



# 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 ( $\delta^{15}\text{N}$ ) 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  in Antarctic Zone surface waters is extremely low ( $-61 \pm 20\text{‰}$ ), 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  is relatively constant ( $5.5 \pm 0.6\text{‰}$ ) across the Antarctic Zone, shows no relationship with mixed layer depth, and is in agreement with sediment trap  $\delta^{15}\text{N}$  measurements. Estimates of the N isotope effect derived from nitrate-only  $\delta^{15}\text{N}$  are higher and more variable ( $7.9 \pm 1.5\text{‰}$ ), consistent with an artifact from nitrate–nitrite isotope exchange. In the case of the Southern Ocean, we conclude that the  $\delta^{15}\text{N}$  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 the 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

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the “major nutrients” nitrate and phosphate are fully used by phytoplankton. The incomplete consumption of major nutrients in Southern Ocean surface waters has been attributed to co-limitation of phytoplankton growth by iron and light (Martin et al., 1990; Mitchel et al., 1991) and allows (i) CO<sub>2</sub> 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 (NO<sub>3</sub><sup>-</sup>) assimilation by phytoplankton preferentially incorporates <sup>14</sup>N into new biomass, leaving the residual NO<sub>3</sub><sup>-</sup> pool enriched in <sup>15</sup>N (Wada and Hattori, 1978; Montoya and McCarthy, 1995; Granger et al., 2004, 2010). The kinetic isotope effect (ε) expresses the degree of isotopic discrimination and is commonly defined as the ratio of reaction rates at which the two isotopes are converted from reactant to product (i.e.  $\epsilon (\text{‰}) = 1 - k^{15}/k^{14}$ ; where <sup>x</sup>k is the rate constant for the <sup>x</sup>N-containing reactant). The isotopic fractionation of NO<sub>3</sub><sup>-</sup> assimilation links the degree of NO<sub>3</sub><sup>-</sup> consumption to the δ<sup>15</sup>N ( $= (^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{reference}} - 1$ , with atmospheric N<sub>2</sub> as the reference) of both the NO<sub>3</sub><sup>-</sup> and the newly produced organic matter (Altabet and Francois, 1994a,b; Sigman et al., 1999a). Thus, the δ<sup>15</sup>N 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 the changes in atmospheric CO<sub>2</sub> 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 δ<sup>15</sup>N 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 NO<sub>3</sub><sup>-</sup> 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 pCO<sub>2</sub> (Francois et al., 1997; Sigman et al., 2010).

The δ<sup>15</sup>N of the NO<sub>3</sub><sup>-</sup> supply and the isotope effect of NO<sub>3</sub><sup>-</sup> assimilation are two key parameters required for estimating the degree of past NO<sub>3</sub><sup>-</sup> 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 δ<sup>15</sup>N 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 (NO<sub>2</sub><sup>-</sup>) would have been largely lost during storage, due to the volatility of HNO<sub>2</sub> followed by its rapid conversion into gaseous nitrogen oxide (NO and NO<sub>2</sub>) (Park and Lee, 1988; Rayson et al., 2012). NO<sub>2</sub><sup>-</sup> can be removed prior to isotope analysis through the addition of sulfamic acid or sulfanilamide, allowing the measurement of both NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>-only δ<sup>15</sup>N and δ<sup>18</sup>O 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 NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> δ<sup>15</sup>N and NO<sub>3</sub><sup>-</sup> δ<sup>15</sup>N in Antarctic Zone surface waters, with NO<sub>3</sub><sup>-</sup> δ<sup>15</sup>N typically being 0.5–1.0‰ higher than NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> δ<sup>15</sup>N. They suggested that NO<sub>3</sub><sup>-</sup>–NO<sub>2</sub><sup>-</sup> interconversion occurs in the euphotic zone, leading to the expression of an N equilibrium isotope effect between NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup>, enriching NO<sub>3</sub><sup>-</sup> and depleting NO<sub>2</sub><sup>-</sup> in <sup>15</sup>N. Seasonal mixed layer deepening was suggested to entrain nitrite oxidizers from the subsurface into the late-summer mixed layer, discouraging NO<sub>2</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup> 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 NO<sub>3</sub><sup>-</sup>–NO<sub>2</sub><sup>-</sup> interconversion with subsequent NO<sub>2</sub><sup>-</sup> 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 (Fig. 1) (e.g., Sigman et al., 2009a). The boundary between these two zones is also roughly delineated by either the northernmost extent of winter sea ice or the southernmost extent of Upper Circumpolar Deep Water (UCDW) (Orsi et al., 1995).

Across the Antarctic Zone, NO<sub>3</sub><sup>-</sup> is supplied by Ekman pumping and vertical mixing across the base of the winter mixed layer. Nutrient-rich Circumpolar Deep Water (CDW) upwells in the Antarctic Zone. Part of the upwelled CDW gains buoyancy due to warming and freshening, causing it to flow northward in the Ekman layer, ultimately sinking north of the Polar Front into the main oceanic pycnocline (Marshall and Speer, 2012). The remainder loses buoyancy near Antarctica, inducing deep convection over the continental shelves and leading to Antarctic Bottom Water (AABW) formation (Fig. 2). Deep ventilation may also occur in the Antarctic Zone away from the coasts, for example, due to mixing by mesoscale eddies (Abernathey and Ferreira, 2015).

In summer, the upper halocline layer in most of the Antarctic Zone (hereafter referred as Antarctic Surface Water, AASW) is characterized by both a relatively fresh, well-mixed surface layer, and a subsurface temperature minimum (T<sub>min</sub>) layer below (Fig. 2c and A1c; hereafter referred to as the ‘T<sub>min</sub>’ stations) (e.g., Park et al., 1998). The latter is also known as “Winter Water” because it is the remnant of the previous winter mixed layer that has become isolated in the shallow subsurface by spring-to-summer warming and freshening of the overlying water. AASW tends to be thicker in the OAZ due to higher wind stress and thus deeper vertical mixing near the Polar Front, with generally shallower mixed layers in the seasonally sea-ice covered areas to the south (Fig. 1) (Pellichero et al., 2017).

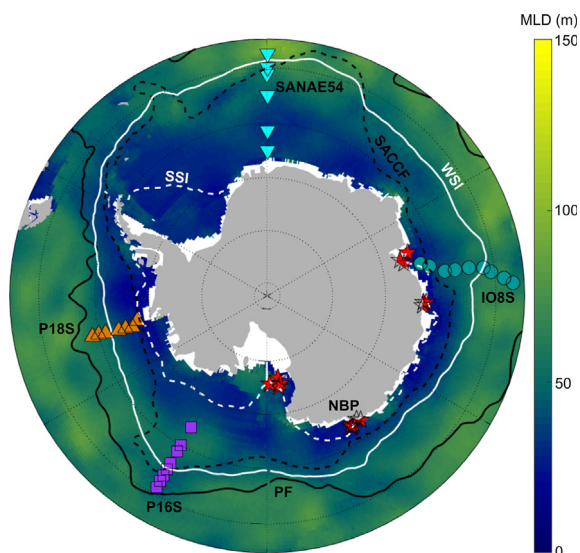


Fig. 1. Location of the stations (symbols) overlaid on mixed layer depth (MLD) climatology for February (Pellichero et al., 2017). The Polar Front (PF) and the Southern Antarctic Circumpolar Current Front (SACCF) according to Orsi et al. (1995) are indicated by the solid and dashed black lines, respectively. The solid and dashed white lines represent the mean winter maximal (WSI) and summer minimal (SSI) sea ice extent (1979–2008,  $\geq 15\%$  ice cover) (Raymond, 2014). The cyan inverted triangles indicate the stations for SANA54, 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). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Near and above the continental shelves (hereafter referred as to the ‘margin’ stations), a shallow mixed layer usually caps a relatively homogeneous, dense, near-freezing shelf water mass. The latter has undergone a brine rejection-driven increase in salinity, a prerequisite for the formation of AABW (e.g., Orsi et al., 1999). As a result of lateral exchange, intru-

sion of AASW and CDW southward toward the continental shelves can generate a distinct subsurface temperature maximum between the summertime mixed layer and the shelf waters below (hereafter referred to as the ‘ $T_{\max}$ ’ stations) (e.g., DiFiore et al., 2009).

## 2.2. Sample collection

New and previously published  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  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 SANA54 (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 section).

A total of 50 hydrocasts (24 unpublished) were collected between the Antarctic continental shelf and the Polar Front. Most of the hydrocasts ( $n = 46$ ) were collected between December 12th and April 9th during the various years (Table 1). This summer–early fall condition captures the major extent of annual  $\text{NO}_3^-$  depletion in the mixed layer. Four hydrocasts from the Ross Sea (NBP01–01) were sampled in November at the onset of the growing period (DiFiore et al., 2009). Unfiltered water samples were collected and immediately frozen ( $-20\text{ }^\circ\text{C}$ ) until their analysis at home-based laboratories (Max Planck Institute for Chemistry (MPIC) for IO8S and P18S and Princeton University for SANA54).

## 2.3. Nitrate isotope analysis

$\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  were determined using the denitrifier method (Sigman et al., 2001; Casciotti et al.,

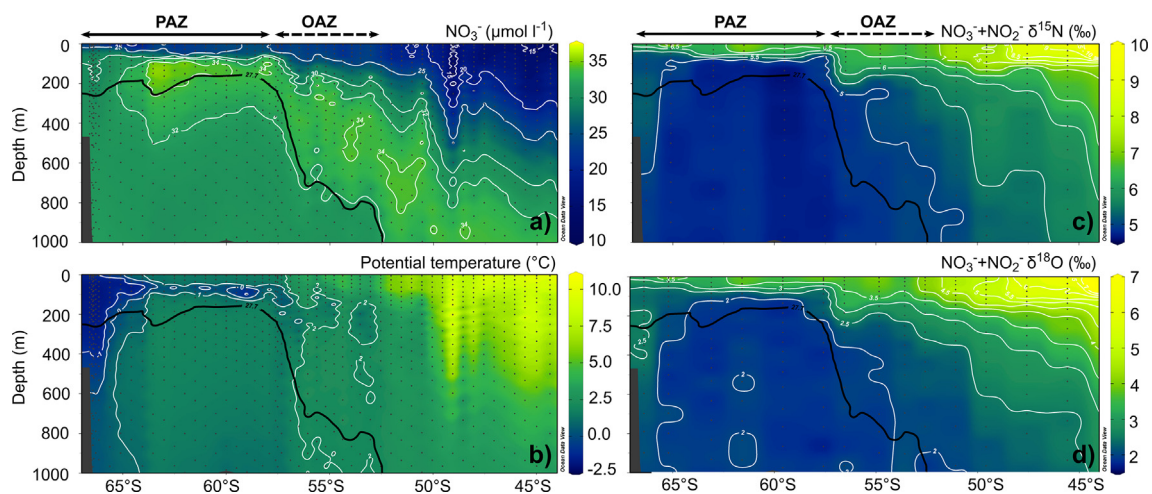


Fig. 2. Meridional depth sections of  $\text{NO}_3^-$  concentration (a), potential temperature (b),  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  (c) and  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{18}\text{O}$  (d) in the Indian Sector at  $78\text{--}95^\circ\text{E}$  (IO8S). The thin white lines are contours of  $\text{NO}_3^-$  concentration (a), potential temperature (b),  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  (c) and  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{18}\text{O}$  (d). The isopycnal delimiting Upper and Lower Circumpolar Deep Water is indicated with the thick black solid line. The sections were generated with Ocean Data View [Available at <http://odv.awi.de>].

Table 1

Averages ( $\pm$ 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.

Cruise Name (Sector)	Location	Date	PAZ	OAZ	Regional $\epsilon$ average (‰)				Station $\epsilon$ average <sup>c</sup> (‰)			
					$\text{NO}_3^- + \text{NO}_2^-$		$\text{NO}_3^-$ -only		$\text{NO}_3^- + \text{NO}_2^-$		$\text{NO}_3^-$ -only	
					$^{15}\epsilon$	$^{18}\epsilon$	$^{15}\epsilon$	$^{18}\epsilon$	$^{15}\epsilon$	$^{18}\epsilon$	$^{15}\epsilon$	$^{18}\epsilon$
IO8 (Indian)	83°E	Feb. 2016	6	3	5.9 $\pm$ 0.4	4.4 $\pm$ 0.3	7.6 $\pm$ 0.6	4.2 $\pm$ 0.3	6.1 $\pm$ 0.5	4.3 $\pm$ 0.8	8.0 $\pm$ 0.6	4.5 $\pm$ 1.0
P16 (Pacific) <sup>a</sup>	150°W	Apr. 2014	6	2	4.9 $\pm$ 0.3	4.6 $\pm$ 0.3	6.7 $\pm$ 0.5	4.5 $\pm$ 0.3	5.3 $\pm$ 0.9	4.2 $\pm$ 0.9	8.4 $\pm$ 2.2	4.0 $\pm$ 0.7
P18 (Pacific)	103°W	Jan. 2017	2	5	5.8 $\pm$ 0.5	4.8 $\pm$ 0.5	8.4 $\pm$ 0.7	4.2 $\pm$ 0.5	6.1 $\pm$ 1.6	3.5 $\pm$ 1.0	10.8 $\pm$ 3.0	3.3 $\pm$ 1.4
SANAE54 (Atlantic)	0°W	Dec-Feb 2014–2015	7	1	4.8 $\pm$ 0.3	4.3 $\pm$ 0.2	6.2 $\pm$ 0.2	4.8 $\pm$ 0.3	4.5 $\pm$ 1.3	4.3 $\pm$ 0.8	6.4 $\pm$ 1.1	5.2 $\pm$ 1.7
NBP01-01 (DDU) <sup>b,c</sup>	146°E	Feb. 2001	6	0	3.2 $\pm$ 0.4 <sup>d</sup>	1.9 $\pm$ 0.9 <sup>d</sup>			5.7 $\pm$ 2.7	5.9 $\pm$ 4.6		
NBP01-01 (Davis Sea) <sup>c</sup>	93°E	Feb. 2001	3	0	4.9 $\pm$ 0.4	4.8 $\pm$ 0.8			4.9 $\pm$ 0.9	4.7 $\pm$ 1.6		
NBP01-01 (Prydz Bay) <sup>c</sup>	76°E	Mar. 2001	5	0	3.8 $\pm$ 0.6 <sup>d</sup>	4.2 $\pm$ 0.7 <sup>d</sup>			6.7 $\pm$ 2.8	4.5 $\pm$ 1.2		
NBP06-08 (Ross Sea) <sup>c</sup>	174°E	Nov. 2006	4	0	5.8 $\pm$ 0.5	6.3 $\pm$ 0.8			5.7 $\pm$ 0.4	6.2 $\pm$ 0.2		
AASW average			39	11	5.2 $\pm$ 0.1	4.2 $\pm$ 0.1	7.3 $\pm$ 0.3	4.5 $\pm$ 0.2	5.7 $\pm$ 1.5	4.4 $\pm$ 1.5	8.5 $\pm$ 2.0	4.2 $\pm$ 1.1
AASW average (without DDU and Prydz Bay) <sup>d</sup>			28	11	5.4 $\pm$ 0.2	4.6 $\pm$ 0.1						

<sup>a</sup> Kemeny et al. (2016).

<sup>b</sup> Dumont D’Urville Sea.

<sup>c</sup> DiFiore et al. (2009).

<sup>d</sup> The variability in the AASW for these areas is close to the analytical precision.

<sup>e</sup> Only the stations with a vertical isotopic gradient greater than 0.2‰ are used.



2002; Weigand et al., 2016). Briefly, 10–20 nmol of  $\text{NO}_3^- + \text{NO}_2^-$  was quantitatively converted to  $\text{N}_2\text{O}$  gas by a strain of denitrifying bacteria (*Pseudomonas chlororaphis* f. sp. *aureofaciens* ATCC n°13985) that lacks an active  $\text{N}_2\text{O}$  reductase enzyme. In both laboratories, the isotopic composition of  $\text{N}_2\text{O}$  was measured by gas chromatography/isotope ratio mass spectrometry using a purpose-built online  $\text{N}_2\text{O}$  extraction and purification system and Thermo MAT 253 mass spectrometer (Weigand et al., 2016). Measurements are referenced to air  $\text{N}_2$  for  $\delta^{15}\text{N}$  and Vienna Standard Mean Ocean Water (VSMOW) for  $\delta^{18}\text{O}$  using the  $\text{NO}_3^-$  reference materials IAEA-NO3, with a  $\delta^{15}\text{N}$  of 4.7‰ and a  $\delta^{18}\text{O}$  of 25.6‰, and USGS-34, with a  $\delta^{15}\text{N}$  of -1.8‰ and a  $\delta^{18}\text{O}$  of -27.9‰ (Böhlke et al., 2003).

During the reduction of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  to  $\text{N}_2\text{O}$  by the denitrifier method, O atoms are either transferred to the resulting nitrogen oxide pool (ultimately to  $\text{N}_2\text{O}$ ) or lost as water, and isotopic fractionation is known to occur during these branching reactions (Casciotti et al., 2002). For  $\text{NO}_3^-$ , this effect is accounted for by calibration with  $\text{NO}_3^-$  isotopic reference materials. Because  $\text{NO}_2^-$  reduction to  $\text{N}_2\text{O}$  represents a smaller fractional loss of oxygen atoms than  $\text{NO}_3^-$  reduction (3/4 vs. 5/6), the  $\text{N}_2\text{O}$  generated from  $\text{NO}_2^-$  by the denitrifier method is ~25‰ lower in  $\delta^{18}\text{O}$  than  $\text{N}_2\text{O}$  generated from  $\text{NO}_3^-$  with the same initial  $\delta^{18}\text{O}$  (Casciotti et al., 2007). This methodological bias is corrected for each sample by adding to  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{18}\text{O}$  the product of 25‰ and the relative contribution of  $\text{NO}_2^-$  to  $\text{NO}_3^- + \text{NO}_2^-$  (Kemeny et al., 2016). In order to isolate and measure the  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , samples with detectable  $\text{NO}_2^-$  concentrations were treated with sulfamic acid prior to  $\text{NO}_3^-$  isotope analysis (Granger and Sigman, 2009).

Replicate analyses (100% of the samples) at MPIC indicate median 1sd reproducibility of <0.07‰ and <0.13‰ for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , respectively (i.e., similar to <0.05‰ and <0.14‰ for P16S and SANA54 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‰ and <0.12‰ for  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , 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 1sd reproducibility of 0.17‰ for  $\delta^{15}\text{N}$  and 0.26‰ for  $\delta^{18}\text{O}$  (DiFiore et al., 2009).

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

If  $\text{NO}_3^-$  assimilation proceeds with a constant isotope effect and if the reactant N pool ( $\text{NO}_3^-$ ) is neither replenished nor subject to loss other than consumption, then the isotopic evolution of the residual  $\text{NO}_3^-$ , 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):

$$R_{\text{NO}_3^-} = R_{\text{NO}_3^-}^0 \cdot f^{-\varepsilon} \quad (1)$$

$$R_{\text{PN}}^{\text{inst}} = (1 - \varepsilon) \cdot R_{\text{NO}_3^-} \quad (2)$$

$$R_{\text{PN}}^{\text{acc}} = R_{\text{NO}_3^-}^0 \cdot \frac{1 - f^{(1-\varepsilon)}}{1 - f} \quad (3)$$

where  $f$  is the fraction of  $\text{NO}_3^-$  remaining (i.e.  $f = [\text{NO}_3^-] / [\text{NO}_3^-]_{\text{initial}}$ ), the superscript 0 is the initial condition,  $R$  is the  $^{15}\text{N}/^{14}\text{N}$  or  $^{18}\text{O}/^{16}\text{O}$  ratio, and  $\varepsilon$  is the isotope effect for either N or O isotopes, but opposite to the sign convention of Mariotti (1981) so that positive values indicate preferential processing of  $^{14}\text{N}$  or  $^{16}\text{O}$ . Since O atoms are not incorporated into biomass, only Eq. (1) is valid for the O isotopes. Because phytoplankton discriminate against  $^{15}\text{N}$  and  $^{18}\text{O}$  to the same extent during  $\text{NO}_3^-$  assimilation ( $^{15}\varepsilon \approx ^{18}\varepsilon$ ; Granger et al., 2004, 2010; Karsh et al., 2012, 2014), the residual  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  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  $^{15}\text{N}$  and  $^{18}\text{O}$  isotope are low, implying that  $^{14}\text{N} \sim ^{14}\text{N} + ^{15}\text{N}$  and  $^{16}\text{O} \sim ^{16}\text{O} + ^{17}\text{O} + ^{18}\text{O}$  (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  $\varepsilon$  as its slope and initial conditions ( $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$ ) as intercept (Mariotti et al., 1981), as shown here for the N isotopes:

$$\begin{aligned} \ln(\delta^{15}\text{N}_{\text{NO}_3^-} + 1) &= -\varepsilon \cdot \ln([\text{NO}_3^-]) \\ &+ \left( \ln(\delta^{15}\text{N}_{\text{NO}_3^-}^0 + 1) + \varepsilon \cdot \ln([\text{NO}_3^-]_0) \right) \end{aligned} \quad (4)$$

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):

$$\delta^{15}\text{N}_{\text{NO}_3^-} = -\varepsilon \cdot \ln([\text{NO}_3^-]) + \left( \delta^{15}\text{N}_{\text{NO}_3^-}^0 + \varepsilon \cdot \ln([\text{NO}_3^-]_0) \right) \quad (5)$$

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

### 3. RESULTS

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

In all the Southern Ocean stations analyzed, there is a small but significant accumulation of  $\text{NO}_2^-$  near the surface, increasing from near-zero concentrations in deep water to ~0.25  $\mu\text{mol l}^{-1}$  (Fig. 3). This is in contrast to low-latitude areas, where a primary  $\text{NO}_2^-$  maximum is typically found at the base of the euphotic layer, with <0.02  $\mu\text{mol l}^{-1}$  in

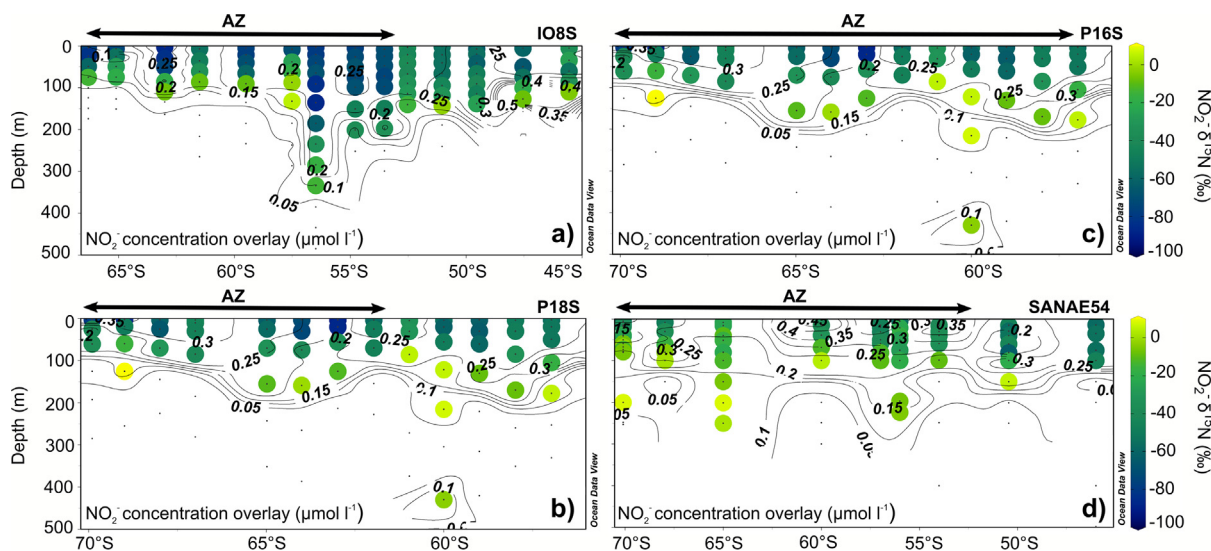


Fig. 3. Meridional depth sections of  $\text{NO}_2^- \delta^{15}\text{N}$  (colored dots) with  $\text{NO}_2^-$  concentration overlay (black contours) for IO8S eastern Indian section (a), P18S eastern Pacific section (b), P16S western Pacific section (c) and SANAE54 Atlantic section (d).  $\text{NO}_2^- \delta^{15}\text{N}$  is shown when the  $\text{NO}_2^-$  contribution to the  $\text{NO}_3^- + \text{NO}_2^-$  pool is larger than 0.25%. The black horizontal arrows above each panel indicate the Antarctic Zone. The sections were generated with Ocean Data View [Available at <http://odv.awi.de>]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the surface mixed layer (Lomas and Lipschultz, 2006; Fawcett et al., 2015), but consistent with a recent compilation of observations for the high latitude ocean showing similarly elevated levels of  $\text{NO}_2^-$  throughout the mixed layer (Zakem et al., 2018). The removal of  $\text{NO}_2^-$  from samples with detectable  $\text{NO}_2^-$  concentrations has a significant impact on the nitrate isotope distribution (Figs. 4 and 5a).  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  is higher than  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  by  $\sim 0.7 \pm 0.2\text{‰}$  in the summer surface mixed layer, but it is not significantly different deeper in the water column. In contrast, there is no clear difference ( $0.0 \pm 0.2\text{‰}$ ) between  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^- \delta^{18}\text{O}$  throughout the upper water column (Figs. 4 and 5b).

### 3.2. Nitrate isotope distribution in the Antarctic Zone

The  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of both  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^-$  increase toward the surface in concert with the upward decline in  $\text{NO}_3^-$  concentration, reflecting the preferential assimilation of  $^{14}\text{N}$ - and  $^{16}\text{O}$ -bearing  $\text{NO}_3^-$  by phytoplankton (Figs. 2 and 4). In the OAZ,  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  increases from 5.0‰ in the deeper layers to 6.6‰ at the surface on average, and from 5.0‰ to 7.4‰ for  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$ . Both  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^- \delta^{18}\text{O}$  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,  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  increases from 4.8‰ to 6.2‰ on average, and from 4.8‰ to 6.9‰ for  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$ . Similar to the OAZ, both  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^- \delta^{18}\text{O}$  increase to the same extent, from 2.0‰ to 3.4‰. The northward increases in  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  result from progressive  $\text{NO}_3^-$  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).

Deep convection over the shelves mixes lower  $[\text{NO}_3^-]$  and higher  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  surface waters down into the ocean interior, causing these dense shelf waters to be depleted in  $\text{NO}_3^-$  concentration and elevated in the  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only relative to deep waters further north (Figs. 2 and 4). Because of the lower degree of  $\text{NO}_3^-$  consumption at the PAZ margin stations, the increase in  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  toward the surface is small and close to the analytical precision. At the PAZ  $T_{\max}$  stations, greater scatter is observed toward the surface but with similar mean properties as for the PAZ  $T_{\min}$  stations (Fig. 4). For these stations, the  $\delta^{15}\text{N}$  or  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$ -only were not measured (DiFiore et al., 2009).

## 4. DISCUSSION

### 4.1. Nitrate supply to Antarctic surface waters

Below AASW lie two distinct water masses and, therefore, two different sources of  $\text{NO}_3^-$  to Antarctic Zone surface waters: Lower Circumpolar Deep Water (LCDW) and Upper Circumpolar Deep Water (UCDW), which supply  $\text{NO}_3^-$  to the PAZ and OAZ, respectively. UCDW is characterized on average by higher  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$  than LCDW (by  $1.5 \mu\text{mol l}^{-1}$  and 0.2–0.3‰, respectively; Sigman et al., 2000; DiFiore et al., 2010) but is similar in  $\text{NO}_3^- \delta^{18}\text{O}$  (Figs. 2, 4, and A1).

The  $\text{NO}_3^-$  concentration maximum in UCDW results from exchange with the Indian and Pacific Oceans, where regenerated nutrients accumulate along the mid-depth

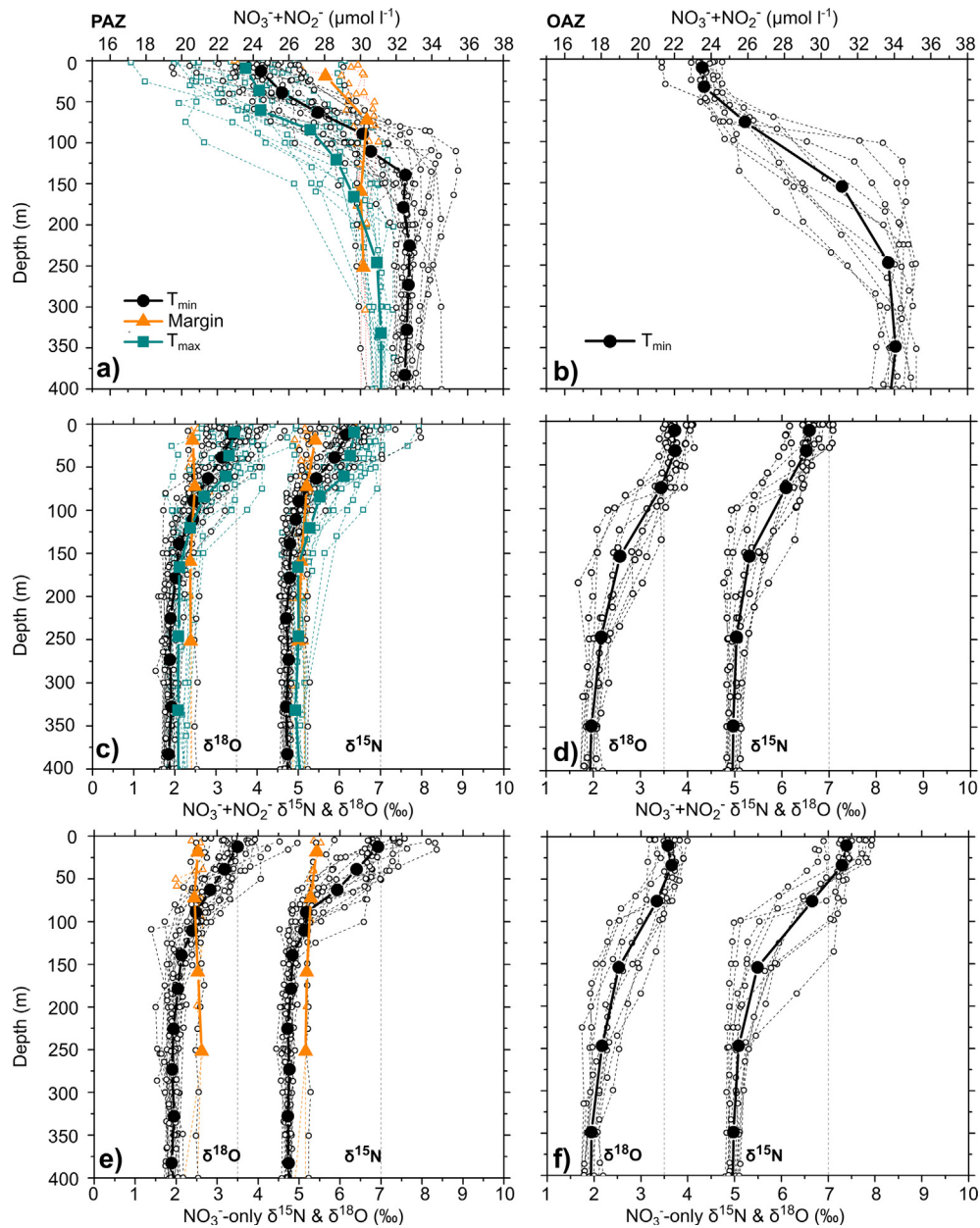


Fig. 4. Profiles of (a, b)  $\text{NO}_3^- + \text{NO}_2^-$  concentration, (c, d)  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ , and (d, e)  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for the Polar Antarctic Zone (PAZ) (a, c, e) 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<sub>min</sub>’ stations (white circles), ‘margin’ stations (orange triangles) or ‘T<sub>max</sub>’ stations (green-blue squares). The mean profiles for each category are given by the corresponding solid symbols. All stations in the OAZ fall into the T<sub>min</sub> category. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

return flow of the ocean’s “conveyor belt” circulation (Sarmiento et al., 2007; Talley, 2013). The  $^{15}\text{N}$  enrichment in UCDW ultimately derives from the transfer (via Ekman transport) of  $^{15}\text{N}$ - and  $^{18}\text{O}$ -enriched residual  $\text{NO}_3^-$  from the polar ocean to lower latitude intermediate, thermocline, and surface waters (Fig. 2; Sigman et al., 1999a; Rafter et al., 2013; Sigman et al., 2009a). In low-latitude areas where  $\text{NO}_3^-$  consumption is complete, export production and remineralization produce regenerated  $\text{NO}_3^-$  with the

same  $\delta^{15}\text{N}$  as the  $\text{NO}_3^-$  originally supplied to the euphotic zone from the underlying thermocline. The  $\text{NO}_3^-$  in the thermocline was, in turn, elevated in  $\delta^{15}\text{N}$  (and  $\delta^{18}\text{O}$ ) by partial  $\text{NO}_3^-$  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  $\delta^{15}\text{N}$  of UCDW and its deep Pacific and Indian precursors (e.g., Pacific Deep Water). The same processes do not elevate the  $\text{NO}_3^-$   $\delta^{18}\text{O}$

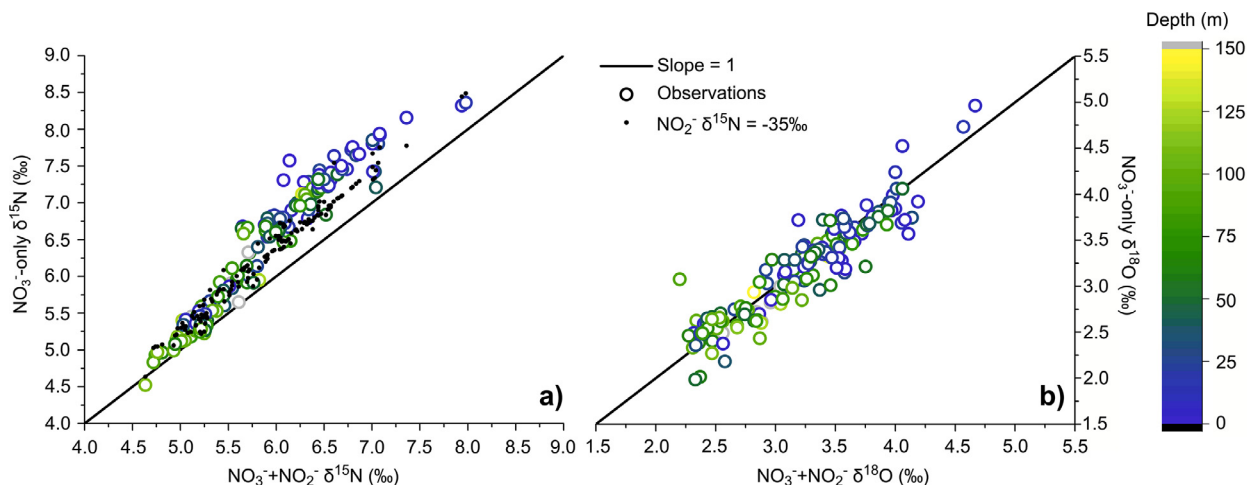


Fig. 5. Relationship between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  (a) and  $\delta^{18}\text{O}$  (b). In (a), the black dots show the expected  $\text{NO}_3^-$   $\delta^{15}\text{N}$  if  $\text{NO}_2^-$   $\delta^{15}\text{N}$  was  $-35\text{‰}$ . This  $\text{NO}_2^-$   $\delta^{15}\text{N}$  value represents the minimum value expected from nitrification,  $\text{NO}_2^-$  assimilation, or an imbalance in assimilatory  $\text{NO}_3^-$  reduction (Fripiat et al., 2015a).

of UCDW because regenerated  $\text{NO}_3^-$  has a  $\delta^{18}\text{O}$  equal to that of ambient water plus  $\sim 1.1\text{‰}$  (Sigman et al., 2009a; Buchwald et al., 2012), which is lower than the ambient  $\text{NO}_3^-$   $\delta^{18}\text{O}$  ( $>1.8\text{‰}$ ). Water column denitrification in the tropical oxygen deficient zones also works to increase the  $\text{NO}_3^-$   $\delta^{15}\text{N}$  in this mid-depth return flow, again causing a much weaker increase in  $\text{NO}_3^-$   $\delta^{18}\text{O}$  due to the low latitude  $\text{NO}_3^-$  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  $\text{NO}_3^-$  isotopes by  $\text{N}_2\text{O}$ -based methods, both  $\text{NO}_3^-$  and  $\text{NO}_2^-$  are converted to  $\text{N}_2\text{O}$ , but the contribution from  $\text{NO}_2^-$  to the measured isotopic composition is often considered negligible due to its typically low concentration relative to  $\text{NO}_3^-$  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  $\text{NO}_2^-$  near the surface. Most of the previous  $\text{NO}_3^-$  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  $\text{NO}_2^-$ . Under acidic conditions, nitrous acid ( $\text{HNO}_2$ ) forms, some of which may be lost to the gas phase. In addition,  $\text{HNO}_2$  can decompose to nitrogen oxides ( $\text{NO}$  and  $\text{NO}_2$ ), with some of the  $\text{NO}_2$  being hydrolyzed to form  $\text{NO}_3^-$  and the rest escaping from the high-density polyethylene sample bottles typically used for storage (Park and Lee, 1988; Rayson et al., 2012).  $\text{NO}_2^-$  disappearance during storage could plausibly be argued to be beneficial for the understanding of the isotope dynamics associated with  $\text{NO}_3^-$  assimilation, with the caveat that a portion of the  $\text{HNO}_2$  breakdown can yield  $\text{NO}_3^-$ . Freezing of seawater samples

is thought to better preserve both  $\text{NO}_3^-$  and  $\text{NO}_2^-$  concentrations as well as their  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values, a view that is supported by the stability of  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  of  $\text{NO}_3^- + \text{NO}_2^-$  measurements in frozen samples over time (e.g., Smart et al., 2015; Kemeny et al., 2016; this study).

Because of the small  $\text{NO}_2^-$  contribution to the  $\text{NO}_3^- + \text{NO}_2^-$  pool ( $<1\%$ ) in the Southern Ocean, prior studies assumed  $\text{NO}_2^-$  to have a negligible effect on  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  (DiFiore et al., 2009). However, applying  $\text{NO}_2^-$  removal to Southern Ocean surface mixed layer samples has shown that  $\text{NO}_2^-$  can have a significant effect on  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$ , 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  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  is higher than  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  by  $\sim 0.7 \pm 0.2\text{‰}$  in the summer surface mixed layer but is not significantly different deeper in the water column (Fig. 5a). Given the small contribution of  $\text{NO}_2^-$  to the  $\text{NO}_3^- + \text{NO}_2^-$  pool (median =  $0.9\%$ ), the  $\delta^{15}\text{N}$  difference implies that  $\text{NO}_2^-$   $\delta^{15}\text{N}$  is extremely low, ranging from  $-90\text{‰}$  to  $-17\text{‰}$  (averaging  $-61 \pm 20\text{‰}$ ) in the mixed layer and increasing with depth toward  $\sim 0\text{‰}$  at the  $T_{\min}$  (Fig. 3).

$\text{NO}_2^-$  is thought to be generated in the subsurface largely as a result of the low-light conditions. Here,  $\text{NO}_2^-$  is produced during the first step of nitrification ( $\text{NH}_4^+ \rightarrow \text{NO}_2^-$ ) and as a result of efflux of  $\text{NO}_2^-$  out of phytoplankton cells due to an imbalance between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction during assimilatory  $\text{NO}_3^-$  uptake.  $\text{NO}_2^-$  is consumed by both the second step of nitrification ( $\text{NO}_2^- \rightarrow \text{NO}_3^-$ ) and  $\text{NO}_2^-$  assimilation by phytoplankton (Ward, 1985; Lomas and Lipschultz, 2006). This combination of processes can yield  $\text{NO}_2^-$  with a  $\delta^{15}\text{N}$  between  $\sim 0\text{‰}$  and  $-35\text{‰}$ , depending on the isotope effects of each process and their relative contributions to  $\text{NH}_4^+$  and  $\text{NO}_2^-$  removal (Fripiat et al., 2015a). We observe  $\text{NO}_2^-$   $\delta^{15}\text{N}$  values that fall roughly within this range in the subsurface (i.e., below the mixed layer), consistent with  $\text{NO}_2^-$  being produced and consumed by the processes outlined above.



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

Most of the stations in both the PAZ and OAZ are found to have low  $\text{NO}_2^-$   $\delta^{15}\text{N}$  in the mixed layer, suggesting that  $\text{NO}_3^-$ – $\text{NO}_2^-$  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 ~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 following their entrainment into it such that they spend a significant 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  $\text{NO}_2^-$  oxidizers and, therefore, the unidirectional oxidation of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (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  $\text{NO}_3^-$  and depleting  $\text{NO}_2^-$  in  $^{15}\text{N}$ . The effective co-occurrence of  $\text{NO}_2^-$  oxidation and  $\text{NO}_3^-$  reduction in the same intracellular (or periplasmic) space could allow for the full expression of the equilibrium isotope effect at the environmental scale. While the biological catalysis of  $\text{NO}_3^-$ – $\text{NO}_2^-$  equilibrium has previously been observed in culture studies (Sundermeyer-Klinger et al., 1984; Friedman et al., 1986; Brunner et al., 2013; Wunderlich et al., 2013), the existence and operation of specific microbial consortia and enzymatic machinery that could explain our observations remains to be demonstrated.

From the perspective of the N atoms in  $\text{NO}_3^- + \text{NO}_2^-$ , interconversion operates as a closed system, with N atoms exchanged between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  without the  $\text{NO}_3^- + \text{NO}_2^-$  pool experiencing any N loss or gain. This means that  $^{15}\text{E}$  associated with the assimilation of  $\text{NO}_3^- + \text{NO}_2^-$  should be unaffected by  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion. However, as interconversion enriches  $\text{NO}_3^-$  in  $^{15}\text{N}$ , the  $\text{NO}_3^-$ -only  $^{15}\text{E}$  will be affected, and will be larger than the  $\text{NO}_3^- + \text{NO}_2^-$   $^{15}\text{E}$  (Table 1; Fig. 6a and b). Consistent with Kemeny et al. (2016), we observe that the  $^{15}\text{E}$  derived from  $\text{NO}_3^-$ -only profiles is higher than the  $^{15}\text{E}$  derived from profiles of  $\text{NO}_3^- + \text{NO}_2^-$  (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  $^{15}\text{E}$  estimated from the  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only data

( $R^2 = 0.01$ ; p-value = 0.63) (Fig. 7a). The  $^{15}\text{E}$  difference has been suggested to reflect the extent of mixed layer  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion at any given location. However, 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 average MLD. This is likely to lead to conditions favorable for  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion. Moreover, the mean ( $\pm 1\text{sd}$ )  $^{15}\text{E}$  difference (for  $\text{NO}_3^- + \text{NO}_2^-$  versus  $\text{NO}_3^-$ ) is similar for stations in the OAZ ( $2.9 \pm 1.3\text{‰}$ ) and the PAZ ( $2.8 \pm 1.9\text{‰}$ ) despite the greater MLD in the OAZ. 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 nitrification (Priscu et al., 1990; Fripiat et al., 2014), and the nitrifiers of this community may also facilitate  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion in the mixed layer.

From the perspective of the O atoms in  $\text{NO}_3^- + \text{NO}_2^-$ , nitrate-nitrite interconversion implies an open system in which both the  $\text{NO}_3^-$ -only and  $\text{NO}_3^- + \text{NO}_2^-$  pools are continuously supplied with O deriving predominantly from water (Kemeny et al., 2016). This is illustrated by comparing  $^{15}\text{E}$  with  $^{18}\text{E}$  for either  $\text{NO}_3^-$ -only or  $\text{NO}_3^- + \text{NO}_2^-$ . The estimates of  $^{18}\text{E}$  tend to fall below the 1:1 relationship with  $^{15}\text{E}$  expected from  $\text{NO}_3^-$  assimilation alone (Fig. 8; Granger et al., 2004, 2010; Karsh et al., 2012). This can be explained by the incorporation of low- $\delta^{18}\text{O}$  O atoms from ambient  $\text{H}_2\text{O}$  into either  $\text{NO}_2^-$  or  $\text{NO}_3^-$  during  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion (Kemeny et al., 2016). This process would propagate the low- $\delta^{18}\text{O}$  anomaly into both the  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $^{18}\text{E}$ , consistent with the similar values of  $^{18}\text{E}$  that we estimate from the  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only data (Table 1; Fig. 6c and 6d). However, the very low concentration ratios of  $\text{NO}_2^-$  to  $\text{NO}_3^-$  may mask a large range in  $\text{NO}_2^-$   $\delta^{18}\text{O}$  (~50‰), which would yield no significant difference ( $\pm 0.2\text{‰}$ ) in  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$ , precluding an assessment of the O isotope systematics of  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion. We expect kinetic isotope effects during the incorporation and removal of O atoms, as well as equilibrium isotope effects between both  $\text{NO}_3^-$  and  $\text{NO}_2^-$  and  $\text{NO}_3^- + \text{NO}_2^-$  and water (e.g., Buchwald et al., 2012).

We conclude that, in the AZ, the N isotopic composition of  $\text{NO}_3^- + \text{NO}_2^-$  is more representative than that of  $\text{NO}_3^-$ -only with regard to the true isotope effect of  $\text{NO}_3^-$  assimilation because  $\text{NO}_3^-$ -only  $^{15}\text{E}$  is altered during the putative  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion due to  $^{15}\text{N}$ -enrichment. The  $^{18}\text{E}$  values estimated from both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only are vulnerable to alteration during interconversion, with O atoms likely being exchanged with water and then redistributed between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in the process.

#### 4.2.2. Isotopic fractionation during nitrate assimilation in Antarctic Surface Waters

The negative correlation between  $[\text{NO}_3^-]$  and both  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  reflects the link between  $\text{NO}_3^-$  consumption and the  $\text{NO}_3^-$  isotopes in the Antarctic Zone (Fig. 6), whereby preferential assimilation of  $^{14}\text{N}$  and  $^{16}\text{O}$  by phytoplankton leaves the residual  $\text{NO}_3^-$  pool enriched in  $^{15}\text{N}$  and  $^{18}\text{O}$  (Sigman et al., 1999a). There is strong seasonality

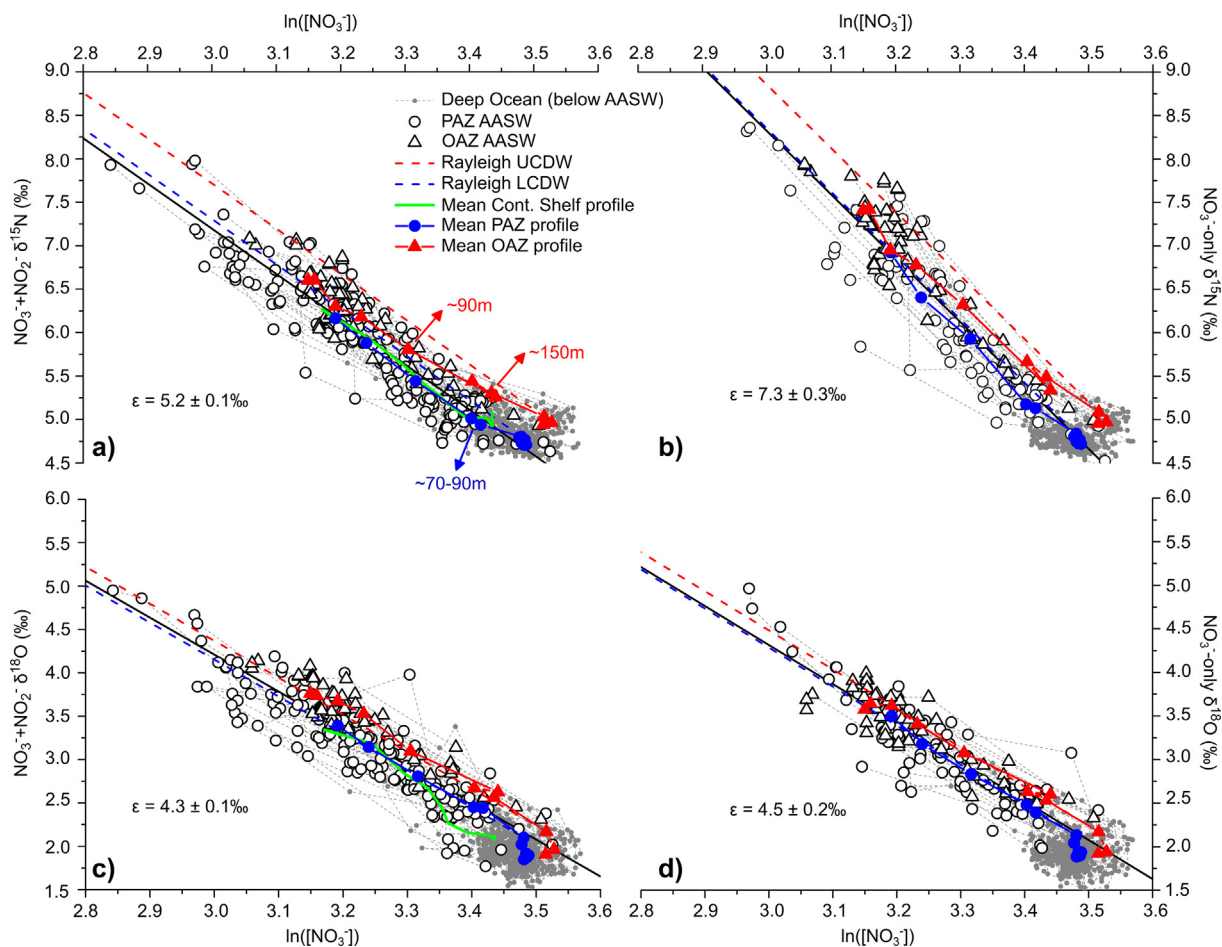


Fig. 6. Relationship between  $\text{NO}_3^- + \text{NO}_2^-$  (a, c) and  $\text{NO}_3^-$ -only (b, d) concentrations and  $\delta^{15}\text{N}$  (a, b) and  $\delta^{18}\text{O}$  (c, d) for AASW (empty black circles and triangles for the PAZ and OAZ, respectively) and deep water (gray circles). Red triangles and blue circles correspond to the mean OAZ and PAZ profiles (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). Regressions for AASW are indicated with the black solid lines (AASW average in Table 1), and Rayleigh fractionation trends (with 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  $\text{NO}_3^- \delta^{15}\text{N}/[\text{NO}_3^-]$  profiles at the more pelagic ‘ $T_{\min}$ ’ PAZ stations. In terms of  $\text{NO}_3^- \delta^{18}\text{O}/[\text{NO}_3^-]$ , the stations near the continental shelf have similar surface values to the pelagic PAZ stations, but with a lower  $\text{NO}_3^-$  concentration deeper in the water column (i.e., below ~140 m). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

associated with both the supply of  $\text{NO}_3^-$  to the mixed layer and consumption by phytoplankton. Primary production and  $\text{NO}_3^-$  assimilation are restricted to the late spring and summer 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 2.4) (DiFiore et al., 2010; Fripiat et al., 2012). Accordingly,  $\text{NO}_3^-$  assimilation in AASW should generate a linear trend in  $\text{NO}_3^- \delta^{15}\text{N}$  vs.  $\ln([\text{NO}_3^-])$  and  $\delta^{18}\text{O}$  vs.  $\ln([\text{NO}_3^-])$  space starting from LCDW and UCDW values in the PAZ and OAZ, respectively (blue and red dashed lines in Fig. 6).

The data generally fall on a single  $\delta^{18}\text{O}/(\ln[\text{NO}_3^-])$  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 similar progressive  $^{18}\text{O}$  enrichment ( $\sim 0.2\text{‰}$ ) in the mean profiles from the deeper layers up to below AASW, despite the lack of a clear decline in  $\text{NO}_3^-$  concentration. This small  $^{18}\text{O}$  enrichment may be caused by the co-occurrence of partial assimilation and subsurface nitrification (Fawcett et al., 2015; Peng et al., 2018), which will cause the  $\delta^{18}\text{O}$  of  $\text{NO}_3^-$  in the upper ocean to increase upwards because the assimilated  $\text{NO}_3^-$  is initially lower in  $\delta^{18}\text{O}$  ( $\sim -3\text{‰}$  to  $-2\text{‰}$ ) than the regenerated  $\text{NO}_3^-$  produced by nitrification (i.e.,  $\sim \text{H}_2\text{O} \delta^{18}\text{O} + 1.1\text{‰}$ ; Sigman et al., 2009a).

The AASW  $\text{NO}_3^- \delta^{15}\text{N}/(\ln[\text{NO}_3^-])$  relationship displays upward concavity that causes  $T_{\min}$  samples to fall below a Rayleigh fractionation trend with CDW as the  $\text{NO}_3^-$  source,

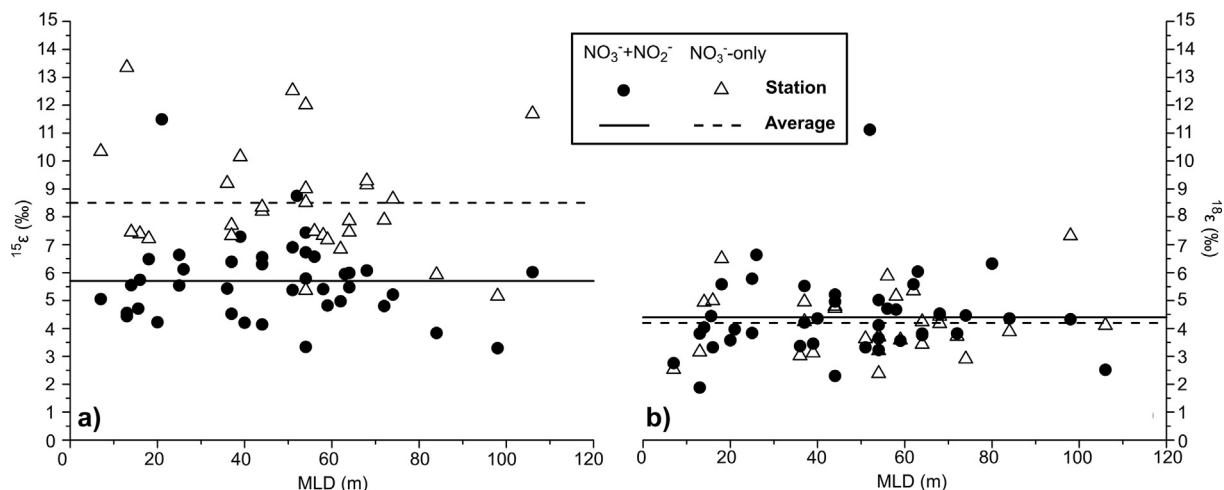


Fig. 7.  $^{15}\epsilon$  (a) and  $^{18}\epsilon$  (b) vs. mixed layer depth (MLD) for  $\text{NO}_3^- + \text{NO}_2^-$  (filled circles) and  $\text{NO}_3^-$ -only (open triangles). The solid line is for the average value of  $\text{NO}_3^- + \text{NO}_2^-$   $^{15}\epsilon$  (5.7‰) and  $^{18}\epsilon$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

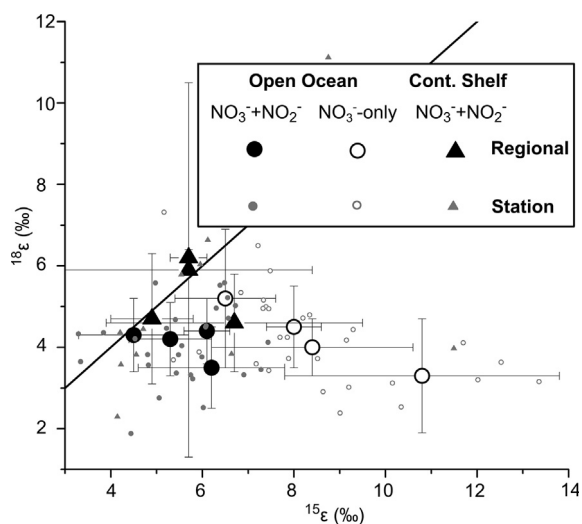


Fig. 8.  $^{15}\epsilon$  vs.  $^{18}\epsilon$  for  $\text{NO}_3^- + \text{NO}_2^-$  (filled symbols) and  $\text{NO}_3^-$ -only (open symbols) (Table 1). Small gray symbols are station averages, while large black symbols with error bars are regional averages. NBP stations near and on the continental shelf are shown with the gray triangles (i.e., no  $\text{NO}_3^-$ -only measurements). The solid line is for  $^{15}\epsilon = ^{18}\epsilon$ .

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  $\text{NO}_3^- \delta^{15}\text{N}/(\ln[\text{NO}_3^-])$  relationship than CDW has been put forward to explain the OAZ  $T_{\min}$   $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  is also observed in the PAZ, arguing that lateral exchange cannot be the only process at play. Remineralization of low- $\delta^{15}\text{N}$  PN represents an alternative hypothesis (Sigman et al., 1999a; Smart et al., 2015). It has been suggested that regenerated

$\text{NO}_3^-$  with a much lower  $\delta^{15}\text{N}$  ( $\sim -5 \pm 5\text{‰}$ ) than expected from  $\text{NO}_3^-$  assimilation is required to explain the  $T_{\min}$  anomaly (Smart et al., 2015). Low suspended PN  $\delta^{15}\text{N}$  has been reported for the Antarctic Zone ( $-4$  to  $-2\text{‰}$ ) (Altabet and Francois, 1994a,b, 2001), with values as low as  $-5\text{‰}$  observed in late summer in the Polar Frontal Zone (Lourey et al., 2003). The low PN  $\delta^{15}\text{N}$  in late summer is likely due to the assimilation of regenerated  $^{14}\text{N}$ -rich ammonium (Altabet, 1988; Fawcett et al., 2011) but requires further investigation. When this low- $\delta^{15}\text{N}$  PN is regenerated to  $\text{NO}_3^-$  upon wintertime deep mixing, it lowers  $\text{NO}_3^- \delta^{15}\text{N}$  throughout the (winter) mixed layer (Smart et al., 2015). A related possibility is that sinking PN is remineralized with net isotopic fractionation as it is exported through the  $T_{\min}$ , generating low- $\delta^{15}\text{N}$   $\text{NH}_4^+$  that is subsequently oxidized to  $\text{NO}_3^-$ ; this possibility will be addressed in a separate manuscript.

Compared to the variations observed in downcore diatom-bound  $\delta^{15}\text{N}$  records (up to  $\sim 4\text{‰}$ ) (Robinson and Sigman, 2008; Studer et al., 2015), the  $\text{NO}_3^- \delta^{15}\text{N}/(\ln[\text{NO}_3^-])$  anomaly is small ( $\sim < 0.5\text{‰}$ ). Nevertheless, it may have repercussions for paleoceanographic reconstructions (Kemeny et al., 2018). Within AASW from the  $T_{\min}$  upward, a strong negative linear correlation is observed for  $\text{NO}_3^-$  isotopes vs.  $\ln([\text{NO}_3^-])$  (p-value  $< 0.001$ ;  $R^2 > 0.76$ ), indicating that the dominant biogeochemical process at play is  $\text{NO}_3^-$  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  $T_{\min}$   $\delta^{15}\text{N}$  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 aver-

age” 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 averaging these estimates for each cruise or regional setting. All estimates were calculated using Eq. (4). The AASW  $^{15}\epsilon$  average ( $\pm 1\text{sd}$ ) is estimated to be  $5.2 \pm 0.1\text{‰}$ , with the regional  $^{15}\epsilon$  averages varying from  $3.2\text{‰}$  to  $5.9\text{‰}$  (Table 1). A low  $^{15}\epsilon$  is reported for Dumont D’Urville Sea and Prydz Bay, both of which are characterized by small amplitudes of  $\text{NO}_3^-$  depletion ( $\sim 1.7 \mu\text{mol l}^{-1}$ ) and  $^{15}\text{N}$ -enrichment ( $\sim 0.3\text{‰}$ ). These amplitudes are relatively close to the analytical precision ( $\pm 2\text{sd}$ ) at the time when the samples from the NBP cruises were measured. Excluding these two areas, the AASW  $^{15}\epsilon$  average ( $\pm 1\text{sd}$ ) is  $5.4 \pm 0.2\text{‰}$ , with the regional  $^{15}\epsilon$  averages varying between  $4.8\text{‰}$  and  $5.9\text{‰}$ . Consistent with the expected effect of  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion, the  $^{15}\epsilon$  estimate is higher and more variable for  $\text{NO}_3^-$ -only, with an AASW  $^{15}\epsilon$  average of  $7.3 \pm 0.3\text{‰}$ , which varies regionally from  $6.2\text{‰}$  to  $8.4\text{‰}$ , 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  $^{18}\epsilon$  that are lower than  $^{15}\epsilon$ , counter to expectations for  $\text{NO}_3^-$  assimilation (Granger et al., 2004, 2010; Rohde et al., 2015). No significant difference is observed between  $\text{NO}_3^- + \text{NO}_2^-$  ( $4.6 \pm 0.1\text{‰}$ ) and  $\text{NO}_3^-$ -only ( $4.5 \pm 0.2\text{‰}$ )  $^{18}\epsilon$  estimates. We interpret both estimates to be artificially low, likely as a result of the  $\text{NO}_3^-$ - $\text{NO}_2^-$  interconversion process.

Compared to the PAZ, the OAZ  $\text{NO}_3^- \delta^{15}\text{N}$  vs.  $\ln([\text{NO}_3^-])$  relationship is indistinguishable in slope but is shifted upward by  $\sim 0.3\text{‰}$  (Fig. 6), reflecting the  $\delta^{15}\text{N}$  difference in the sources of  $\text{NO}_3^-$  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  $T_{\text{min}}$  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  $T_{\text{min}}$ , the isotope effect will be

underestimated. To estimate the maximal bias associated with this decoupling, we first calculate the average  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$  of the  $T_{\text{min}}$  and mixed layer for the PAZ and OAZ. An equal-volume mixture of PAZ and OAZ water is also considered for the  $T_{\text{min}}$ . Using this range of scenarios for  $T_{\text{min}}$  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  $0.2\text{‰}$  ( $\pm 2\text{sd}$ ), only the stations with a vertical isotopic gradient greater than  $0.2\text{‰}$  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 ( $p\text{-value} > 0.32$ ;  $R^2 < 0.04$ ; Fig. 7), nor with sampling date ( $p\text{-value} > 0.34$ ;  $R^2 < 0.03$ , 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  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$ . We use the method of least-squares to solve for these two parameters based on the calculated intercept of each profile in Rayleigh space. The least-square solutions for the more pelagic stations, for which both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  are available (P16S, P18S, IO8S, and SANAE54), are  $29.6 \pm 1.1 \mu\text{mol l}^{-1}$ ,  $5.2 \pm 0.2\text{‰}$ , and  $5.6 \pm 0.3\text{‰}$  for  $\text{NO}_3^-$  concentration,  $\text{NO}_3^- + \text{NO}_2^- \delta^{15}\text{N}$ , and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$ , respectively. These values are indistinguishable from the mean ( $\pm 1\text{sd}$ )  $T_{\text{min}}$  conditions inferred from the observations at these stations:  $29.6 \pm 1.9 \mu\text{mol l}^{-1}$ ,  $5.2 \pm 0.3\text{‰}$ , and  $5.4 \pm 0.4\text{‰}$ . This analysis further confirms that the  $T_{\text{min}}$  conditions are likely to be representative of the source conditions to the summer AZ surface waters.

Table 2

The effect of varying PAZ and OAZ conditions for both the  $T_{\text{min}}$  and mixed layer on the N isotope effect (Eq. (4)). In order to quantify this effect, we calculated the average  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$  for both PAZ and OAZ  $T_{\text{min}}$  (column 1) and ML (column 2). A mixture between PAZ and OAZ (50–50%) is also considered for the  $T_{\text{min}}$ . These different conditions are inserted in Eq. (4) to calculate the N isotope effect for  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$  only (column 3 and 4, respectively).

WW conditions	ML conditions	$\text{NO}_3^- + \text{NO}_2^- \text{ }^{15}\epsilon$ (‰)	$\text{NO}_3^-$ -only $^{15}\epsilon$ (‰)
PAZ	PAZ	5.4	10.4
OAZ	OAZ	5.4	8.0
PAZ	OAZ	6.8	12.6
OAZ	PAZ	4.3	5.9
Mixture PAZ-OAZ	PAZ	4.8	7.8
Mixture PAZ-OAZ	OAZ	6.7	11.1
	Average	5.6	9.3
	sd	1.0	2.5



For the NBP cruises, which are more representative of coastal Antarctic conditions and for which only  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  is available, the least-square solutions are  $23.1 \pm 2.9 \mu\text{mol l}^{-1}$  and  $6.2 \pm 0.4\text{‰}$  for  $\text{NO}_3^-$  concentration and  $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$ , respectively. These values are in agreement with the lower  $T_{\text{max}}$   $\text{NO}_3^-$  concentration ( $25.8 \pm 3.0 \mu\text{mol l}^{-1}$ ) and higher  $T_{\text{max}}$   $\text{NO}_3^- + \text{NO}_2^-$   $\delta^{15}\text{N}$  ( $5.9 \pm 0.6\text{‰}$ ) observed at these stations.

Averaging ( $\pm$ sd) both the regional and station  $^{15}\epsilon$  averages, we estimate a nitrate assimilation isotope effect of  $5.5 \pm 0.6\text{‰}$  for the Antarctic Zone, based on the  $\text{NO}_3^- + \text{NO}_2^-$  measurements (Table 1). An isotope effect of  $7.8 \pm 1.5\text{‰}$  is derived from the  $\text{NO}_3^-$ -only measurements; we interpret this to be artificially high due to the isotopic impacts of  $\text{NO}_3^- - \text{NO}_2^-$  interconversion described above. The relative stability of the isotope effect calculated from  $\text{NO}_3^- + \text{NO}_2^-$  measurements, as well as the absence of any correlation with MLD (Fig. 7a) are in disagreement with previous studies. Using a compilation of  $\text{NO}_3^-$  isotope data from the Australian sector of the Southern Ocean, DiFiore et al. (2010) observed a poleward decrease in  $^{15}\epsilon$  from the SAZ to the PAZ (i.e., from  $\sim 9\text{‰}$  to  $5\text{‰}$ ). However, except for the PAZ dataset, the OAZ-SA Z 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  $\text{NO}_3^-$  assimilation and cellular  $\text{NO}_3^-$  efflux to light availability (Needoba et al., 2004). Given our new understanding of the role of  $\text{NO}_2^-$ , we now propose that the higher OAZ  $^{15}\epsilon$  estimates were due to  $\text{NO}_3^- - \text{NO}_2^-$  interconversion, leading to higher apparent values of  $^{15}\epsilon$  driven by the subsequent loss of  $\text{NO}_2^-$  during acidified storage in the OAZ samples (Table 1; Fig. 6a, b). 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  $^{15}\epsilon$  simplifies paleoceanographic interpretation of N isotope data in terms of the degree of  $\text{NO}_3^-$  consumption (Robinson and Sigman, 2008).

#### 4.3. Comparison of nitrate-based $^{15}\epsilon$ estimates with sinking PN $\delta^{15}\text{N}$ data

Based on the observed Rayleigh fractionation trend in the AASW  $\text{NO}_3^- + \text{NO}_2^-$  data and the N isotope effect of  $\text{NO}_3^-$  consumption that we estimate from them (Table 1), we infer an exported PN  $\delta^{15}\text{N}$  of  $\sim 0.4\text{‰}$  ( $-0.2\text{‰}$  to  $1.3\text{‰}$ ), which is in the range of the annual average sinking PN  $\delta^{15}\text{N}$  from sediment traps in the Antarctic Zone,  $-0.1\text{‰}$  to  $1.7\text{‰}$  (Altabet and Francois, 2001). In contrast, if  $\text{NO}_3^-$ -only data are used to estimate  $^{15}\epsilon$ , then the resulting export production  $\delta^{15}\text{N}$  is too low ( $-4.4\text{‰}$  to  $-0.2\text{‰}$ ) given the constraints offered by the sediment trap data. This supports our argument that the  $\text{NO}_3^- + \text{NO}_2^-$  pool is more representative of the N available for consumption and is thus the appropriate substrate pool from which to estimate  $^{15}\epsilon$ . This is confirmed more broadly using sediment trap data from both the OAZ and the Polar Frontal Zone (Altabet and

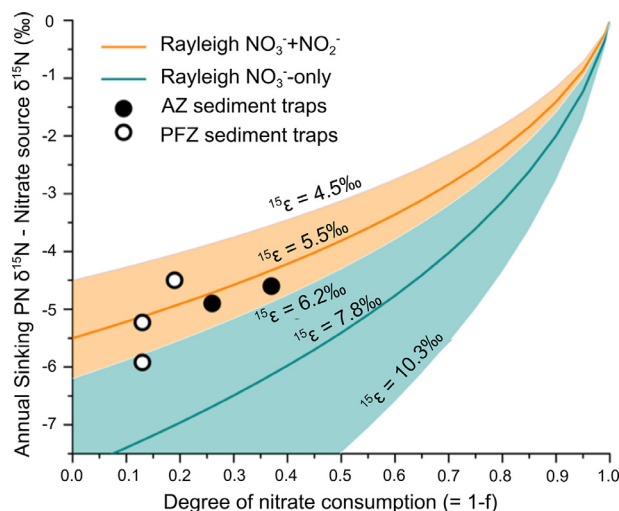


Fig. 9. Degree of nitrate consumption ( $=1 - f$ ) vs the difference between annual sinking PN and  $\text{NO}_3^-$ -source  $\delta^{15}\text{N}$ . Black circles show the annual average sinking flux for the Antarctic Zone and white circles for the Polar Front Zone (Altabet and Francois, 2001; Lourey et al., 2003). The curved lines and shading denote AASW Rayleigh fractionation trends with varying isotope effects. The isotope effects for  $\text{NO}_3^- + \text{NO}_2^-$  (mean =  $5.5\text{‰}$ , varying from  $4.5$  to  $6.2\text{‰}$ ) are shown in orange and for  $\text{NO}_3^-$ -only in blue-green (mean =  $7.8\text{‰}$ , varying from  $6.2$  to  $10.3\text{‰}$ ) (Table 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Francois, 2001; Lourey et al., 2003). To account for the effect of varying source  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$ , the difference between annual average sinking PN  $\delta^{15}\text{N}$  and initial  $\delta^{15}\text{N}$  of  $\text{NO}_3^-$  is plotted against the degree of nitrate consumption ( $=1 - f$ ) (Fig. 9) (Altabet and Francois, 2001). Estimates of the degree of nitrate consumption are based on winter to summer seasonal  $\text{NO}_3^-$  consumption, and initial  $\text{NO}_3^-$   $\delta^{15}\text{N}$  on winter observations in the mixed layer at the corresponding sediment trap locations. The values for both degree of nitrate consumption and initial  $\text{NO}_3^-$   $\delta^{15}\text{N}$  are given in Altabet and Francois (2001) and Lourey et al. (2003). Annual average sinking PN  $\delta^{15}\text{N}$  falls on the AASW Rayleigh fractionation trend for the accumulated product if the  $^{15}\epsilon$  estimated from the  $\text{NO}_3^- + \text{NO}_2^-$  pool is used (Fig. 9); in contrast, the trend underestimates sinking PN  $\delta^{15}\text{N}$  if the  $^{15}\epsilon$  estimated from the  $\text{NO}_3^-$ -only data is used. This analysis confirms a tight connection between the degree of  $\text{NO}_3^-$  consumption and the  $\delta^{15}\text{N}$  of both  $\text{NO}_3^- + \text{NO}_2^-$  and export production (Sigman et al., 1999a; Altabet and Francois, 2001).

#### 4.4. Paleoceanographic implications

The strong and consistent relationship between  $\text{NO}_3^- + \text{NO}_2^-$  concentration and  $\delta^{15}\text{N}$ , which yields a relatively constant isotope effect in the lower range of previous estimates ( $\sim 5.5 \pm 0.6\text{‰}$ ; from  $4.5\text{‰}$  to  $6.2\text{‰}$ , Table 1), has implications for reconstructions of the past degree of  $\text{NO}_3^-$  consumption and thus the  $\text{NO}_3^-$  concentration in Antarctic Zone surface waters (Table 3). The  $\delta^{15}\text{N}$  of coral- and

Table 3

Surface  $\text{NO}_3^-$  utilization during the last glacial maximum inferred with both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$  only  $\delta^{15}\text{N}$  and  $^{15}\epsilon$ , as well as variations in the parameters being used to estimate this values.

	$\text{NO}_3^- + \text{NO}_2^-$	$\text{NO}_3^-$ -only
N isotope effect <sup>a</sup>	4.5–6.7‰	6.2–10.8‰
Source $\text{NO}_3^-$ $\delta^{15}\text{N}^b$	5.0–6.2‰	5.1–6.0‰
Source $\text{NO}_3^-$ concentration <sup>b</sup>	24–32 $\mu\text{mol l}^{-1}$	24–32 $\mu\text{mol l}^{-1}$
Summer surface $\text{NO}_3^-$ concentration <sup>c</sup>	~24 $\mu\text{mol l}^{-1}$	~24 $\mu\text{mol l}^{-1}$
Modern surface $\text{NO}_3^-$ consumption <sup>d</sup>	~20%	~20%
Core-top diatom-bound $\delta^{15}\text{N}^e$	2.2‰	2.2‰
Expected sinking PN $\delta^{15}\text{N}^f$	0.4‰	–2.1‰
Isotopic offset <sup>g</sup>	1.8‰	4.3‰
LGM diatom-bound $\delta^{15}\text{N}^e$	6.2‰	6.2‰
LGM Expected sinking PN $\delta^{15}\text{N}^h$	4.4‰	1.9‰
LGM surface $\text{NO}_3^-$ consumption	79–97%	53–87%
LGM summer surface $\text{NO}_3^-$ concentration	0.7–6.7 $\mu\text{mol l}^{-1}$	3.1–15.4 $\mu\text{mol l}^{-1}$

<sup>a</sup> Range given by the regional and station  $^{15}\epsilon$  averages (Table 1).

<sup>b</sup> Range given by the T<sub>min</sub> average for each individual cruises.

<sup>c</sup> Average for the Antarctic Zone summer mixed layer.

<sup>d</sup> Based on the average for the Antarctic Zone T<sub>min</sub> and summer mixed layer.

<sup>e</sup> Based on Studer et al. (2015).

<sup>f</sup> Estimated based on the AASW Rayleigh fractionation trends.

<sup>g</sup> Difference between core-top diatom-bound and expected sinking PN  $\delta^{15}\text{N}$ .

<sup>h</sup> LGM diatom-bound  $\delta^{15}\text{N}$  minus the isotopic offset.

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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$ , 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  $\delta^{15}\text{N}$  in the modern Antarctic Zone (~0.4‰) and core-top diatom-bound  $\delta^{15}\text{N}$  (~2.2‰; Studer et al., 2015), and apply this correction to the down-core diatom-bound  $\delta^{15}\text{N}$  record (Table 3). This isotopic offset is relatively consistent with the mean  $\delta^{15}\text{N}$  offset reported for sinking PN and core-top diatom-bound  $\delta^{15}\text{N}$  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 ( $2.6 \pm 2.5\%$ ) (Morales et al., 2014). A similar offset (~3‰) was also recovered in a frustule cleaning study of diatoms grown for aquaculture (Morales et al., 2013), while a culture study of various species of diatoms yielded a range of offsets (Horn et al., 2011a). The higher isotope effect range suggested by  $\text{NO}_3^-$ -only measurements predicts lower expected sinking PN  $\delta^{15}\text{N}$  (–2.1‰) and thus requires a larger isotopic offset (4.3‰) to simulate the core-top diatom-bound  $\delta^{15}\text{N}$ . Changes in diatom assemblage between glacial and inter-

glacial periods are relatively modest in the investigated record (Studer et al., 2015), implying little effect from inter-species variability on the frustule-biomass isotopic offset that may be important in other down-core records from the Atlantic sector of the Antarctic Zone (Jacot des Combes et al., 2008; Horn et al., 2011b). This is further supported by assemblage-specific measurements of diatom-bound  $\delta^{15}\text{N}$  through this record (Studer et al., 2015). However, the controls on the frustule-biomass isotopic offset remain unclear, requiring further study.

Taking into account the variability encountered in the modern Antarctic Zone for the source  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and isotope effect, we infer that the degree of  $\text{NO}_3^-$  consumption increased by ~60–80% during ice ages (Fig. 10; Table 3). The higher isotope effect range estimated from  $\text{NO}_3^-$ -only and acidified samples suggests a weaker increase (~30–65%). Our study suggests that the higher isotope effects implied by the  $\text{NO}_3^-$ -only and acidified samples are likely an artifact derived from mixed layer  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion, which argues for lower ice age Antarctic surface  $\text{NO}_3^-$  concentrations (<7  $\mu\text{mol l}^{-1}$ ) than would have been calculated using the previous best estimates for  $^{15}\epsilon$  in the Antarctic Zone (<15  $\mu\text{mol l}^{-1}$ ; Table 3). A separate issue is the suitability of the Rayleigh model for simulating ice age Antarctic conditions; this is addressed elsewhere (Kemeny et al., 2018).

#### 4.5. Isotope effects from different oceanic regions

If the isotope effect estimates from  $\text{NO}_3^-$ -only data in the high-latitude regions are discarded, our estimate for the isotope effect of nitrate assimilation in the Antarctic Zone ( $5.5 \pm 0.6\%$ ) is similar to estimates based on the  $\text{NO}_3^-$  concentration/ $\delta^{15}\text{N}$  relationship in other oceanic regions (Table 4), particularly for nutrient-replete environments (4.5–6.7‰).

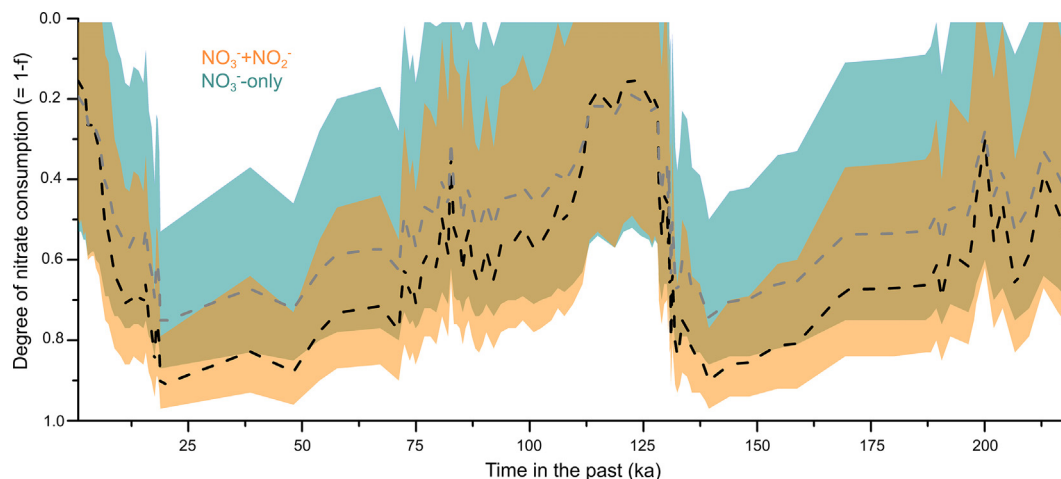


Fig. 10. Past degree of  $\text{NO}_3^-$  consumption ( $=1 - f$ ) inferred from the AASW Rayleigh fraction trends (Fig. 6) for  $\text{NO}_3^- + \text{NO}_2^-$  (orange) and  $\text{NO}_3^-$ -only (green-blue) and a diatom-bound  $\delta^{15}\text{N}$  record from the Pacific sector of the Antarctic Zone, corrected for a constant isotopic offset between diatom biomass and frustule-bound N (Studer et al., 2015). The isotopic offset is taken from the difference between core-top diatom-bound  $\delta^{15}\text{N}$  and the expected export production  $\delta^{15}\text{N}$ , inferred from the AASW Rayleigh accumulated product (Table 3). The source  $\text{NO}_3^-$   $\delta^{15}\text{N}$  ( $T_{\min}$ ) varies from 5.0 to 6.2‰ in the case of  $\text{NO}_3^- + \text{NO}_2^-$  and from 5.1 to 6.0‰ for  $\text{NO}_3^-$ -only. The  $^{15}\epsilon$  varies from 4.5 to 6.7‰ in the case of  $\text{NO}_3^- + \text{NO}_2^-$  and from 6.2 to 10.8‰ for  $\text{NO}_3^-$ -only (Table 3). The dashed black and gray lines represent the mean fractional  $\text{NO}_3^-$  depletion implied by the  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only isotope effects, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4

Estimates of the isotope effects in different oceanic regions based on the relationship between  $\text{NO}_3^-$  concentration and  $\delta^{15}\text{N}$ .

	$\text{NO}_3^- + \text{NO}_2^-$ $^{15}\epsilon$ (‰)	$\text{NO}_3^-$ -only $^{15}\epsilon$ (‰)	Study
<i>Nutrient-replete regions</i>			
Antarctic Zone	4.5–6.1	6.7–10.8	This study
Subantarctic Zone		8.0–9.0 <sup>a</sup>	DiFiore et al. (2006)
Subarctic Pacific	4.7–5.5		Brunelle (2009)
Subarctic Pacific		5.8–6.9	Wu et al. (1997)
Subarctic Pacific		9.1 <sup>a</sup>	Altabet and Francois (1994b)
Equatorial Pacific	5.2	4.5–6.7	Altabet (2001) and Rafter and Sigman (2016)
California Current		4.9–5.2 <sup>a</sup>	Altabet et al. (1999)
Subarctic North Atlantic	5.2–5.3	5.1–5.2	Peng et al. (2018)
<i>Nutrient-depleted regions</i>			
Sargasso Sea	–1.2 to 2.4	2.2–5.0	Fawcett et al. (2015)
Eastern North Atlantic	2.7	3.5	Peng et al. (2018)

<sup>a</sup> Acidified samples only.

Large variations in the MLD in high-latitude regions may provide a mechanism by which subsurface communities of nitrifying microorganisms are entrained into the mixed layer, leading to conditions favorable for  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion and thus a higher  $\text{NO}_3^-$ -only  $^{15}\epsilon$  than expected from  $\text{NO}_3^-$  assimilation alone. In the Subantarctic Zone, this is supported by new measurements showing  $\text{NO}_2^-$   $\delta^{15}\text{N}$  as low as  $-52 \pm 11\text{‰}$  in the mixed layer of the GOSHIP IO8S and P18S sections (data not shown). In the subarctic Pacific, this hypothesis is supported by much lower  $\text{NO}_3^- + \text{NO}_2^-$   $^{15}\epsilon$  (4.7–5.5‰) (Brunelle, 2009) than for  $\text{NO}_3^-$ -only  $^{15}\epsilon$  (5.8–9.1‰) (Altabet and Francois, 1994b; Wu et al., 1997).

In lower latitude regions,  $\text{NO}_3^-$ -only  $^{15}\epsilon$  appears to be more representative than  $\text{NO}_3^- + \text{NO}_2^-$   $^{15}\epsilon$  of the ‘true’ isotope effect for  $\text{NO}_3^-$  assimilation (Table 3) (Fawcett et al., 2015; Rafter and Sigman, 2016), in which case the former

could be compared with  $^{15}\epsilon$  estimates from acidified samples in similar environments (Altabet et al., 1999). The evidence in favor of this view is that (1)  $\text{NO}_3^-$ -only (rather than  $\text{NO}_3^- + \text{NO}_2^-$ )  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  increase in unison from below the euphotic zone toward the surface, in agreement with  $\text{NO}_3^-$  assimilation, and (2)  $\text{NO}_2^-$   $\delta^{15}\text{N}$  is higher than in AASW and thus less clearly affected by  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion (Fawcett et al., 2015; Rafter and Sigman, 2016; Peng et al., 2018). The implication is that, rather than being strongly influenced by  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion, the  $\delta^{15}\text{N}$  of  $\text{NO}_2^-$  at the lower latitude regions is dominantly altered by other  $\text{NO}_2^-$ -oxidizing and -reducing processes. In nutrient-depleted regions, the  $^{15}\epsilon$  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  $[\text{NO}_3^-]$ , there may be a reduction in the  $^{15}\epsilon$  of a given phytoplankton strain (Granger et al., 2004).

The subarctic North Atlantic is characterized by little difference in  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  between  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only (Peng et al., 2018), yielding  $^{15}\epsilon$  estimates of 5.2–5.3‰ and 5.1–5.2‰, respectively. In this environment,  $\text{NO}_2^-$  dynamics thus appear to be relatively unimportant in the isotope dynamics.  $[\text{NO}_2^-]$  is not at appreciably lower concentration through the upper water column than in the Antarctic (Peng et al., 2018). Thus, in the subarctic North Atlantic,  $\text{NO}_2^-$   $\delta^{15}\text{N}$  must be far less distinct from  $\text{NO}_3^-$ . 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 isotopic 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 ( $\sim 1$ –20‰) and the expected variations induced by changing  $\text{NO}_3^-$  metabolism (Montoya and McCarthy, 1995; Granger et al., 2004, 2010; Needoba et al., 2004; Karsh et al., 2012, 2014).  $\text{NO}_3^-$  assimilation has been conceptualized as a stepwise process, in which  $\text{NO}_3^-$  is actively taken up through the membrane into the cell (i.e.,  $\text{NO}_3^-$  uptake,  $^{15}\epsilon_{\text{upt}} \sim 2\%$ ; Karsh et al., 2014). Once inside the cell,  $\text{NO}_3^-$  can either be reduced by the nitrate reductase enzyme, which is thought to be the dominant fractionating step ( $^{15}\epsilon_{\text{NR}} \sim 26.6\%$ ) or released back into the surrounding water with the isotope effect of  $\text{NO}_3^-$  efflux ( $^{15}\epsilon_{\text{eff}} \sim 1.2\%$ ) (Karsh et al., 2012, 2014). Any imbalance between  $\text{NO}_3^-$  uptake and intracellular reduction allows  $^{15}\text{N}$ -enriched  $\text{NO}_3^-$ , which is accumulated inside the cell (e.g., in the vacuole), to be released via  $\text{NO}_3^-$  efflux, transmitting the  $^{15}\text{N}$ -enrichment to seawater  $\text{NO}_3^-$  (Needoba et al., 2004). Given the enzyme-level isotope effects available in the literature, the organism-level isotope effect lies between  $\sim 2\%$  ( $^{15}\epsilon_{\text{upt}}$ ) and  $\sim 25.8\%$  ( $^{15}\epsilon_{\text{upt}} + ^{15}\epsilon_{\text{NR}} - ^{15}\epsilon_{\text{eff}}$ ) for an efflux/uptake ratio of 0 and 1, respectively (Karsh et al., 2012, 2014). An isotope effect between 4.5‰ and 6.7‰ argues 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  $\text{NO}_3^-$  consumption and therefore a succession of different phytoplankton assemblages and  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$  assimilation in the modern Antarctic Zone

( $5.5 \pm 0.6\%$ ), representative of the full seasonal  $\text{NO}_3^-$  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  $\text{NO}_3^-$  consumption and the  $\delta^{15}\text{N}$  of both the  $\text{NO}_3^-$  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  $\text{NO}_3^-$  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  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion in the mixed layer. Although the systematics of  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion are relatively straightforward for the N isotopes, operating as a closed system where N atoms are exchanged between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  and expressing a large equilibrium N isotope effect, the systematics applicable to the O isotopes remain unknown. No clear difference is reported between  $\text{NO}_3^-$ – $\text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$ . However, the small contribution ( $0.7 \pm 0.2\%$ ) of  $\text{NO}_2^-$  to the  $\text{NO}_3^- + \text{NO}_2^-$  pool may mask large differences in  $\text{NO}_2^-$   $\delta^{18}\text{O}$  ( $\sim 50\%$ ) that yield no significant differences ( $\pm 0.2\%$ ) in  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{18}\text{O}$ . Kinetic isotope effects during the incorporation and removal of O atoms are expected, as well as equilibrium isotope effects between  $\text{NO}_3^-$  and  $\text{NO}_2^-$  and between  $\text{NO}_3^- + \text{NO}_2^-$  and water. However, full elucidation of these systematics requires measurement of the  $\delta^{18}\text{O}$  of  $\text{NO}_2^-$  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  $^{15}\epsilon$  for nitrate assimilation, as observed in culture experiments (Needoba et al., 2004). However, new measurements of both  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{NO}_3^-$ -only  $\delta^{15}\text{N}$  on the GOSHIP IO8S and P18S sections indicate that  $\text{NO}_3^-$ – $\text{NO}_2^-$  interconversion also occurs in the SAZ mixed layer, with  $\text{NO}_2^-$   $\delta^{15}\text{N}$  estimated to be as low as  $-52 \pm 11\%$  (data not shown). Thus, artifacts related to  $\text{NO}_2^-$  loss during storage have likely also raised the measured  $\text{NO}_3^-$   $\delta^{15}\text{N}$  and thus the estimates of  $^{15}\epsilon$  in this zone of the Southern Ocean as well. A model taking into account the  $\text{NO}_3^-$  supplies from both the south and the underlying SAZ thermocline is required to tackle this question.

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## APPENDIX A. SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gca.2018.12.003>.

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