

The subarctic North Atlantic is characterized by little difference in $^{15}$N and $^{18}$O between NO$_3$ + NO$_2$ and NO$_3$-only.
NO₃-only (Peng et al., in press), yielding 15ε estimates of 5.2–5.3‰ and 5.1–5.2‰, respectively. In this environment, NO₃ dynamics thus appear to be relatively unimportant in the isotope dynamics. [NO₃] is not at appreciably lower concentration through the upper water column than in the Antarctic (Peng et al., in press). Thus, in the subarctic North Atlantic, NO₃ δ¹⁵N must be far less distinct from NO₃. One possible explanation is that mixed layer depth is less variable subsequent to spring/summer mixed layer shoaling, leading to less entrainment of nitrifiers into the euphotic zone and thus an overall lack of the isotope processing that applies in the summertime Antarctic.

The overall stability of the isotope effect in nutrient-replete regions (4.5–6.7‰) contrasts with the large range of values reported from culture studies (~1–20‰) and the expected variations induced by changing NO₃ metabolisms (Montoya and McCarthy, 1995; Granger et al., 2004, 2010; Needoba et al., 2014; Karsh et al., 2012, 2014). NO₃ assimilation has been conceptualized as a stepwise process, in which NO₃ is actively taken up through the membrane into the cell (i.e., NO₃ uptake, $\delta_{\text{upt}} \approx 2$‰; Karsh et al., 2014).

Once inside the cell, NO₃ can either be reduced by the nitrate reductase enzyme, which is thought to be the dominant fractionating step ($\delta^{15}N_{\text{NR}} \approx 26.6$‰) or released back into the surrounding water with the isotope effect of NO₃ efflux ($\delta^{15}N_{\text{eff}} \approx 1.2$‰) (Karsh et al., 2012, 2014). Any imbalance between NO₃ uptake and intracellular reduction allows 15N-enriched NO₃, which is accumulated inside the cell (e.g., in the vacuole), to be released via NO₃ efflux, transmitting the 15N-enrichment to seawater NO₃ (Needoba et al., 2004). Given the enzyme-level isotope effects available in the literature, the overall isotope effect lies between $\sim 2$‰ ($\delta_{\text{upt}}$) and $\sim 25.8$‰ ($\delta_{\text{eff}}$) for an efflux/uptake ratio of 0 and 1, respectively (Karsh et al., 2012, 2014). An isotope effect between 4.5‰ and 6.7‰ suggests for a small and relatively constant (0.10–0.27) efflux/uptake ratio in nutrient-replete regions (Table 3), with the caveat that the isotope effect provides an integrative measure of seasonal NO₃ consumption and therefore a succession of different phytoplankton assemblages and NO₃ metabolisms. Lower isotope effects in nutrient-depleted regions may point to an efflux/uptake ratio less than 0.05, with most of the isotope fractionation driven by NO₃ uptake. However, as described above, we cannot rule out underestimation of the isotope effect due to artifacts resulting from mixing in these low-nutrient settings.

5. Conclusions and Remaining Questions

We report a relatively constant isotope effect for summertime NO₃ assimilation in the modern Antarctic Zone (5.5 ± 0.6‰), representative of the full seasonal NO₃ depletion and the associated range of physical properties encountered in this region of the Southern Ocean. This implies a tight connection between the degree of NO₃ consumption and the $\delta^{15}N$ of both the NO₃ pool and the export flux. These findings contradict previous studies that reported variability in the isotope effect due to varying environmental conditions (i.e., mixed layer depth), and simplify the effort to reconstruct the degree of NO₃ consumption in the Antarctic Zone at times in the past.

We suggest that the variability reported in previous studies is due to a methodological artifact, induced by putative NO₃–NO₂ interconversion in the mixed layer. Although the systematics of NO₃–NO₂ interconversion are relatively straightforward for the N isotopes, operating as a closed system where N atoms are exchanged between NO₃ and NO₂ and expressing a large equilibrium N isotope effect, the systematics applicable to the O isotopes remain unknown. No clear difference is reported between NO₃–NO₂ and NO₃-only $\delta^{18}O$. However, the small contribution (0.7 ± 0.2‰) of NO₃ to the NO₃ + NO₂ pool may mask large differences in NO₃ $\delta^{18}O$ (~50‰) that yield no significant differences (~±0.2‰) in NO₃ + NO₂ and NO₃-only $\delta^{18}O$. Kinetic isotope effects during the incorporation and removal of O atoms are expected, as well as equilibrium isotope effects between NO₃ and NO₂ as well as between NO₃–NO₂ and water. However, the full elucidation of these systematics requires the measurement of the $\delta^{18}O$ of NO₂ immediately upon sample collections (e.g., Buchwald et al., 2015).

Another open question for future studies concerns the higher N isotope effect estimates previously reported for the Subantarctic Zone, also based on measurements from acidified samples (DiFiore et al., 2006, 2010). It remains possible that the particularly deep mixed layers of the SAZ are adequate for light limitation to cause a higher 15ε for nitrate assimilation, as observed in culture experiments (Needoba et al., 2004). However, new measurements of both NO₃ + NO₂ and NO₃-only $\delta^{15}N$ on the GOSHIP IOSS and P18S sections indicate that NO₃–NO₂ interconversion also occurs in the SAZ mixed layer, with NO₃ $\delta^{15}N$ estimated to be as low as −52 ± 11‰ (data not shown). Thus, artifacts related to NO₂ loss during storage have likely also raised the measured NO₃ $\delta^{15}N$ and thus the estimates of 15ε in this zone of the Southern Ocean as well. A model taking into account the NO₃ supplies from both the south and the underlying SAZ thermocline is required to tackle this question.

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