Global Biogeochemical Cycles

Supporting Information for

Enzyme-level Interconversion of Nitrate and Nitrite in the Fall Mixed Layer of the Antarctic Ocean

P.C. Kemeny[a,b]*, M. A. Weigand[a], R. Zhang[c], B.R. Carter[d], K.L. Karsh[e], S.E. Fawcett[f], D.M. Sigman[a]

[a] Department of Geosciences, Princeton University, Princeton, New Jersey, USA

[b] Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, USA

[c] College of Ocean and Earth Sciences, Xiamen University, Xiamen, China

[d] Joint Institute for the Study of the Atmosphere and Ocean, University of Washington,, Seattle, Washington, USA

[e] Antarctic Climate & Ecosystems Cooperative Research Centre, University of Tasmania, Hobart, Tasmania, Australia

[f] Department of Oceanography, University of Cape Town, Rondebosch, South Africa

*pkemeny@princeton.edu

Contents of this file

Introduction

This supporting information provides additional context for the analyses reported in the main text. *S1* is a discussion of the possible isotopic impacts of brine loss, *S2* presents a figure showing the impacts of NO_2^- removal on measured values of $\delta^{15}N$ and $\delta^{18}O$, *S3* presents the derivation of isotope effects for each station, and *S4* considers the impacts of NO_3^--NO_2^- interconversion on the $\delta^{18}O$ of NO_3^- and NO_2^-.

S1: Isotopic Impacts of Brine Loss

Results from the first two mass spectrometer runs revealed that certain samples had NO_3^- concentrations significantly lower than reported shipboard values. Subsequent refractometer testing found that these samples also had reduced salinity, with measured-to-reported salinity and NO_3^- ratios falling close to a 1:1 line. One possible explanation for these observations is that captured phytoplankton or bacteria continued to use NO_3^- after sample collection. This scenario would require that samples remain unfrozen and, at least in the case of phytoplankton assimilation, be exposed to light. The 1:1 relationship of NO_3^- loss and salt loss does not support post-collection NO_3^- assimilation, however, as this would preferentially consume NO_3^- relative to salt. A more likely explanation is that the samples underwent brine loss sometime between collection and analysis. When seawater freezes, the salt is excluded from the forming crystalline ice structure and is concentrated into a brine. If a sample container were overfilled or lying on its side during freezing, the brine could have leaked out and left the sample depleted in NO_3^- and salinity relative to shipboard measurements. Indeed, the sample collection report from the cruise details consistent problems correctly filling the sample containers. In at least one profile, sample bottles were overfilled, leakage was observed upon freezing, and the samples were discarded.

Comparing $\delta^{15}N$ and $\delta^{18}O$ from deep samples in our southernmost profiles, where we expect the isotopic variability between samples to be very small, allows us to assess the potential isotopic impacts of brine loss. In water from below 150 m there is no clear relationship between the intensity of brine loss and $\delta^{15}N$ or $\delta^{18}O$ in either $NO_3^-+NO_2^-$ or NO_3^--only measurements (figure *S1*). These findings suggest that brine loss is not an

isotopically-fractionating process, a conclusion subsequently tested and confirmed though freezing and brine extraction experiments (V. Luu, personal communication).

S2. Impacts of NO_2^- removal on $\delta^{15}N$ and $\delta^{18}O$

NO_2^--removal consistently increased the measured values of $\delta^{15}N$ and had a minor, inconsistent impact on $\delta^{18}O$ (figure *S2*). The average difference in $\delta^{15}N$ and $\delta^{18}O$ between NO_3^--only and NO_3^-+NO_2^- samples (for all samples with detectable NO_2^- concentrations) was 0.3‰ and 0.0‰, respectively.

S3: Derivation of Isotope Effects

The N and O isotope effects for NO_3^- assimilation, $^{15}\varepsilon$ and $^{18}\varepsilon$, were derived by regressing $\delta^{15}N$ and $\delta^{18}O$ against $\ln(NO_3^-)$ for both NO_3^-+NO_2^- and NO_3^--only data (figure S3). When regressing against $\ln(NO_3^-+NO_2^-)$ instead of $\ln(NO_3^-)$, the derived values of $^{15}\varepsilon$ and $^{18}\varepsilon$ in NO_3^-+NO_2^- samples change by an average of -0.08‰ and -0.06‰, respectively. However, as shown below, these changes are small relative to uncertainty in the derived isotope effects.

Error in the calculated $^{15}\varepsilon$ and $^{18}\varepsilon$ for each station was estimated using a Monte Carlo simulation of the linear regression. For each sample from the surface through the core of the T_{min} layer in a given profile, NO_3^- concentration, $\delta^{15}N$, and $\delta^{18}O$ were independently shifted by terms drawn randomly from normal distributions with characteristic standard deviations. For NO_3^- concentration, the normal distribution had a standard deviation of 0.21 μmol/kg, as reported from shipboard measurements. For the $\delta^{15}N$ and $\delta^{18}O$ adjustments, the normal distributions had standard deviations equal to the

pooled standard deviation of repeat measurements. For $\delta^{15}N$, pooled standard deviation was 0.05‰ in untreated samples and 0.04‰ in treated samples. For $\delta^{18}O$, pooled standard deviation was 0.14‰ in both treated and untreated samples. We then took a linear regression through the shifted data and repeated this process 10,000 times. Here we report the standard deviation of the resultant set of possible regression slopes as the uncertainty in the $^{15}\varepsilon$ and $^{18}\varepsilon$ of each profile. For $NO_3^-+NO_2^-$ data, the mean 1σ uncertainty in $^{15}\varepsilon$ is 0.4‰ and in $^{18}\varepsilon$ is 0.8‰, regardless of whether the concentration used in the Monte Carlo simulation is $NO_3^-+NO_2^-$ or only NO_3^-. For NO_3^--only data, the mean 1σ uncertainty in $^{15}\varepsilon$ is 0.5‰ and in $^{18}\varepsilon$ is 0.8‰. Lastly, the uncertainty in the difference between two isotopes effects is reported as the square root of the sum of the squares of the two constituent uncertainties.

S4: Oxygen Isotope Impacts of the Proposed Interconversion

Buchwald et al. [2012] use ^{18}O-labeled H_2O in both field cultures and mixed co-cultures of ammonia-oxidizing archaea, ammonia-oxidizing bacteria, and nitrite-oxidizing bacteria to calculate that oxygen from H_2O and O_2 is incorporated into NO_2^- with a normal isotope effect of 10-22‰ during the oxidation of NH_4^+, the first step of nitrification. Furthermore, *Buchwald & Casciotti* [2013] show that the incorporation of O atoms from H_2O into NO_2^- can also occur abiotically with a 13-16‰ equilibrium isotope effect that concentrates ^{18}O in NO_2^-. However, the expression of this equilibrium isotope effect depends on the duration of NO_2^- accumulation during nitrification (i.e., the extent of equilibration), which is in turn linked to the degree of coupling of NH_4^+ and NO_2^- oxidation [*Buchwald et al.,* 2012; *Wunderlich et al.,* 2013]. Furthermore, *Buchwald et al.*

[2012] observe that the incorporation of O from H_2O into NO_3^- occurs during the oxidation of NO_2^- to NO_3^- with a normal isotope effect of 1-27‰.

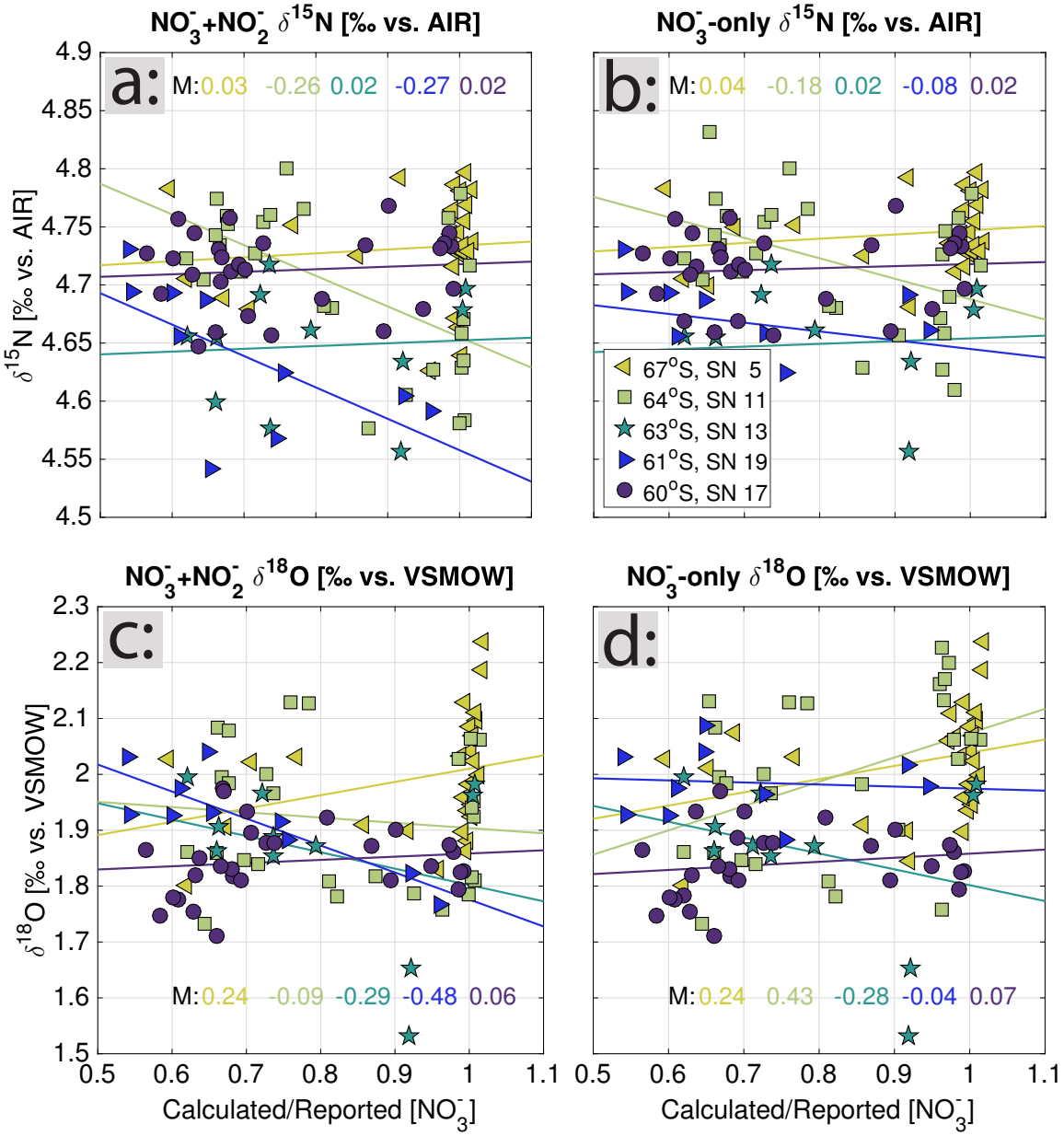

Figure S1: $\delta^{15}N$ and $\delta^{18}O$ of NO_3^-+NO_2^- and NO_3^--only samples from below 150 m plotted against the measured-to-reported NO_3^- concentration ratios in the five southernmost stations. The slopes of regression lines (M) are given in corresponding colored text and show no relationship between the intensity of brine loss and $\delta^{15}N$ or $\delta^{18}O$, strongly suggesting that brine loss is not an isotopically-fractionating process and that it did not compromise our samples.

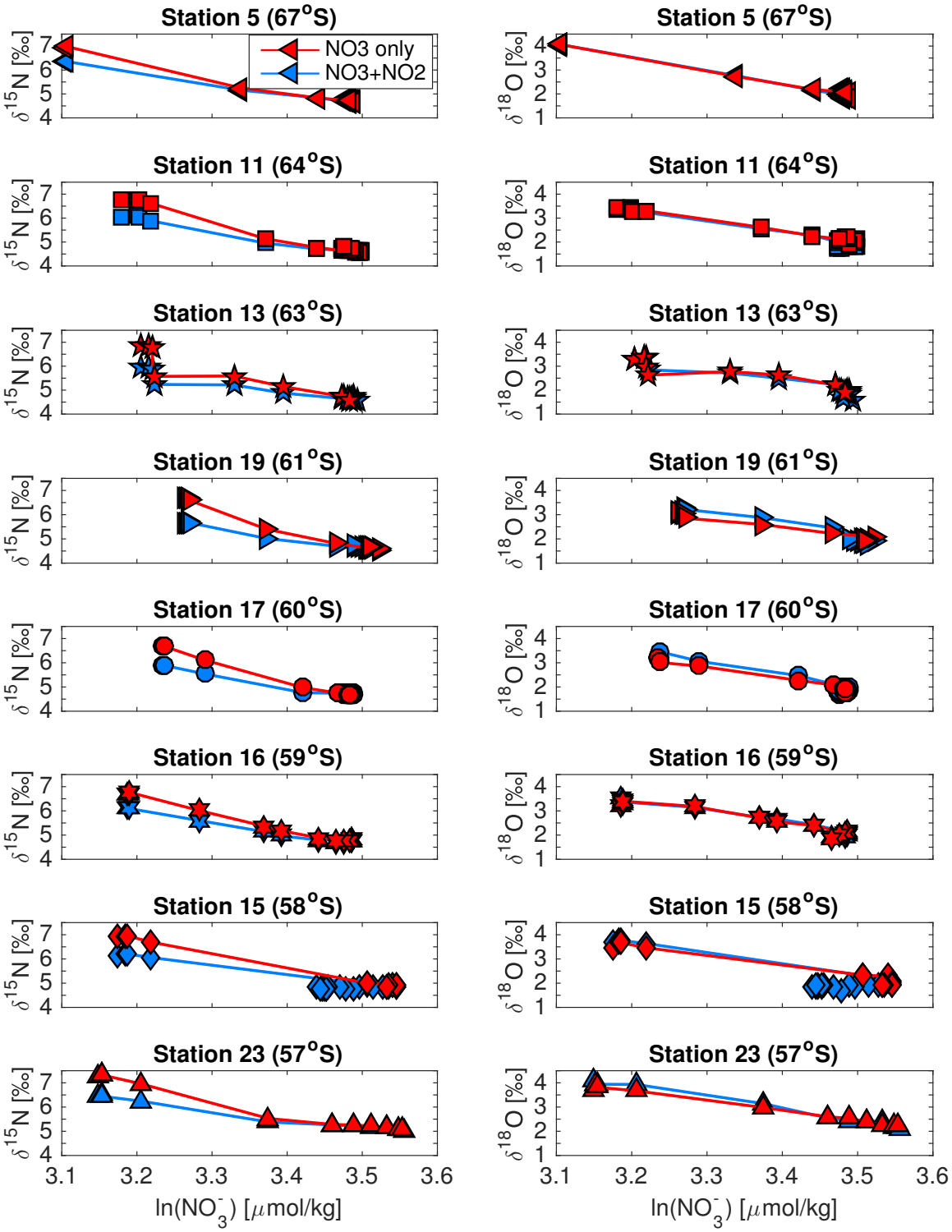

Figure S2: $\delta^{15}N$ and $\delta^{18}O$ in Rayleigh space for $NO_3^-+NO_2^-$ (blue) and NO_3^--only (red) samples. NO_2^- removal increased measured values of $\delta^{15}N$ but only moderately impacted $\delta^{18}O$. All data are plotted against $\ln(NO_3^-)$.

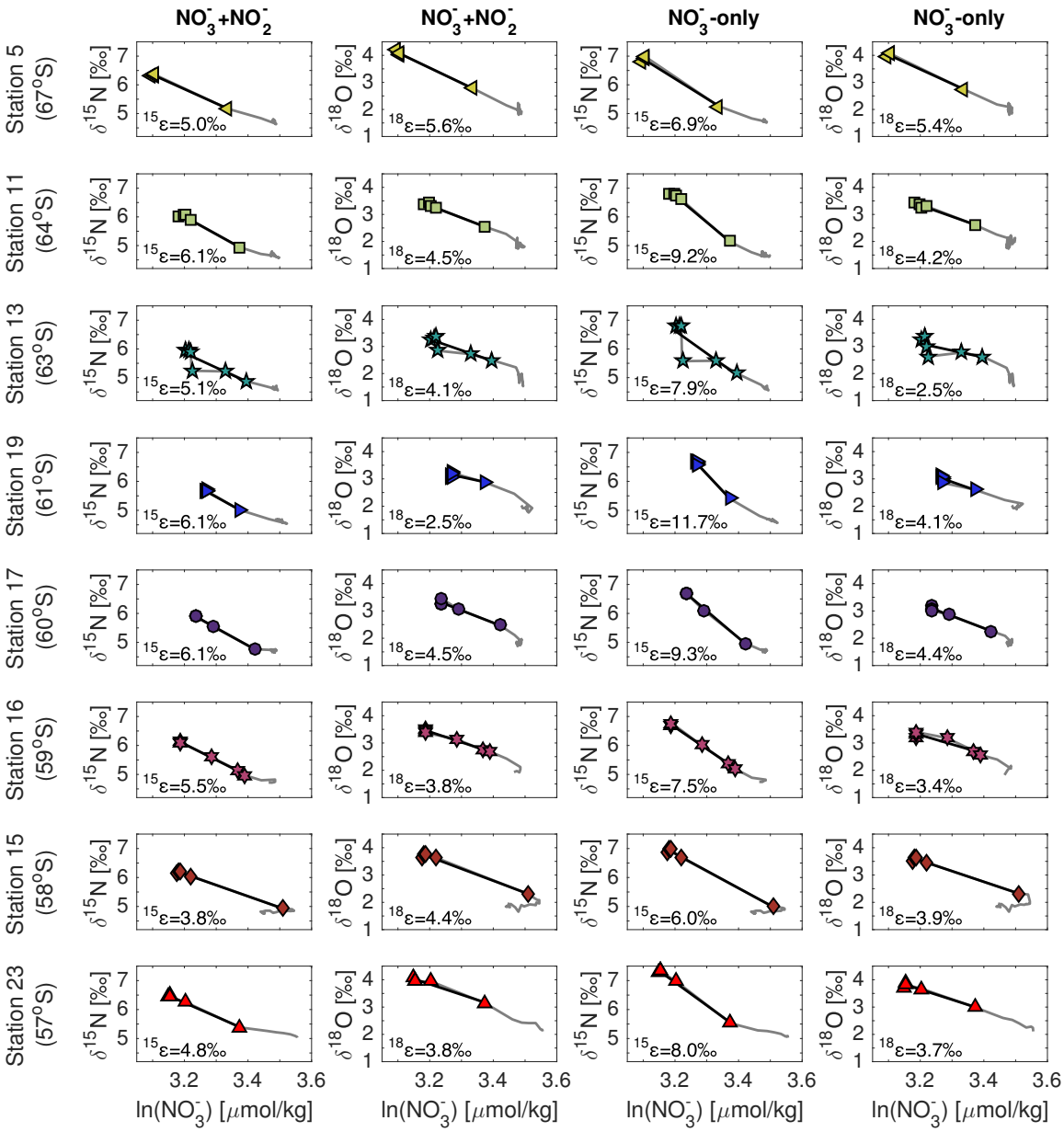

Figure S3: Linear regressions in Rayleigh space on $NO_3^-+NO_2^-$ and NO_3^--only $\delta^{15}N$ and $\delta^{18}O$. The regressed data are shown in color, the regression is in black, and a line connecting all samples for each profile is in grey. The slope of each regression approximates $^{15}\varepsilon$ or $^{18}\varepsilon$ and is indicated on each panel.