

Author's response to reviewers

Reviewer #2

While the authors have done a fair job in considering the specific comments to the earlier version of their manuscript during revision, the general criticism still applies. The introduction has not changed significantly and still lacks clear structure/focus that would allow the non-expert reader to fully comprehend the broader context and aims of the current study.

Unfortunately, this also applies to large parts of the results and discussion sections. Despite being well familiar with N cycling-related OMZ research, I often found it difficult to follow the authors' train of thought or to extract the main implications of their results.

Our response: We did our best to address this concern during revision and re-writing the manuscript at this point would represent major revisions. The two other reviewers and Associate Editor did not raise this concern, and now agree that the manuscript is almost ready for publication. We therefore decided to keep the main structure of the manuscript as it is.

An important result of the current study appears to be rapid nitrite turnover in the Peruvian coastal OMZ and a major role of nitrite oxidation in OMZ N cycling – which basically confirms recently published results from ¹⁵N-labelling experiments in the same area (Kalvelage et al. 2013). Confusingly, however, the authors estimate a turnover time for nitrite of at least 40 days - compared to 12 days based on the coastal OMZ nitrite production rates provided by Kalvelage et al. (2013) – which, to my knowledge, is sufficiently long for significant nitrite-water oxygen exchange.

Our response: We agree with reviewer #2 that since $\delta^{18}\text{O}$ -nitrite is not fully equilibrated with water, the turnover time should be “up to” 40 days, not “at least”. We accordingly corrected the revised version of the manuscript.

The implications of a lower fractionation factor for coastal water column N loss remain equally vague. I am missing a more in-depth discussion on how the global oceanic N (im-)balance changes under the assumption of highly suppressed isotope fraction in oxygen-depleted shelf areas.

Our response: We already discuss this point in the introduction, page 4, lines 19-29 of revised manuscript: “Most recently, a detailed study in a region of extreme N-loss associated with a Peru coastal mode-water eddy confirmed a value for ϵ for N-loss of $\sim 14\%$ (Bourbonnais et al., 2015). Applying such a lowered value to global budgets would bring the global N budget closer to balance.”

We also added a sentence in the discussion (section 4.2, lines 15-18, page 18) to better discuss this point: “As discussed in Bourbonnais et al. (2015), a lower overall ϵ for net N-loss could help resolve any imbalance in the oceanic N-budget, by decreasing the ratio of sedimentary and water-column N-loss necessary to account for the observed $\delta^{15}\text{N}$ of mean ocean NO_3^- .”

Further, I feel the authors' interpretations are in part overly simplified or highly speculative. For example, the role of aerobic ammonium oxidation on nitrite $\delta^{15}\text{N}$ values is not evaluated,

despite this process being a significant source of nitrite at oxygen levels considered here.

Our response: We do consider the effect of aerobic ammonium oxidation with an isotope effect of 13‰, as estimated by Casciotti (2009), on nitrite $\delta^{15}\text{N}$ values. See page 15, lines 19-31.

Also, there are still instances where relevant literature is either not considered/cited, cited incorrectly (not in a technical sense), or irrelevant literature is cited. Last but not least, the manuscript still suffers from a number of spelling and grammatical errors as well as often missing punctuation.

Our response: We thoroughly revised the manuscript for incorrect citations and spelling and grammatical errors (see track change version).

Reviewer #3

The authors have done a good job responding to my comments and this manuscript is almost ready for publication. I only have a couple of minor things.

Regarding their response to my comment about Page 7260 Line 11. In their response the section starting with "In older studies....." and ending witha bit problematic.", this is an interesting point and perhaps should be mentioned in the text.

Our response: We agree with reviewer #3, but as estimates for the ϵ associated with NO_3^- reduction from these older studies (Brandes et al. (1998) and Voss et al. (2001)) are overall comparable to values estimated by Granger et al. (2009), that did remove NO_2^- before $\delta^{15}\text{N}$ - NO_3^- analysis, we prefer not mention this in the introduction as it would distract from our main point and possibly add confusion. Nitrite concentrations in these prior studies were thus probably not significant. It is now well known in the field that it is necessary to remove NO_2^- prior to $\delta^{15}\text{N}$ - NO_3^- analysis and all recent studies have generally done so.

Regarding their response to my comment about Page 7265 Line 20-22. The word "huge" seems a little exaggerated to me. I would say "large" or "significant" or something like that. To me, to be huge it would have to be something like the sulfate background of 25,000 μM .

Our response: We changed "huge" for "large".

Regarding their response to my comment on Page 7275 Line 16-18. I think this is a semantic problem. Definition of dominate: "to rule over, control, or govern; in mathematics, to have terms or components greater in absolute value than the corresponding terms or components of a given series" There is less NO_2^- oxidation than NO_2^- reduction, i.e., the NO_2^- oxidation rate is less than the reduction rate. How can the smaller rate dominate. I suggest they reword this to say something straightforward like the " NO_2^- oxidation is clearly a major pathway because the most of the nitrate reduced is reoxidized".

Our response: We changed the word "dominance" for "effect": "Our results suggest a turnover

time for NO_2^- faster than the equilibration time with water and the effect of NO_2^- oxidation over NO_2^- reduction in these highly productive coastal waters.”

Regarding their response to my comment about Page 7276 Line13-15. They say “we discuss this point on Page 7277, Lines 8 to 10.” The explanation on page 7277 is very brief and basically says the discrepancy is due to anammox. If anammox is the explanation, what are the implications? How much OM has to be oxidized to produce the needed NH_4^+ (or is there another source)? How is the OM oxidized? How is the overall epsilon value affected potential anammox isotopic fractionations?

Our response: Anammox is an autotrophic process, and hence does not involve OM remineralization. NH_4^+ can come from microaerobic OM remineralization or DNRA. Under purely anaerobic conditions, an electron acceptor (other than O_2) for OM oxidation is unclear. This point was also previously raised by reviewer #1 and we consequently added the following clarification, that address this issue, page 17, lines 15-23 of the revised manuscript: “The 4‰ difference in ϵ calculated from changes in $\delta^{15}\text{N}$ of biogenic N_2 vs. $\delta^{15}\text{N}$ of DIN may arise from the contribution of NH_4^+ derived from organic matter to biogenic N_2 via the anammox process. Supporting this hypothesis, NH_4^+ accumulation (5.3-7.5 μM) associated with a relatively low $\delta^{15}\text{N}\text{-NH}_4^+$ of 3.8‰ to 6.1‰ was observed at 125 and 200 m bottom water depths at shallow stations located in the studied area ($\sim 12.3^\circ\text{S}$ and 77.3°W) in January 2013 (unpublished results). A contribution of NH_4^+ from organic material and consumption by anammox could therefore supply comparatively lower $\delta^{15}\text{N}$ to the biogenic N_2 pool, increasing ϵ that must be larger to account for the observed isotopic enrichment.”

1 **Nitrogen cycling in shallow low oxygen coastal waters off**
2 **Peru from nitrite and nitrate nitrogen and oxygen isotopes**

3

4 **Happy Hu^{a,1}, Annie Bourbonnais^{a,1}, Jennifer Larkum¹, Hermann W. Bange², Mark**
5 **A. Altabet¹**

6 [a] These authors contributed equally to this work.

7 [1] {School for Marine Science and Technology, University of Massachusetts Dartmouth, 706
8 South Rodney French Blvd, New Bedford, MA 02744-1221, USA}

9 [2] {GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105
10 Kiel, Germany}

11 Correspondence to: Annie Bourbonnais (abourbonnais@umassd.edu)

12

13 **Abstract**

14 O₂ deficient zones (ODZs) of the world's oceans are important locations for microbial
15 dissimilatory nitrate (NO₃⁻) reduction and subsequent loss of combined nitrogen (N) to biogenic
16 N₂ gas. ODZs are generally coupled to regions of high productivity leading to high rates of N-
17 loss as found in the coastal upwelling region off Peru. Stable N and O isotope ratios can be
18 used as natural tracers of ODZ N-cycling because of distinct kinetic isotope effects associated
19 with microbially-mediated N-cycle transformations. Here we present NO₃⁻ and nitrite (NO₂⁻)
20 stable isotope data from the nearshore upwelling region off Callao, Peru. Subsurface oxygen
21 was generally depleted below about 30 m depth with concentrations less than 10 μM, while
22 NO₂⁻ concentrations were high, ranging from 6 to 10 μM, and NO₃⁻ was in places strongly
23 depleted to near 0 μM. We observed for the first time, a positive linear relationship between
24 NO₂⁻ δ¹⁵N and δ¹⁸O at our coastal stations, analogous to that of NO₃⁻ N and O isotopes during
25 NO₃⁻ uptake and dissimilatory reduction. This relationship is likely the result of rapid NO₂⁻
26 turnover due to higher organic matter flux in these coastal upwelling waters. No such
27 relationship was observed at offshore stations where slower turnover of NO₂⁻ facilitates
28 dominance of isotope exchange with water. We also evaluate the overall isotope fractionation
29 effect for N-loss in this system using several approaches that vary in their underlying

1 assumptions. While there are differences in apparent fractionation factor (ϵ) for N-loss as
2 calculated from the $\delta^{15}\text{N}$ of NO_3^- , dissolved inorganic N, or biogenic N_2 , values for ϵ are
3 generally much lower than previously reported, reaching as low as 6.5%. A possible
4 explanation is the influence of sedimentary N-loss at our inshore stations which incurs highly
5 suppressed isotope fractionation.

6

7 **1 Introduction**

8 Chemically combined nitrogen (N), e.g., nitrate (NO_3^-), is an important phytoplankton nutrient
9 limiting primary productivity and carbon export throughout much of the ocean (e.g. Gruber,
10 2008). The marine nitrogen cycle involves a series of microbial processes, which transfer N
11 between a number of chemical forms. These include N_2 fixation, nitrification (ammonium
12 (NH_4^+) and nitrite (NO_2^-) oxidation), and loss of combined N to N_2 via denitrification and
13 anaerobic ammonium oxidation (anammox). Of particular importance is the global balance
14 between sources of combined N (N_2 fixation) and N-loss processes which ultimately control the
15 combined N content of the ocean and thus its productivity and strength of the biological carbon
16 pump. N-loss typically occurs under nearly anoxic conditions where the first step, dissimilatory
17 NO_3^- reduction to NO_2^- , active at oxygen (O_2) concentrations less than $\sim 25 \mu\text{M}$ (Kalvelage et
18 al., 2011), is used by heterotrophic microbes in lieu of O_2 for respiration. Canonically, the
19 denitrification pathway of successive reduction of NO_3^- , NO_2^- , nitric oxide (NO), and nitrous
20 oxide (N_2O) to N_2 was considered as the dominant pathway for N-loss. However, since the
21 early 2000s, anammox ($\text{NO}_2^- + \text{NH}_4^+ \rightarrow \text{N}_2$) was found to be widespread in the ocean (Kuypers
22 et al. 2003; 2005; Hamersley et al., 2007; Dalsgaard et al., 2012; Kalvelage et al., 2013). While
23 it is still a matter of debate whether denitrification or anammox is the dominant pathways for
24 N-loss in Oxygen Minimum Zones (OMZ's) (e.g., Lam et al., 2009; Ward et al., 2009), both N-
25 loss processes have been shown to strongly vary spatially and temporally and are linked to
26 organic matter export and composition (Kalvelage et al., 2013; Babbitt et al., 2014). It follows
27 that there is still considerable uncertainty as to the controls on N-loss as well as the role for
28 other linking processes such as DNRA (NO_3^- to NH_4^+) and NO_2^- oxidation in the absence of O_2 .
29 Marine N-loss to N_2 occurs predominately in reducing sediments and the O_2 deficient water
30 columns of OMZ's as found in the Arabian Sea and Eastern Tropical North and South Pacific
31 (Ulloa et al., 2012; Lam et al., 2011 and references therein). NO_2^- is an important intermediate
32 during N-loss and generally accumulates at concentrations up to $\sim 10 \mu\text{M}$ in these regions

1 (Codispoti et al., 1986; Casciotti et al., 2013). The depletion of NO_3^- is typically quantified as a
2 dissolved inorganic N ($\text{DIN}=\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) deficit relative to phosphate (PO_4^{3-})
3 assuming Redfield stoichiometry and the accumulation of biogenic N_2 (when measured) is
4 detected as anomalies in N_2/Ar relative to saturation with atmosphere (Richards and Benson,
5 1961; Chang et al., 2010; Bourbonnais et al., 2015).

6 NO_3^- and NO_2^- N and O isotopes represent a useful tool to study N cycle transformations as
7 they respond to in situ processes and integrate over their characteristic time and space scales.
8 Biologically mediated reactions are generally faster for lighter isotopes. For instance, both NO_3^-
9 uptake and dissimilatory NO_3^- reduction produce a strong enrichment in both ^{15}N ($\delta^{15}\text{N} =$
10 $[(^{15}\text{N}/^{14}\text{N}_{\text{sample}})/(^{15}\text{N}/^{14}\text{N}_{\text{standard}})-1]\times 1000$) and ^{18}O ($\delta^{18}\text{O} = [(^{18}\text{O}/^{16}\text{O}_{\text{sample}})/(^{18}\text{O}/^{16}\text{O}_{\text{standard}})-$
11 $1]\times 1000$) in the residual NO_3^- (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001;
12 Granger et al., 2004; 2008; Sigman et al., 2005).

13 Canonical values for the N isotope effect ($\epsilon \approx \delta^{15}\text{N}_{\text{substrate}} - \delta^{15}\text{N}_{\text{product}}$, without significant
14 substrate depletion) associated with microbial NO_3^- reduction during water-column
15 denitrification range from 20 to 30‰ (Brandes et al., 1998; Voss et al., 2001; Granger et al.
16 2008). In contrast, the expression of the isotope effect of sedimentary denitrification is highly
17 suppressed as compared to the water-column (generally <3‰) mostly due to near complete
18 consumption of the porewater NO_3^- and diffusion limitation (Brandes and Devol, 1997;
19 Lehmann et al., 2007; Alkhatib et al., 2012). The $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- are affected in
20 fundamentally different ways during NO_3^- consumption and production processes. The ratio of
21 the ^{15}N and ^{18}O fractionation factors ($^{18}\epsilon:^{15}\epsilon$) during NO_3^- consumption during denitrification or
22 assimilation by phytoplankton in surface waters is close to 1:1 (Casciotti et al., 2002; Granger
23 et al., 2004; 2008). While the $\delta^{15}\text{N}$ of the newly nitrified NO_3^- depends on the $\delta^{15}\text{N}$ of the
24 precursor molecule being nitrified, the O atom is mostly derived from water (with a $\delta^{18}\text{O}$ of ~0
25 ‰), with significant isotopic fractionation associated with O incorporation during NO_2^- and
26 NH_4^+ oxidation (Casciotti, 2002; Buchwald and Casciotti, 2010; Casciotti et al., 2010).
27 Therefore, any deviation from this 1:1 ratio in the field has been interpreted as evidences that
28 NO_3^- regeneration is co-occurring with NO_3^- consumption (Sigman et al., 2005; Casciotti and
29 McIlvin, 2007; Bourbonnais et al., 2009). NO_2^- oxidation is associated with an inverse N
30 isotope effect (Casciotti, 2009), atypical of biogeochemical reactions, and can cause both lower
31 and higher ratios for $^{18}\epsilon:^{15}\epsilon$ compared to pure NO_3^- assimilation or denitrification, depending on

LocalAdmin 1/13/2016 1:40 PM

Deleted: at no

LocalAdmin 1/13/2016 1:43 PM

Deleted: ,

Unknown

Field Code Changed

1 the initial isotopic compositions of the NO_2^- and NO_3^- and the ^{18}O added back (Casciotti et al.,
2 2013).

3 Additional information on N-cycling processes can be obtained from the isotopic composition
4 of NO_2^- . For example, because of its inverse N isotope effect, NO_2^- oxidation results in a lower
5 NO_2^- $\delta^{15}\text{N}$ than initially produced by NH_4^+ oxidation and NO_3^- reduction (Casciotti, 2009;
6 Brunner et al., 2013). Logically, NO_2^- reduction would be expected to produce a positive
7 relationship between $\delta^{15}\text{N}\text{-NO}_2^-$ and $\delta^{18}\text{O}\text{-NO}_2^-$ though there are no quantitative observations in
8 the literature. Analogous to NO_3^- reduction, it also involves enzymatic breakage of the N-O
9 bond. However, O-isotope exchange of NO_2^- with water (as a function of pH and temperature)
10 would reduce the slope of a NO_2^- $\delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ relationship toward zero. NO_2^- turnover time
11 can therefore be assessed from this observed relationship and in situ pH and temperature
12 (Buchwald and Casciotti, 2013)

13 It is still under discussion whether the global ocean N budget is in balance. Current estimates
14 from direct observations and models for N_2 fixation, considered the primary marine N source,
15 range from 110-330 Tg N yr^{-1} (Brandes and Devol, 2002; Gruber, 2004; Deutsch et al., 2007;
16 Eugster and Gruber, 2012; Großkopf et al., 2012). Estimates for major marine N-sinks, i.e.,
17 denitrification and anammox in the water-column of oxygen deficient zones and sediments
18 account for 145-450 Tg N yr^{-1} (Gruber, 2004; Codispoti, 2007; DeVries et al., 2012; Eugster
19 and Gruber, 2012). Large uncertainties are associated with this budget, mainly in constraining
20 the proportion of sedimentary denitrification, which is typically estimated from ocean's N
21 isotope balance and the expressed isotope effects for water-column versus sedimentary NO_3^-
22 reduction during denitrification (e.g. Brandes and Devol, 2002; Altabet, 2007; DeVries et al.,
23 2012). Liu (1979) was first to suggest a lower ϵ for denitrification in the Peru ODZ as
24 compared to the subsequently accepted canonical range for NO_3^- reduction of 20‰ to 30‰
25 (Brandes et al., 1998; Voss et al., 2001; Granger et al., 2008). Ryabenko et al. (2012) provided
26 a more widely distributed set of data in support. Most recently, a detailed study in a region of
27 extreme N-loss associated with a Peru coastal mode-water eddy confirmed a ϵ value for N-loss
28 of ~14‰ (Bourbonnais et al., 2015). Applying such a lowered value to global budgets would
29 bring the global N budget closer to balance.

30 Ryabenko et al. (2012) also suggested that ϵ values were even lower in the shelf region of the
31 Peru ODZ. To investigate further, we present here N and O isotope data for NO_2^- and NO_3^-
32 from shallow coastal waters near Callao, off the coast of Peru. These waters are highly

Annie Bourbonnais 1/11/2016 3:40 PM

Formatted: Font color: Black, German, Do not check spelling or grammar

Annie Bourbonnais 1/11/2016 3:31 PM

Deleted: Casciotti and Buchwald 2012

LocalAdmin 1/13/2016 1:45 PM

Deleted: ,

LocalAdmin 1/13/2016 1:45 PM

Deleted: for ϵ

1 productive as a consequence of active upwelling that is also responsible for shoaling of the
2 oxycline. We determine the relationship between NO_2^- $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ and its implication for
3 NO_2^- cycling in these shallow waters as compared to offshore stations. We finally derive
4 isotope effects for N-loss and infer the likely influence of sedimentary N-loss, which incurs a
5 highly suppressed isotope effect, at our relatively shallow sites.

6

7 **2 Material and Methods**

8 **2.1 Sampling**

9 The R/V Meteor 91 research cruise (M91) to the eastern tropical South Pacific Ocean off Peru
10 in December 2012 was part of the SOPRAN program and the German SFB 754 project. It
11 included an along shore transect of seven inner shelf stations located between 12°S to 14°S that
12 were chosen for this study (Fig. 1). These stations had a maximum depth of 150 m except for
13 station 68 (250 m depth). We additionally sampled deep offshore stations during the M90
14 cruise in November 2012. Samples for NO_3^- and NO_2^- isotopic composition and N_2/Ar ratio
15 were collected using Niskin bottles mounted on a CTD/Rosette system, which was equipped
16 with pressure, temperature, conductivity and oxygen sensors. O_2 concentrations were
17 determined using a Seabird sensor, calibrated using the Winkler method (precision of 0.45
18 $\mu\text{mol L}^{-1}$) with a lower detection limit of 2 $\mu\text{mol L}^{-1}$. Nutrients concentrations were measured
19 on board using standard methods as described in Stramma et al. (2013).

20 **2.2 NO_2^- and NO_3^- isotope analysis**

21 NO_2^- samples were stored in 125 ml HDPE bottles preloaded with 2.25 ml 6 M NaOH to
22 prevent microbial activity as well as alteration of $\delta^{18}\text{O}-\text{NO}_2^-$ by isotope exchange with water
23 (Casciotti et al., 2007). Bottles were kept frozen after sample collection, though we have
24 subsequently determined in the laboratory that seawater samples preserved in this way can be
25 kept at room temperature for at least a year without alteration of NO_2^- $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$
26 (unpublished data). Samples were analyzed by continuous He flow isotope-ratio mass
27 spectrometry (CF-IRMS; see below) after chemical conversion to N_2O using acetic acid
28 buffered sodium azide (McIlvin & Altabet 2005). Because of high sample pH, the reagent was
29 modified for NO_2^- isotope analysis by increasing the acetic acid concentration to 7.84 M. In-
30 house (i.e., MAA1, $\delta^{15}\text{N} = -60.6\%$; MAA2, $\delta^{15}\text{N} = 3.9\%$; Zh1, $\delta^{15}\text{N} = -16.4\%$) and other

1 laboratory calibration standards (N23, $\delta^{15}\text{N} = 3.7\text{‰}$ and $\delta^{18}\text{O} = 11.4\text{‰}$; N7373, $\delta^{15}\text{N} = -79.6\text{‰}$
2 and $\delta^{18}\text{O} = 4.5\text{‰}$; and N10219; $\delta^{15}\text{N} = 2.8\text{‰}$ and $\delta^{18}\text{O} = 88.5\text{‰}$; see Casciotti and McIlvin,
3 2007) were used for NO_2^- $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ analysis.
4 NO_3^- samples were stored in 125 ml HDPE bottles preloaded with 1 ml of 2.5 mM sulfamic
5 acid in 25 % HCl to both act as a preservative and to remove NO_2^- (Granger and Sigman,
6 2009). Samples were also kept at room temperature and we have found that they can be stored
7 in this way for many years without alteration of NO_3^- $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$. Cadmium reduction was
8 used to convert NO_3^- to NO_2^- prior to conversion to N_2O using the “azide method” (McIlvin &
9 Altabet 2005) and IRMS analysis. Standards for NO_3^- isotope analysis were N3 ($\delta^{15}\text{N} = 4.7\text{‰}$
10 and $\delta^{18}\text{O} = 25.6\text{‰}$), USGS34 ($\delta^{15}\text{N} = -1.8\text{‰}$ and $\delta^{18}\text{O} = -27.9\text{‰}$) and USGS35 ($\delta^{15}\text{N} = 2.7\text{‰}$
11 and $\delta^{18}\text{O} = 57.5\text{‰}$) (Casciotti et al., 2007). The lowest concentration of NO_2^- or NO_3^- analyzed
12 for isotopic composition was 0.5 μM , thus $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{15}\text{N}\text{-NO}_2^-$ could not be measured
13 below 37 m at station 63.
14 A GV Instruments IsoPrime Isotope Ratio Mass Spectrometer (IRMS) coupled to an on-line He
15 continuous-flow purge/trap preparation system was used for isotope analysis (Sigman et al.,
16 2001; Casciotti et al., 2002; McIlvin & Altabet 2005). N_2O produced by the azide reaction was
17 purged with He from the septum sealed 20 ml vials and trapped, cryofocused and purified prior
18 to transfer to the IRMS. Total run time was 700 s/sample (McIlvin & Altabet 2005). Isotopic
19 values are referenced against atmospheric N_2 for $\delta^{15}\text{N}$ and VSMOW for $\delta^{18}\text{O}$. Reproducibility
20 was 0.2‰ and 0.5‰, respectively.

21 **2.3 N_2/Ar IRMS analysis and calculation of biogenic N_2 and $\delta^{15}\text{N}$ biogenic**

22 **N_2**

23 The accumulation of biogenic N_2 from denitrification and anammox can be measured directly
24 from precise N_2/Ar measurements (see above; Richards and Benson, 1961; Chang et al., 2010;
25 Bourbonnais et al., 2015). As described in Charoenpong et al. (2014), N_2/Ar samples were
26 collected from Niskin bottles using 125 ml serum bottles, and all samples were treated with
27 HgCl_2 as a preservative and filled without headspace. When cavitation bubbles formed from
28 cooling of warm, near-surface samples, these bubbles were collapsed and reabsorbed by
29 warming samples in the laboratory, in a 30-35°C water bath before analysis. N_2/Ar was
30 measured using an automated dissolved gas extraction system coupled to a multicollector
31 IRMS (Charoenpong et al., 2014). Excess N_2 was calculated first from anomalies relative to

Annie Bourbonnais 1/14/2016 10:52 AM
Formatted: Font:Symbol
Annie Bourbonnais 1/14/2016 10:52 AM
Formatted: Superscript
Annie Bourbonnais 1/14/2016 10:52 AM
Formatted: Subscript

LocalAdmin 1/13/2016 1:55 PM
Deleted: to
LocalAdmin 1/13/2016 1:55 PM
Deleted: 30 to 35°C

1 N₂/Ar expected at saturation with atmosphere at *in situ* temperature and salinity. Locally
2 produced biogenic N₂ was obtained by subtracting excess N₂ at the corresponding density
3 surface for waters outside of the ODZ (O₂ > 10 μM) not affected by N-loss (Chang et al., 2010;
4 Bourbonnais et al., 2015). ~~δ¹⁵N biogenic N₂ was calculated from the δ¹⁵N-N₂ anomaly as in~~
5 ~~Bourbonnais et al. (2015).~~ Reproducibility was better than 0.7 μM for excess N₂ and 0.03 ‰
6 for δ¹⁵N-N₂. δ¹⁵N of biogenic N₂ was calculated by mass balance as in Bourbonnais et al.
7 (2015).

LocalAdmin 1/13/2016 1:53 PM

Deleted:

8 2.4 Isotope effect (ε) calculations

9 Isotope effects are estimated using the Rayleigh equations describing the change in isotope
10 ratio as a function of fraction of remaining substrate. The following equations are used for a
11 closed system (Mariotti et al., 1981):

$$12 \delta^{15}\text{N-NO}_3^- = \delta^{15}\text{N-NO}_3^- (f=1) - \epsilon \times \ln[f_1] \quad \text{or} \quad (1)$$

$$13 \delta^{15}\text{N-DIN} = \delta^{15}\text{N-DIN} (f=1) - \epsilon \times \ln[f_2] \quad (2)$$

14 where f_1 is the fraction of remaining NO₃⁻ and f_2 is the fraction of remaining DIN (NO₃⁻ + NO₂⁻
15 concentrations). δ¹⁵N-DIN is the average δ¹⁵N for NO₃⁻ and NO₂⁻ weighted by their
16 concentrations. The fraction of remaining DIN is a better estimation of the overall effective
17 isotope effect for N-loss (Bourbonnais et al., 2015), while using NO₃⁻ as the basis to calculate
18 ε specifically targets NO₃⁻ reduction. See below for details of f value calculation.

19 The overall isotope effect for N-loss can also be estimated from the δ¹⁵N of biogenic N₂
20 produced:

$$21 \delta^{15}\text{N-biogenic N}_2 = \delta^{15}\text{N-DIN} (f=1) + \epsilon \times f_2 / [1 - f_2] \times \ln[f_2] \quad (3)$$

22 Whereas the closed system equations assume no addition or loss of substrate or product,
23 corresponding steady-state open system equations can account for such effects (Altabet, 2005):

$$24 \delta^{15}\text{N-NO}_3^- = \delta^{15}\text{N-NO}_3^- (f=1) + \epsilon [1 - f_1] \quad \text{or} \quad (4)$$

$$25 \delta^{15}\text{N-DIN} = \delta^{15}\text{N-DIN} (f=1) + \epsilon \times [1 - f_2] \quad (5)$$

$$26 \delta^{15}\text{N-biogenic N}_2 = \delta^{15}\text{N-DIN} (f=1) - \epsilon \times f_2 \quad (6)$$

27 For all equations, the slope represents ε and the y-intercept is the initial δ¹⁵N prior to N-loss.

28 For calculations using equations 3 and 6 we only used δ¹⁵N values associated with biogenic N₂
29 greater than 7.5 μM because of increasing noise below this level due to the ~~large~~ atmospheric
30 dissolved N₂ background (typically up to ~500 μM).

Annie Bourbonnais 1/11/2016 3:05 PM

Deleted: huge

1 Since the closed system equations assume no loss or resupply of substrate or production in a
2 water parcel, they are appropriate where there is little mixing and/or advection is dominant over
3 mixing. The open system equations take into account supply from or loss to surrounding water
4 parcels, e.g. mixing dominance. Both cases represent extreme situations. In the next section, we
5 will estimate and compare ϵ using both sets of equations.

6 To do so, we need to estimate the fraction of NO_3^- or DIN remaining (f). The assumption of
7 Redfield stoichiometry (as in eq. 9) in source waters is typically made:

$$8 \quad f_{1p} = [\text{NO}_3^-] / N_{p_{\text{expected}}} \text{ or} \quad (7)$$

$$9 \quad f_{2p} = ([\text{NO}_3^-] + [\text{NO}_2^-]) / N_{p_{\text{expected}}} \quad (8)$$

$$10 \quad N_{p_{\text{expected}}} = 15.8 * ([\text{PO}_4^{3-}] - 0.3) \quad (9)$$

$$11 \quad N_{\text{observed}} = [\text{NO}_3^-] + [\text{NO}_2^-] + [\text{NH}_4^+] \quad (10)$$

12 where $N_{p_{\text{expected}}}$ is the concentration expected assuming Redfield stoichiometry. Equation 9 was
13 derived in Chang et al. (2010) from stations to the west of the ETSP ODZ (143-146°W) and
14 takes into account preformed nutrient concentrations. In our study, NH_4^+ generally did not
15 significantly accumulate, except at station 63, and was thus not included. This has been the
16 traditional approach to quantify N-loss in ODZ's (N deficit, $N_{p_{\text{def}}}$), by comparing observed
17 DIN concentrations (N_{observed}) to $N_{p_{\text{expected}}}$:

$$18 \quad N_{p_{\text{def}}} = N_{p_{\text{expected}}} - N_{\text{observed}} \quad (11)$$

19 However, the assumption of Redfield stoichiometry may not be appropriate in this shallow
20 environment due to preferential release of PO_4^{3-} following iron and manganese oxyhydroxide
21 dissolution in anoxic sediments (e.g., Noffke et al., 2012). An alternative method of calculating
22 f makes use of our biogenic N_2 measurements to estimate expected N prior to N-loss (N_{expected}
23 bio N_2) and f values based on it:

$$24 \quad N_{\text{expected bio N}_2} = [\text{NO}_3^-] + [\text{NO}_2^-] + 2 \times [\text{Biogenic N}_2] \quad (12)$$

$$25 \quad f_{1\text{bioN}_2} = [\text{NO}_3^-] / N_{\text{expected bio N}_2} \text{ or} \quad (13)$$

$$26 \quad f_{2\text{bioN}_2} = ([\text{NO}_3^-] + [\text{NO}_2^-]) / N_{\text{expected bio N}_2} \quad (14)$$

27 A third way to estimate f is to use NO_3^- or DIN concentrations divided by observed maximum
28 NO_3^- or DIN concentrations for the source of the upwelled waters (see red rectangles in Fig. 2).

29

LocalAdmin 1/13/2016 2:00 PM

Deleted: or

LocalAdmin 1/13/2016 2:01 PM

Deleted: also

LocalAdmin 1/13/2016 2:01 PM

Deleted: a

LocalAdmin 1/13/2016 2:02 PM

Deleted: by

LocalAdmin 1/13/2016 2:04 PM

Deleted: or

1 3 Results

2 3.1 Hydrographic characterization

3 During the study period, there was active coastal upwelling especially at station 63 as seen by
4 relatively low satellite sea surface temperatures, higher chlorophyll a concentrations, and a
5 shallow oxycline (Fig. 1). A common relationship and narrow range for T and S were found,
6 comparable to T/S signatures for offshore ODZ waters between ~100 and 200 m depths
7 (Bourbonnais et al. 2015), indicating a common source of water upwelling at these inner shelf
8 stations (Fig. 2). This is expected in these shallow waters, where upwelling of the Peru Coastal
9 Current with low O_2 and high nutrients plays a dominant role (Penven et al., 2005). O_2
10 increased only in warmer near-surface waters as a consequence of atmospheric exchange. There
11 was a change in surface water temperature from 15 °C to 20 °C (Fig. 1 B) with distance along
12 the coast (from 12.0°S to 14.0°S, about 222 km) that indicates corresponding changes in
13 upwelling intensity. Stronger local wind forcing likely brought up colder deep water near
14 station 63.

15 3.2 Dissolved O_2 and nutrient concentrations

16 As a consequence of active upwelling sourced from the offshore ODZ, the oxycline was very
17 shallow at our in-shore stations. O_2 was generally depleted below 10 to 20 m (Fig. 3 A) and
18 was always less than 10 μM below 30 m. Because we are focusing on N-transformations that
19 occur in the absence of O_2 , our data analyses will be mainly restricted to samples where O_2
20 concentration is below this value. Whereas a recent study indicates that denitrification and
21 anammox are reversibly suppressed at nanomolar O_2 levels (Dalsgaard et al., 2014), CTD
22 deployed Seabird O_2 sensors are not sufficiently sensitive to detect such low concentrations and
23 hence our choice of a 10 μM threshold. In contrast, NO_2^- oxidation, an aerobic process, was
24 shown to occur even at low to non-detectable O_2 (Füssel et al., 2012).

25 Both $\text{Si}(\text{OH})_4$ and PO_4^{3-} concentrations had very similar vertical and along section distributions
26 (Fig. 3 C and D). Concentrations were at a minimum at the surface, presumably due to
27 phytoplankton uptake, and increased with depth to up to 46 μM and 3.7 μM , respectively.
28 Station 63 had the highest near-bottom concentrations, a likely result of release from the
29 sediments, which is further supported by high near-bottom NH_4^+ concentrations (up to ~4 μM) as
30 compared to the other stations (Fig. 3 B, C, and D).

Annie Bourbonnais 1/14/2016 10:32 AM

Deleted: α

Annie Bourbonnais 1/14/2016 10:32 AM

Formatted: Font:Italic

LocalAdmin 1/13/2016 2:09 PM

Deleted: (

Annie Bourbonnais 1/14/2016 10:36 AM

Deleted: as

Annie Bourbonnais 1/14/2016 10:33 AM

Deleted: coastal,

LocalAdmin 1/13/2016 2:10 PM

Deleted: ,

LocalAdmin 1/13/2016 2:13 PM

Deleted: As

1 In contrast to other nutrients, NO_3^- and NO_2^- concentrations were lowest near-bottom at station
2 63, only reaching their maxima above 60 m. Across most of our stations, NO_3^- concentration
3 was 22 μM at 20 to 40 m depth but decreased to near zero deeper within the O_2 -depleted zone
4 due to microbially mediated NO_3^- reduction (Fig. 4 A). NO_2^- concentrations correspondingly
5 ranged from 6 to 11 μM for O_2 concentrations less than 10 μM (Fig. 4 B). The highest NO_2^-
6 concentration (11 μM) was found at around 50 m (station 64), but only reached 6 μM at all
7 other stations.

8 3.3 NO_2^- and NO_3^- isotope compositions

9 As a consequence of kinetic isotope fractionation during N-loss, the N and O isotope
10 composition of NO_3^- and NO_2^- varied inversely with NO_3^- and NO_2^- concentrations, with
11 maximum $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values near the bottom at each station. $\delta^{15}\text{N}\text{-NO}_3^-$ increased from
12 about 10‰ in surface waters to up to 50‰ in the O_2 -depleted zone (Fig. 4 C), with near bottom
13 values at station 64 significantly higher (50‰) than at the other stations which ranged from 20
14 to 30‰. $\delta^{15}\text{N}\text{-NO}_2^-$ varied from -25 to about 10‰ (Fig. 4 D), with maximum values also in
15 deeper waters at station 64.

16 As expected for NO_3^- reduction, $\delta^{18}\text{O}\text{-NO}_3^-$ positively co-varied with $\delta^{15}\text{N}\text{-NO}_3^-$ and ranged
17 from 12 to 46‰. We observed an overall linear relationship between $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$
18 with a slope of 0.86, which was significantly different than 1 (p-value<0.05), and a y-intercept
19 of 1.90 ($r^2= 0.996$, see Fig. 5 A). NO_3^- $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ have been shown to increase equally
20 (ratio 1:1) during assimilatory and dissimilatory NO_3^- reduction (Casciotti et al., 2002; Granger
21 et al., 2004; 2008). However, deviations from this trend have been observed in the ocean and
22 interpreted as evidence for co-occurring NO_3^- production processes (Sigman et al. 2005;
23 Casciotti and McIlvin, 2007 ; Bourbonnais et al., 2009; 2015). In this study, we observed a
24 NO_3^- $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ relationship less than 1, likely originating from NO_2^- re-oxidation to NO_3^- in
25 our environmental setting as in Casciotti and McIlvin (2007). We also observed, for the first
26 time, a significant correlation between $\delta^{15}\text{N}\text{-NO}_2^-$ and $\delta^{18}\text{O}\text{-NO}_2^-$ in the ODZ for our in-shore
27 water stations (Fig. 5 B). As in prior studies (Casciotti and McIlvin 2007; Casciotti et al.,
28 2013), no such relationship was observed by us for a nearby set of offshore stations (see Fig. 5
29 C) where longer NO_2^- turnover times likely facilitated O isotope exchange with water. We will
30 discuss implications of this unique finding in the next section.

Annie Bourbonnais 1/14/2016 10:40 AM

Deleted: (Casciotti et al. 2002;
Sigman et al. 2003

1 3.4 The $\delta^{15}\text{N}$ difference between NO_3^- and NO_2^-

2 The difference in $\delta^{15}\text{N}$ between NO_3^- and NO_2^- ($\Delta\delta^{15}\text{N}$) reflects the combined isotope effects of
3 simultaneous NO_3^- reduction, NO_2^- reduction, and NO_2^- oxidation. For NO_3^- reduction alone,
4 highest $\Delta\delta^{15}\text{N}$ values would be around 25‰ at steady-state (Cline and Kaplan, 1975; Brandes et
5 al., 1998; Voss et al., 2001; Granger et al., 2004; 2008). The effect of NO_2^- reduction would be
6 to increase the $\delta^{15}\text{N}$ of the residual NO_2^- , thus decreasing $\Delta\delta^{15}\text{N}$. In contrast, NO_2^- oxidation is
7 associated with an inverse kinetic isotope effect (Casciotti, 2009) which acts to decrease the
8 residual $\delta^{15}\text{N}$ of NO_2^- , and hence overall increases the $\Delta\delta^{15}\text{N}$. Therefore, following NO_2^-
9 oxidation, $\Delta\delta^{15}\text{N}$ may be larger than expected from NO_3^- and NO_2^- reduction alone, especially
10 if the system is not at steady-state (Casciotti et al., 2013). $\Delta\delta^{15}\text{N}$ ranged from 15‰ to 40‰
11 (average = 29.78‰ and median = 32.5‰) for samples with $\text{O}_2 < 10 \mu\text{M}$. These results confirm
12 the presence of NO_2^- oxidation for at least some of our depth intervals.

Annie Bourbonnais 1/14/2016 10:44 AM
Deleted: ; Sigman et al., 2005

LocalAdmin 1/13/2016 2:27 PM
Deleted: and would

LocalAdmin 1/13/2016 2:27 PM
Deleted: ,

13 3.5 N deficit, biogenic N_2 and $\delta^{15}\text{N}-\text{N}_2$

14 N deficits, biogenic N_2 concentrations, and $\delta^{15}\text{N}-\text{N}_2$ anomalies relative to equilibrium with
15 atmosphere were overall greater in the O_2 -depleted zone reaching highest values near the
16 bottom of station 63 (Fig. 7). N deficit, calculated assuming Redfield stoichiometry (Eqs. 9 to
17 11), ranged from 17 to 59 μM in this region. The concentration of biogenic N in N_2 ranged
18 from 12 to 36 $\mu\text{M}-\text{N}$ and, as expected, was strongly linearly correlated with N deficit ($r^2 = 0.87$;
19 Fig. 8 C). However, the slope of 0.45 for the linear relationship shows biogenic N in N_2 to be
20 only half that expected from N_{pdef} , a possible consequence of benthic PO_4^{3-} release. The linear
21 relationship ($r^2 = 0.91$) observed between biogenic N in N_2 and DIN (Fig. 8 A) supports a
22 single initial DIN value for the source waters to our stations and hence validates, using this as a
23 basis for calculating f . The slope of the correlation (0.74) is much closer to 1 as compared to
24 the correlation with N_{pdef} , further supporting excess PO_4^{3-} as a contributor to the latter.
25 However this value is still significantly less than 1, suggesting that biogenic N in N_2 may also
26 be underestimated. Because our data are restricted to O_2 -depleted depths, it is unlikely that
27 biogenic N_2 was lost to the atmosphere. Alternatively, mixing of water varying in N_2/Ar can
28 result in such underestimates of biogenic N_2 when N_2/Ar anomalies are used to calculate excess
29 N_2 (see Charoenpong et al., 2014). As seen below, our estimates of ϵ are rather insensitive to
30 choice of N_{pdef} , biogenic N in N_2 , or DIN concentration changes as the basis for calculation of
31 f .

LocalAdmin 1/13/2016 2:35 PM
Deleted: as

LocalAdmin 1/13/2016 2:37 PM
Deleted: the

LocalAdmin 1/13/2016 2:37 PM
Deleted: ity

LocalAdmin 1/13/2016 2:38 PM
Deleted: of

LocalAdmin 1/13/2016 2:38 PM
Deleted: , though

1 The $\delta^{15}\text{N-N}_2$ anomaly, i.e., the difference between the $\delta^{15}\text{N-N}_2$ observed and at equilibrium,
2 derived as in Charoenpong et al. (2014), ranged from -0.2 to 0.1‰ (Fig. 7 C). The
3 corresponding range in $\delta^{15}\text{N}$ biogenic N_2 at $\text{O}_2 < 10 \mu\text{M}$ was from -9.0 to 3.2‰ . Negative $\delta^{15}\text{N-}$
4 N_2 anomaly (i.e., lower $\delta^{15}\text{N}$ -biogenic N_2) is produced at the onset of N-loss, because
5 extremely depleted $^{15}\text{N-N}_2$ is first produced. At a more advanced N-loss stage, we expect $\delta^{15}\text{N-}$
6 N_2 anomaly and $\delta^{15}\text{N}$ -biogenic N_2 to increase, which we observed in this study, as heavier ^{15}N
7 is added to the biogenic N_2 pool. The $\delta^{15}\text{N-N}_2$ anomaly signal appears small when compared to
8 the isotopic composition of NO_3^- and NO_2^- but is 1) analytically significant and 2) the result of
9 dilution by the large background of atmospheric N_2 (400 to $500 \mu\text{M N}_2$).

10 3.6 Isotope effect (ϵ)

11 Isotope effects were calculated using eqs. 1 to 6 to compare closed versus open system
12 assumptions as well as different approaches to estimating f . Examples of plots of the closed
13 system equations with f calculated using biogenic N_2 , are shown in Fig. 6. Comparison of
14 results using all 3 approaches for calculating f (i.e. Redfield stoichiometry, biogenic N_2 and
15 observed substrate divided by maximum “upwelled” concentration, (see section 2.4)) are shown
16 in Table 1 (closed system) and 2 (open system). In the case of the closed system, ϵ values were
17 in all cases lower than canonical ones, ranging narrowly from $\sim 6\text{‰}$ for changes in the $\delta^{15}\text{N}$ of
18 DIN to $\sim 14\text{‰}$ for changes in $\delta^{15}\text{N-NO}_3^-$ (Table 1). For the open system equations, estimated ϵ
19 was higher and covered a large and unrealistic range from $\sim 12\text{‰}$ for changes in the biogenic N_2
20 to $\sim 63\text{‰}$ for changes in the $\delta^{15}\text{N}$ of NO_3^- . For our inshore water stations, where we observed a
21 single water mass (Fig. 2), a closed system should be a more realistic approximation of ϵ . The
22 Rayleigh equations’ y-intercepts, where $f=1$, represent the initial $\delta^{15}\text{N}$ of NO_3^- or DIN, and
23 varied from -0.5 to 3.7‰ and -18.4 to 6.2‰ for closed and open systems, respectively. The
24 higher end of this range is more realistic based on prior isotopic measurements for source
25 waters (e.g. Bourbonnais et al., 2015).

26

LocalAdmin 1/13/2016 2:39 PM
Deleted: and

Annie Bourbonnais 1/14/2016 10:50 AM
Deleted: ,

Annie Bourbonnais 1/14/2016 10:50 AM
Deleted: calculated from the $\delta^{15}\text{N-N}_2$ anomaly as in Bourbonnais et al. (2015),

LocalAdmin 1/13/2016 2:42 PM
Deleted: as

LocalAdmin 1/13/2016 2:40 PM
Deleted: we

LocalAdmin 1/13/2016 2:42 PM
Deleted: as

LocalAdmin 1/13/2016 2:45 PM
Deleted: s.

LocalAdmin 1/13/2016 2:46 PM
Deleted: ,

LocalAdmin 1/13/2016 2:50 PM
Deleted: about

LocalAdmin 1/13/2016 2:49 PM
Deleted: about

Annie Bourbonnais 1/11/2016 4:04 PM
Deleted:

LocalAdmin 1/13/2016 2:49 PM
Deleted: about

LocalAdmin 1/13/2016 2:49 PM
Deleted: about

LocalAdmin 1/13/2016 2:48 PM
Deleted: , respectively

LocalAdmin 1/13/2016 2:48 PM
Deleted: the

LocalAdmin 1/13/2016 2:49 PM
Deleted: see

1 4 Discussion

2 4.1 Behavior of NO_2^-

3 NO_2^- is an important intermediate during either oxidative or reductive N-cycle pathways and
4 can accumulate at relatively high concentrations through the ocean. While NO_2^- is generally
5 elevated at the base of the sunlit euphotic zone (i.e. primary NO_2^- maximum; Dore and Karl,
6 1996; Lomas and Lipschultz, 2006), highest concentrations are found in ODZ's as part of the
7 secondary NO_2^- maximum (Codispoti and Christensen 1985; Lam et al. 2011). Accordingly,
8 high NO_2^- concentrations ranging from 7.2 to 10.7 μM were observed at 50-75 m depth in
9 coastal O_2 -depleted waters in this study as a likely consequence of dissimilatory NO_3^- reduction
10 (e.g., Lipschultz et al., 1990; Lam et al., 2009; Kalvelage et al., 2013).

11 To assess the influence of the various N cycle processes that have NO_2^- as either a substrate or
12 product, we first examined the relationship between the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_2^- . Several
13 processes can influence the isotopic composition of NO_2^- . NO_3^- reduction to NO_2^- is associated
14 with a ϵ of 20 to 30‰ (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001; Granger
15 et al., 2004; 2008) and acts to produce NO_2^- depleted in ^{15}N and ^{18}O . In contrast, NO_2^-
16 reduction as part of either anammox, denitrification or DNRA increases both the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$
17 of residual NO_2^- , with laboratory and field estimates for ϵ clustering around 12‰ to 16‰
18 (Bryan et al., 1983; Brunner et al., 2013; Bourbonnais et al., 2015). However, NO_2^- oxidation to
19 NO_3^- at low or non-detectable O_2 has been shown to be an important sink for NO_2^- in ODZs
20 (e.g. Füssel et al., 2012). Anammox bacteria can also use NO_2^- as an electron donor during CO_2
21 fixation under anaerobic conditions (Strous et al., 2006).

22 Nitrite oxidation has its own unique set of isotope effects (Casciotti, 2009; Buchwald and
23 Casciotti, 2010). Nitrite oxidation incurs an unusual inverse N isotope effect varying from -
24 13‰ for aerobic (Casciotti, 2009) to -30‰ for anammox-mediated [NO₂⁻ oxidation](#) (Brunner et
25 al., 2013), resulting in lower $\delta^{15}\text{N}$ for NO_2^- as it is oxidized to NO_3^- , and increasing $\Delta\delta^{15}\text{N}$.
26 Moreover, enzyme catalysis associated with NO_2^- oxidation is readily reversible (Friedman et
27 al., 1986) also causing O isotope exchange between NO_2^- and water (Casciotti et al., 2007). O
28 atom incorporation during both NH_4^+ and NO_2^- oxidation have also been shown to occur with
29 significant isotope effect, such that the $\delta^{18}\text{O}$ of newly microbially produced NO_3^- in the ocean
30 range from -1.5‰ and 1.3‰ (Buchwald and Casciotti, 2012).

Annie Bourbonnais 1/14/2016 10:45 AM
Deleted: ; Sigman et al., 2005

LocalAdmin 1/13/2016 2:58 PM
Deleted: NO_2^- oxidation

1 Past studies have found $\text{NO}_2^- \delta^{18}\text{O}$ values in ODZ's in isotope equilibrium with water as a
2 likely consequence of relatively long turnover time (e.g., Buchwald and Casciotti, 2013;
3 Bourbonnais et al., 2015). O isotope exchange involves the protonated form, HNO_2 , but
4 because of its high pKa as compared to NO_3^- , this process can occur even at neutral to alkaline
5 ocean pH on a time scale of 2 to 3 months at environmentally relevant temperatures (Casciotti
6 et al., 2007). $\text{NO}_2^- \delta^{18}\text{O}$ isotopic composition at equilibrium with water is a function of the $\delta^{18}\text{O}$
7 of water and temperature (+14‰ for seawater at 22 °C) (Casciotti et al., 2007; Buchwald and
8 Casciotti, 2013) and is independent of its $\delta^{15}\text{N}$ value such that plots of $\text{NO}_2^- \delta^{18}\text{O}$ vs $\delta^{15}\text{N}$
9 usually have a slope of near zero. This is seen in our NO_2^- data from offshore stations occupied
10 during M90 (Fig. 5 C).

11 We observed, for the first time, a significant linear relationship for $\text{NO}_2^- \delta^{18}\text{O}$ vs. $\delta^{15}\text{N}$ at our
12 inshore stations (slope = 0.64 ± 0.07 , $r^2 = 0.59$, p-value= 3×10^{-6}) where $\text{O}_2 < 10 \mu\text{M}$ (Fig. 5 B).
13 Coupled $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ effects for NO_2^- have not been as well studied as NO_3^- . Nevertheless, if
14 NO_2^- turnover was faster than equilibration time with water, NO_3^- and NO_2^- reduction whether
15 as part of the denitrification, anammox or DNRA pathways, should also produce a positive
16 relationship between $\text{NO}_2^- \delta^{15}\text{N}$ and $\delta^{18}\text{O}$. In contrast to our offshore stations (Fig. 5 C), this
17 positive relationship thus demonstrates that the oxygen isotopic composition of NO_2^- is not in
18 equilibrium with water due to both rapid NO_2^- turnover and the dominance of NO_2^- reduction
19 over oxidation in Peru coastal waters. Higher rates for aerobic NH_4^+ and NO_2^- oxidation, as
20 well as anaerobic NO_3^- reduction to NO_2^- , and further reduction to NH_4^+ (DNRA) or N_2 , have
21 been reported in shallow waters off Peru presumably due to increased coastal primary
22 production and organic matter supply to the in-shore OMZ (e.g. Codispoti et al., 1986; Lam et
23 al., 2011; Kalvelage et al., 2013). However, as our observations are restricted to anoxic waters,
24 only high rates of N-loss could explain this more rapid NO_2^- turnover.

25 In principal, we can estimate NO_2^- turnover time from knowledge of rates for exchange with
26 water and assumptions of the $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ slope expected in the absence of exchange.
27 Unfortunately, the slope of the relationship between $\text{NO}_2^- \delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ expected in the absence
28 of equilibration with water is not yet known. An upper limit for turnover time for NO_2^- can be
29 estimated based on equilibration time as a function of *in situ* pH and temperature (Buchwald
30 and Casciotti, 2013). During the M91 cruise in December, subsurface temperature was 13 to
31 15°C along our transect and corresponding pH was near 7.8 (Michelle Graco, unpublished
32 data). Assuming the NO_2^- pool is in steady-state, we estimated an equilibration time of up to

Annie Bourbonnais 1/14/2016 11:07 AM

Deleted:

LocalAdmin 1/13/2016 3:04 PM

Deleted: for

Annie Bourbonnais 1/13/2016 4:46 PM

Deleted: , but the corresponding slopes are not yet known

LocalAdmin 1/13/2016 3:08 PM

Deleted: as compared to offshore

LocalAdmin 1/13/2016 3:10 PM

Deleted: ”

LocalAdmin 1/13/2016 3:12 PM

Deleted: However, a

Annie Bourbonnais 1/11/2016 3:54 PM

Deleted: at least

1 ~40 days for pH near 7.8 (estimated from equation 1 and Fig. 2 in Buchwald and Casciotti,
2 2013). A turnover time of up to 40 days implies a flux of N through the NO_2^- pool of at least
3 0.21 $\mu\text{M d}^{-1}$, as estimated from the maximum NO_2^- concentration observed in this study divided
4 by this estimated turnover time. Assuming steady-state, this range also approximates the rates
5 of NO_3^- reduction as well as NO_2^- oxidation plus production of N_2 from NO_2^- . This estimated
6 flux is consistent with measured high NO_3^- reduction and NO_2^- oxidation rates of up to $\sim 1 \mu\text{M}$
7 d^{-1} in Peru coastal waters (<600 m depth, Kalvelage et al.; 2013).

8 NO_2^- oxidation is a chemoautotrophic process that requires a thermodynamically favorable
9 electron acceptor such as O_2 . As mentioned above, NO_2^- oxidation appears to occur in ODZ's at
10 low or non-detectable O_2 (e.g. Füssel et al. 2012) despite lack of knowledge of its
11 thermodynamically favorable redox couple. The difference in $\delta^{15}\text{N}$ between NO_2^- and NO_3^-
12 ($\Delta\delta^{15}\text{N} = \delta^{15}\text{N-NO}_3^- - \delta^{15}\text{N-NO}_2^-$ see Section 3.3) is further evidence for the presence of NO_2^-
13 oxidation in the ODZ (e.g. Casciotti et al., 2013). At steady-state, and in the absence of NO_2^-
14 oxidation, $\Delta\delta^{15}\text{N}$ should be no more than the ϵ for NO_3^- reduction (20 to 30‰) minus the ϵ for
15 NO_2^- reduction by denitrifying or anammox bacteria (12-16‰; Bryan et al., 1983; Brunner et
16 al., 2013; Bourbonnais et al., 2015) or 8-18‰. Our results range from 15-40‰ and average
17 29.8 ‰ for samples with O_2 concentrations <10 μM .

18 The inverse kinetic isotope effect associated with NO_2^- oxidation is likely responsible for these
19 high $\Delta\delta^{15}\text{N}$ values (e.g. Casciotti and Buchwald, 2012; Casciotti et al., 2013). Taking all
20 isotope effects into account, the following equation can be derived to estimate $\Delta\delta^{15}\text{N}$ at steady-
21 state:

$$22 \Delta\delta^{15}\text{N (steady state)} = \epsilon_{\text{NO}_3\text{-red}} - (1-\gamma) \times \epsilon_{\text{NO}_2\text{-red}} - \gamma \times \epsilon_{\text{NO}_2\text{-oxid}} \quad (15)$$

23 where γ is the fraction of NO_2^- oxidized back to NO_3^- . Highest values (over 30‰) are found
24 between 50 and 100 m, implying greater importance for NO_2^- oxidation in deeper waters.

25 Given that $\epsilon_{\text{NO}_2\text{-oxid}}$ has been reported to be -13‰ for aerobic NO_2^- oxidation and using the
26 literature ranges for $\epsilon_{\text{NO}_3\text{-red}}$ and $\epsilon_{\text{NO}_2\text{-red}}$ above, our observed $\Delta\delta^{15}\text{N}$ implies that up to 100 % of
27 NO_2^- produced by NO_3^- reduction could be oxidized back to NO_3^- . This estimate is higher than
28 ratios of NO_2^- oxidation/ NO_3^- reduction of up to 54% for the Peruvian coastal ODZ derived
29 from direct rate measurements (Lam et al., 2009; Kalvelage et al., 2013), and should thus be
30 considered as an upper limit. Alternatively, NO_2^- oxidation also occurs as part of the overall
31 metabolism of anammox bacteria (Strous et al., 2006) which can be the dominant N_2 producers

Annie Bourbonnais 1/11/2016 3:57 PM

Deleted: at least

Annie Bourbonnais 1/11/2016 3:57 PM

Deleted: up to

LocalAdmin 1/13/2016 3:15 PM

Deleted: to

LocalAdmin 1/13/2016 3:15 PM

Deleted: ‰ to

Annie Bourbonnais 1/14/2016 11:12 AM

Deleted: (Casciotti, 2009; Brunner et al., 2013)

LocalAdmin 1/13/2016 3:22 PM

Deleted:

1 in the Peru ODZ (Kalvelage et al., 2013). A large inverse kinetic ϵ for NO_2^- oxidation of ~
2 30‰ has been observed for anammox bacteria in culture (Brunner et al., 2013). If this is the
3 sole pathway for NO_2^- oxidation, our data suggest NO_2^- oxidation up to only ~80% of total
4 NO_3^- reduction. However, anammox bacteria only oxidize a minor fraction of NO_2^- to NO_3^- in
5 culture. At the same time, estimates of NO_2^- oxidation (8.48 to 928 nM d^{-1}) are significantly
6 higher than N-loss rates by anammox (2.84 to 227 $\text{nmol N l}^{-1} \text{d}^{-1}$) on the Peruvian shelf
7 (Kalvelage et al., 2013), clearly indicating non-anammox related nitrite oxidation.

8 The deviations from a 1:1 relationship for $\text{NO}_3^- \delta^{18}\text{O}$ and $\delta^{15}\text{N}$ can also be indicative of NO_2^-
9 oxidation. During NO_3^- uptake or dissimilative NO_3^- reduction, $\text{NO}_3^- \delta^{15}\text{N}$ and $\delta^{18}\text{O}$ increase
10 equally with a ratio of 1:1 (Granger et al., 2004; 2008). We observed a slope of about 0.86 (Fig.
11 5 A) for the $\text{NO}_3^- \delta^{18}\text{O}$ versus $\delta^{15}\text{N}$ relationship in the in-shore Peru ODZ, similar to recent off-
12 shore observations (Bourbonnais et al., 2015). Prior reports of deviations toward higher values
13 for the slope were indicative of addition of newly nitrified NO_3^- from a relatively low $\delta^{15}\text{N}$
14 source (e.g. see Sigman et al., 2005; Bourbonnais et al., 2009). Our observed deviation toward
15 slopes < 1 can instead be explained by the addition of newly nitrified NO_3^- with a lower $\delta^{18}\text{O}$ -
16 NO_3^- , mostly derived from water (Andersson and Hooper, 1983), relative to the high ambient
17 $\delta^{18}\text{O}$ - NO_3^- values. In fact, a slope for $\delta^{18}\text{O}:\delta^{15}\text{N}$ of either greater or less than 1 can be observed,
18 depending on initial environmental NO_3^- isotopic composition relative to any in-situ sources
19 (Casciotti et al., 2013). Casciotti and Buchwald (2012) showed model results where NO_2^-
20 oxidation generally produces a slope < 1 for the $\text{NO}_3^- \delta^{18}\text{O}$ versus $\delta^{15}\text{N}$ relationship, when the
21 $\text{NO}_3^- \delta^{15}\text{N}$ and $\delta^{18}\text{O}$ are higher than ~15‰ as observed in Casciotti et al. (2013) and
22 Bourbonnais et al. (2015).

23 4.2 Isotope effects for N-loss

24 As described above, the Rayleigh fractionation equations (Eqs. 1 to 6) are used here to estimate
25 ϵ values (Mariotti et al., 1981; Altabet, 2005) and examine the significance of calculations
26 using a) different approaches for calculating f (Eqs. 7 and 14), b) changes in the $\delta^{15}\text{N}$ of
27 substrate (DIN) versus changes in the $\delta^{15}\text{N}$ of product, and c) closed versus open system
28 equations. This approach provides redundancy in our estimates of ϵ and tests implied
29 assumptions including N and ^{15}N balance between NO_3^- or DIN loss and the accumulation of
30 biogenic N_2 .

LocalAdmin 1/13/2016 3:23 PM

Deleted: e

Annie Bourbonnais 1/14/2016 11:14 AM

Deleted: for $\text{NO}_3^- \delta^{18}\text{O}$ versus $\delta^{15}\text{N}$

LocalAdmin 1/13/2016 3:25 PM

Deleted: about

1 Linear regression coefficients for ϵ calculated using the different approaches presented in
2 section 2.4 are listed in Tables 1 and 2. For illustration, example Rayleigh closed system plots
3 for $\delta^{15}\text{N-NO}_3^-$, $\delta^{15}\text{N-DIN}$, or $\delta^{15}\text{N}$ biogenic N_2 as a function of $f_{2\text{bioN}_2}$ are shown in Fig. 6.
4 Surprisingly, ϵ values estimated from the slope of these relationships are not sensitive to choice
5 of method for calculating f despite the lack of 1:1 correspondence between different bases
6 ($\text{N}_{\text{pexpected}}$, biogenic N_2 , or $[\text{NO}_3^-]/[\text{NO}_3^-]_{\text{max}}$). In the case of ϵ calculated from changes in $\delta^{15}\text{N-}$
7 DIN , ϵ ranged narrowly with choice of f from 6.3 to 7.4 with standard errors on the slope of
8 <0.6 (Table 1). As there was no significant difference between bases for calculating f , it
9 appears that all three of our approaches are valid for this purpose.

10 However, ϵ for N-loss (closed system) does vary significantly between calculations using
11 changes in $\delta^{15}\text{N-NO}_3^-$, $\delta^{15}\text{N-DIN}$, or $\delta^{15}\text{N}$ biogenic N_2 . ϵ is largest for changes in $\delta^{15}\text{N-NO}_3^-$
12 ($\sim 14\%$) and smallest for changes in $\delta^{15}\text{N-DIN}$ ($\sim 7\%$). ϵ based on $\delta^{15}\text{N}$ biogenic N_2 is
13 intermediate ($\sim 11\%$). The latter two, using DIN or biogenic N_2 as the basis to calculate ϵ , are
14 more representative of N-loss. Calculations based on changes in $\delta^{15}\text{N-NO}_3^-$ are affected by
15 NO_2^- accumulation and isotope effects of NO_2^- oxidation (see above). The 4% difference in ϵ
16 calculated from changes in $\delta^{15}\text{N}$ of biogenic N_2 vs. $\delta^{15}\text{N}$ of DIN may arise from the
17 contribution of NH_4^+ derived from organic matter to biogenic N_2 via the anammox process.
18 Supporting this hypothesis, NH_4^+ accumulation ($5.3\text{-}7.5\ \mu\text{M}$) associated with a relatively low
19 $\delta^{15}\text{N-NH}_4^+$ of 3.8% to 6.1% was observed at 125 and 200 m bottom water depths at shallow
20 stations located in the studied area ($\sim 12.3^\circ\text{S}$ and 77.3°W) in January 2013 (unpublished
21 results). A contribution of NH_4^+ from organic material and consumption by anammox could
22 therefore supply comparatively lower $\delta^{15}\text{N}$ to the biogenic N_2 pool, increasing ϵ that must be
23 larger to account for the observed isotopic enrichment.

24 The different approaches for estimating the ϵ for N-loss can also be evaluated by examining the
25 initial substrate $\delta^{15}\text{N}$ predicted where $f = 1$ for each set of regressions. In the case of changes in
26 $\delta^{15}\text{N-DIN}$ and using $\text{N}_{\text{pexpected}}$ or biogenic N_2 as bases for f , realistic values are found consistent
27 with the source of upwelled waters of 6 to 7% (Table 1; also see Ryabenko et al., 2012). For
28 regressions based on changes in $\delta^{15}\text{N-biogenic N}_2$, initial $\delta^{15}\text{N}$ values are somewhat lower (~ 3
29 $\%$), possibly due to a source from organic N decomposition.

30 Estimates of ϵ using open system equations are generally much higher than for closed system
31 equations particularly for changes in $\delta^{15}\text{N-NO}_3^-$ with unrealistically high values ($39\text{-}63\%$;

Annie Bourbonnais 1/14/2016 11:43 AM

Deleted: and

LocalAdmin 1/13/2016 3:34 PM

Deleted: ”

LocalAdmin 1/13/2016 3:34 PM

Deleted: also

Annie Bourbonnais 1/14/2016 11:44 AM

Deleted:

1 Table 2). However, values for both closed and open systems tended to converge for estimates
2 based on changes in $\delta^{15}\text{N}$ -DIN or $\delta^{15}\text{N}$ -biogenic N_2 with the latter having no significant
3 difference. Estimates of substrate initial $\delta^{15}\text{N}$ using the open system equations range widely and
4 do not consistently reflect realistic values (Table 2).

5 Closed system estimates of ϵ are likely more reliable in our setting because of low likelihood of
6 mixing between water masses of contrasting characteristics on the shelf. Temperature and
7 salinity in the ODZ at our stations narrowly ranged from 13.5 to 15 °C and 34.88 to 34.98 (Fig.
8 2), similar to T/S signatures from offshore source waters (Bourbonnais et al., 2015), and
9 suggestive of a single water mass. Accordingly, as in Bourbonnais et al. (2015), we view the
10 closed system equations as most reliable with a value of $\sim 6.5\%$ for ϵ based on changes in $\delta^{15}\text{N}$
11 DIN as the likely best estimate. However, given the overlap with the results of open system
12 equations for changes in $\delta^{15}\text{N}$ of biogenic N_2 , an upper bound of $\sim 11\%$ appears appropriate.
13 This range in ϵ for N-loss falls below the results of Bourbonnais et al. (2015) for a near-coastal
14 eddy in the same region and time period ($\sim 14\%$) and is much less than the canonical range of
15 20 to 30% (Brandes et al., 1998; Voss et al., 2001; Granger et al., 2008). As discussed in
16 Bourbonnais et al. (2015), a lower overall ϵ for net N-loss could help resolve any imbalance in
17 the oceanic N-budget, by decreasing the ratio of sedimentary and water-column N-loss
18 necessary to account for the observed $\delta^{15}\text{N}$ of mean ocean NO_3^- .

19 There are several reasonable explanations for these relatively low ϵ values in coastal waters.
20 These include higher microbial growth rates associated with higher productivity, which would
21 shift biochemical rate limitation away from enzyme reactions to membrane transport with low
22 fractionation potential (e.g. Wada and Hattori, 1978). Another is greater influence from benthic
23 N cycling processes in our relatively shallow inshore system as compared to deeper waters.
24 Sediment N-loss has been shown to incur low ϵ due to, in analogous fashion to the affect of
25 microbial growth rate, dominance of substrate transport limitation through the sediment
26 (Brandes and Devol, 1997). This possibility will be explored further in the next section.
27 Unlikely explanations for our relatively low ϵ values for N-loss include the effects of
28 decreasing NO_3^- concentration (Kritee et al., 2012) and contributions from organic N via
29 anammox to biogenic N_2 . Lack of curvature in the Rayleigh plots demonstrates a lack of
30 dependence of substrate concentration (Fig. 6 A & B) as the range in f corresponds to a large
31 range in NO_3^- or DIN concentrations. The possible effects of contributions from organic N to

LocalAdmin 1/13/2016 3:35 PM

Deleted: $\delta^{15}\text{N}$

LocalAdmin 1/13/2016 3:36 PM

Deleted:

Annie Bourbonnais 1/11/2016 4:23 PM

Deleted:

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Font:Symbol, Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Font:Symbol, Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Superscript, Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Subscript, Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Superscript, Not Highlight

Annie Bourbonnais 1/11/2016 4:23 PM

Formatted: Not Highlight

Annie Bourbonnais 1/11/2016 4:20 PM

Deleted:

1 biogenic N₂ has already been taken into account in calculations based on changes in the δ¹⁵N of
2 biogenic N₂, as discussed above.

3 4.3 Using ε values for estimating sediment N-loss

4 The low ε value we observe for water column N-loss at our inshore stations may be explained
5 by contributions from sediment N-loss (e.g. see Sigman et al., 2003). If so, observed ε for N-
6 loss in the water-column should be the weighted average of the actual ε values for N-loss in the
7 water column and sediments:

$$8 \epsilon_{\text{obs}} = \epsilon_{\text{wc}} \times (1 - P_{\text{sed}}) + \epsilon_{\text{sed}} \times P_{\text{sed}} \quad (13)$$

9 where ε_{wc} and ε_{sed} are the isotope effect of water column and sediments and P_{sed} is the
10 proportion of water column and sedimentary N-loss, respectively. We take 6.8 ± 0.5‰ as the
11 value for ε_{obs} (Fig. 6, Table 1), a value of 13.8 ± 1.3‰ for ε_{wc} as estimated for offshore waters
12 by Bourbonnais et al. (2015), and a ε_{sed} of 1.5 ‰ as in Sigman et al. (2003). From these
13 numbers, we estimated that the proportion of N-loss due to sedimentary N-loss could be up to
14 ~60% (48 to 64%) at our coastal stations, which is in the same range than previously reported
15 for other marine coastal environments, e.g. Saanich Inlet (also up to 60%; Bourbonnais et al.,
16 2013). Our estimate is higher than the 25% of benthic vs. total N-loss from a reaction-diffusion
17 model and direct flux measurements for the same coastal region off Peru (Kalvelage et al.,
18 2013). However, our comparison to direct measurements of fluxes should be considered
19 tentative as they are made at single locations over relatively short time periods are thus subject
20 to considerable spatial and temporal heterogeneity.

21

22 5 Conclusions

23 The inshore Peru ODZ is distinguished from offshore by its high productivity as a consequence
24 of coastal upwelling as well as possible greater influence from benthic processes. To examine
25 impact on N-loss processes and their isotope effects, we investigated the dynamics of N and O
26 isotope of NO₂⁻ and NO₃⁻ at 6 coastal stations off Peru.

27 We found that N-loss representing the net effect of partial denitrification, anammox and
28 nitrification produced in sum large variations in isotopic composition. NO₂⁻ δ¹⁵N ranged from –
29 20 to 10‰ and NO₃⁻ δ¹⁵N ranged from 10 to 50‰. Generally, NO₃⁻ and NO₂⁻ isotope values
30 varied inversely with their concentrations as expected for Rayleigh-like fractionation effects.

1 Isotope values were usually higher in low-O₂ near bottom waters where N species
2 concentrations were also relatively low.

3 We observed, for the first time, a positive linear relationship between NO₂⁻ δ¹⁵N and δ¹⁸O at
4 our inshore stations. In offshore ODZ waters, such a relationship has never previously been
5 observed as NO₂⁻ δ¹⁸O reflected equilibration with water in these regions (Buchwald and
6 Casciotti, 2013). Our results suggest a turnover time for NO₂⁻ faster than the equilibration time
7 with water and the effect of NO₂⁻ oxidation over NO₂⁻ reduction in these highly productive
8 coastal waters. We estimated a NO₂⁻ turnover time of up to ~40 days from our data.

9 The difference in δ¹⁵N between NO₃⁻ and NO₂⁻ (Δδ¹⁵N) was high, reaching up to 40‰ in deeper
10 waters, and was greater than expected from NO₃⁻ and NO₂⁻ reduction only. The influence of
11 NO₂⁻ oxidation is consistent with this observation due to its inverse fractionation effect
12 (Casciotti, 2009). Additional evidence for NO₂⁻ oxidation is found in the relationship between
13 NO₃⁻ δ¹⁵N and δ¹⁸O. NO₃⁻ reduction alone is expected to produce a 1:1 relationship (Granger et
14 al., 2008). While we observed a linear relationship between NO₃⁻ δ¹⁵N and δ¹⁸O, the slope of
15 0.86 is indicative of simultaneous addition of NO₃⁻ with relatively low δ¹⁸O, also consistent
16 with a role for NO₂⁻ oxidation at our coastal sites. However, a favorable thermodynamic couple
17 for NO₂⁻ oxidation in the absence of O₂ in these waters remains unknown.

18 A number of different approaches for estimating ε for N-loss were compared including choice
19 of N form for changes in δ¹⁵N (NO₃⁻, DIN, or biogenic N₂), closed vs open system Rayleigh
20 equations, and the basis for calculating the denominator in f (N_p_{expected}, biogenic N₂, or
21 maximum NO₃⁻). For the latter, there was little difference in estimated ε despite discrepancies
22 between the removal of NO₃⁻ and appearance of N₂ estimated from them. Observation of a
23 single water mass (T-S plot) in our coastal region as well as more realistic ranges for derived ε
24 and initial δ¹⁵N indicated that closed system assumptions were more realistic. Using closed
25 system equations, relatively low ε values were calculated; ~7 ‰ for changes in the δ¹⁵N of DIN
26 and ~11 ‰ for changes in the δ¹⁵N of biogenic N₂. As in Bourbonnais et al. (2015), ε calculated
27 from changes in the δ¹⁵N of NO₃⁻ alone was not representative of the ε for overall N-loss in
28 consideration of the build up of NO₂⁻ with distinct δ¹⁵N. These estimates for ε for net N-loss are
29 lower than recently reported for a nearby offshore eddy with intense N-loss (~14%;
30 Bourbonnais et al., 2015). This lower ε may be attributed to the influence of sedimentary N-
31 loss on the Peruvian shelf (e.g., Bohlen et al., 2011), with a highly suppressed ε on the overlying

Annie Bourbonnais 1/11/2016 3:46 PM

Deleted: dominance

Annie Bourbonnais 1/11/2016 3:50 PM

Deleted: reduction

Annie Bourbonnais 1/11/2016 3:50 PM

Deleted: oxidation

Annie Bourbonnais 1/11/2016 3:52 PM

Deleted: at least

LocalAdmin 1/13/2016 3:44 PM

Deleted: (

LocalAdmin 1/13/2016 3:47 PM

Deleted: ,

LocalAdmin 1/13/2016 3:47 PM

Deleted: ,

1 water column at our shallow stations. Given this assumption, we estimate that sedimentary N-
2 loss (by both denitrification and anammox) could account for up to 60% of the total N-loss in in
3 shore Peru ODZ waters.

4 Our results further support geographical variations in the ϵ of N-loss in ODZ'S, possibly related
5 to the effects of varying primary productivity and microbial growth rates on the expression of ϵ
6 and partitioning between water-column and sedimentary denitrification. These variations need
7 to be considered in future global isotopic N budget (e.g. see Brandes and Devol, 2002),
8 potentially bringing the global N budget more in balance. This is further supported by the
9 relatively lower ϵ for N-loss of ~14‰ recently observed offshore in the ETSP ODZ by
10 Bourbonnais et al. (2015). A lower water-column ϵ for N-loss also decreases the fraction of
11 sedimentary denitrification needed to balance the global isotopic N budget (Brandes and Devol,
12 2002; Altabet, 2007).

13

14 **Acknowledgements**

15 Data for this paper are available on the Data Management Portal for Kiel Marine Sciences
16 hosted at GEOMAR: <https://portal.geomar.de/>, or upon request to the corresponding
17 author. This research was supported by the Deutsche Forschungsgemeinschaft- project
18 SFB-754 (Climate-Biogeochemistry Interactions in the Tropical Ocean: www.sfb754.de),
19 SOPRAN II (grant # FKZ 03F0611A; Surface Ocean Processes in the Anthropocene:
20 www.sopran.pangaea.de), the Nitrogen Isotope and N₂/Ar Biogeochemistry of the Peru
21 Suboxic Zone project (National Science Foundation award OCE-0851092), the University
22 of Massachusetts Intercampus Marine Science graduate program support to H. Hu and a
23 NSERC Postdoctoral Fellowship to A.B. We would like to thank the captain and crew of
24 R/V Meteor during the M91 cruise and Daniel Kiefhaber, Avi Bernales and Violeta Leon
25 for their help during sampling and/or sample analysis. We thank the authorities of Peru for
26 the permission to work in their territorial waters.

27

28 **References**

29 Alkhatib, M., Lehmann, M. F., and del Giorgio, P. A.: The Nitrogen Isotope Effect of Benthic
30 Remineralization-Nitrification-Denitrification Coupling in an Estuarine Environment.
31 *Biogeosciences*, 9, 1633-1646, 2012.

1

2 Altabet, M. A.: Isotopic tracers of the marine nitrogen cycle: present and past, in The handbook
3 of environmental chemistry, vol. 2, edited by O. Hutzinger, pp. 251–293, Springer-Verlag,
4 Berlin, Heidelberg, 2005.

5

6 Altabet, M.A.: Constraints on oceanic N balance/imbalance from sedimentary ^{15}N records.
7 *Biogeosciences*, 4, 74-86, 2007.

8

9 Altabet, M. A., and Francois, R.: Sedimentary Nitrogen Isotopic Ratio as a Recorder for
10 Surface Ocean Nitrate Utilization. *Global Biogeochem. Cy.*, 8, 103-116, 1994.

11

12 Altabet, M. A., Francois, R., Murray, D. W., and Prell, W. L.: Climate-related variations in
13 denitrification in the Arabian Sea from sediment $^{15}\text{N}/^{14}\text{N}$ ratios, *Nature*, 373 (6514), 506–509,
14 1995.

15

16 Andersson, K. K., and Hooper, A. B.: O_2 and H_2O are each the source of one O in NO_2^-
17 Produced from NH_3 by *Nitrosomonas*: ^{15}N -NMR evidence. *FEBS Lett.*, 164, 236-240, 1983.

18

19 Babbín, A. R., Keil, R. G. Devol, A. H. and Ward, B. B.: Organic Matter Stoichiometry, Flux,
20 and Oxygen Control Nitrogen Loss in the Ocean. *Science*, 344 (6182): 406–8, 2014.

21

22 Bourbonnais, A., Altabet, M. A., Charoenpong, C. N., Larkum, J., Hu, H., Bange, H. W., and L.
23 Stramma: N-loss isotope effects in the Peru oxygen minimum zone studied using a mesoscale
24 eddy as a natural tracer experiment, *Global Biogeochem. Cycles*, 29,
25 [doi:10.1002/2014GB005001](https://doi.org/10.1002/2014GB005001), 2015.

26

27 Bourbonnais, A., Lehmann, M. F., Hamme, R. C., Manning, C. C, and Kim Juniper, S.: Nitrate
28 elimination and regeneration as evidenced by dissolved inorganic nitrogen isotopes in Saanich
29 Inlet, a seasonally anoxic fjord. *Mar. Chem.*, 157, 194–207, 2013.

30

31 Bourbonnais, A., Lehmann, M. F. Waniek, J. J. and Schulz-Bull, D. E.: Nitrate Isotope
32 Anomalies Reflect N_2 Fixation in the Azores Front Region (subtropical NE Atlantic). *J.*

1 Geophys. Res.: Oceans, 114, C03003, doi:10.1029/2007JC004617, 2009.

2 Brandes, J. A., and Devol, A. H. Isotopic Fractionation of Oxygen and Nitrogen in Coastal
3 Marine Sediments. *Geochim. Cosmochim. Acta* 61 (9): 1793–1801, 1997.

4

5 Brandes, J. A. and Devol, A. H.: A global marine-fixed nitrogen isotopic budget: Implications
6 for Holocene nitrogen cycling. *Global Biogeochem. Cy.*, 16 (4), 67–1–67–14, 2002.

7

8 Brandes J. A., Devol, A. H., Yoshinari, T., Jayakumar D. A., and Naqvi, S. W. A.: Isotopic
9 composition of nitrate in the central Arabian Sea and eastern tropical North Pacific: A tracer for
10 mixing and nitrogen cycles. *Limnol. Oceanogr.*, 43 (7), 1680–1689, 1998.

11

12 Brunner, B. Contreras, S., Lehmann, M. F., Matantseva, O., Rollog, M., Kalvelage, T.,
13 Klockgether, G., Lavik, G., Jetten, M. S. M., Kartal, B., and Kuypers, M. M. M: Nitrogen
14 isotope effects induced by anammox bacteria. *PNAS*, 110 (47), 18994–18999, 2013.

15

16 Bryant, J. P., Chapin, F. S., and Klein, D. R. Carbon nutrient balance of boreal plants in relation
17 to vertebrate herbivory. *Oikos*, 40 (3): 357–68, 1983.

18

19 Buchwald, C. and Casciotti, K. L.: Isotopic ratios of nitrite as tracers of the sources and age of
20 oceanic nitrite. *Nat. Geosci.*, 6 (4), 308–313, 2013.

21

22 Buchwald, C. and Casciotti, K. L.: Oxygen isotopic fractionation and exchange during bacterial
23 nitrite oxidation. *Limnol. Oceanogr.*, 55 (3), 1064–1074, 2010.

24

25 Casciotti, K. L.: Inverse kinetic isotope fractionation during bacterial nitrite oxidation.
26 *Geochim. Cosmochim. Acta*, 73 (7), 2061–2076, 2009.

27

28 Casciotti, K. L., Böhlke, J. K., McIlvin, M. R., Mroczkowski, S. J., and Hannon, J. E.: Oxygen
29 isotopes in nitrite: analysis, calibration, and equilibration. *Anal. Chem.*, 79 (6), 2427–2436,
30 2007.

31

1 Casciotti, K. L. and Buchwald, C.: Insights on the marine microbial nitrogen cycle from
2 isotopic approaches to nitrification. *Front. Microbiol.*, 3, 1–14, 356, 2012.
3
4 Casciotti, K. L., Buchwald, C., and McIlvin, M.: Implications of nitrate and nitrite isotopic
5 measurements for the mechanisms of nitrogen cycling in the Peru oxygen deficient zone. *Deep-*
6 *Sea Res. Part I*, 80, 78–93, 2013.
7
8 Casciotti, K. L. and McIlvin, M. R.: Isotopic analyses of nitrate and nitrite from reference
9 mixtures and application to eastern tropical North Pacific waters. *Mar. Chem.*, 107 (2), 184–
10 201, 2007.
11
12 Casciotti, K. L., McIlvin, M. and Buchwald, C. Oxygen Isotopic Exchange and Fractionation
13 during Bacterial Ammonia Oxidation. *Limnol. Oceanogr.*, 55 (2): 753–762, 2010.
14
15 Casciotti, K. L. Sigman, D. M., Hastings, M. G., Böhlke, J. K., and Hilkert, A.: Measurement
16 of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier
17 method. *Anal. Chem.*, 74 (19), 4905–4912, 2002.
18
19 Chang, B. X., Devol, A. H., and Emerson, S. R.: Denitrification and the nitrogen gas excess in
20 the eastern tropical South Pacific oxygen deficient zone. *Deep-Sea Res. Part I: Oceanographic*
21 *Research Papers*, 57 (9), 1092–1101, 2010.
22
23 Cline, J. D., and Kaplan, I. R.: Isotopic Fractionation of Dissolved Nitrate during
24 Denitrification in the Eastern Tropical North Pacific Ocean. *Mar. Chem.*, 3 (4), 271–99, 1975.
25
26 Charoenpong, C. N., Bristow, L. A., and Altabet, M. A.: A continuous flow isotope ratio mass
27 spectrometry method for high precision determination of dissolved gas ratios and isotopic
28 composition, *Limnol. Oceanogr. Methods*, 12, 323–337, 2014.
29
30 Codispoti, L. A.: An oceanic fixed nitrogen sink exceeding 400 Tg N a⁻¹, *Biogeosciences*, 4,
31 233–253, 2007.
32

1 Codispoti, L. A. and Christensen, J. P.: Nitrification, denitrification and nitrous oxide cycling in
2 the eastern tropical South Pacific ocean. *Mar. Chem.*, 16 (4), 277–300, 1985.
3
4 Codispoti, L. A., Friederich, G. E., Packard, T. T., Glover, H. E., Kelly, P. J., Spinrad, R. W.,
5 and Barber, R. T.: High Nitrite Levels off Northern Peru: A Signal of Instability in the Marine
6 Denitrification Rate. *Science*, 233 (4769): 1200–1202, 1986.
7
8 Dalsgaard, T., Thamdrup, B., Fariás, L., and Revsbech, N. P.: Anammox and denitrification in
9 the oxygen minimum zone of the eastern South Pacific. *Limnol. Oceanogr.*, 57, 1331-1346,
10 2012.
11
12 Dalsgaard, T., Stewart, F. J., Thamdrup, B., Brabandere, L. D., Revsbech, P., and Ulloa, O.:
13 Oxygen at Nanomolar Levels Reversibly Suppresses Process Rates and Gene Expression in
14 Anammox and Denitrification in the Oxygen. *mBio*, 5, e01966-14, doi:10.1128/mBio.01966-
15 14, 2014.
16
17 Deutsch, C., Sarmiento, J. L., Sigman, D. M., Gruber, N., and Dunne, J. P.: Spatial
18 coupling of nitrogen inputs and losses in the ocean, *Nature*, 445, 163–167, 2007.
19
20 Dore, J. E., and Karl, D. M.: Nitrification in the Euphotic Zone as a Source for Nitrite, Nitrate,
21 and Nitrous Oxide at Station ALOHA. *Limnol. Oceanogr.*, 41 (8), 1619–1628, 1996.
22
23 Emerson, S., Stump, C., Wilbur, D., and Quay, P.: Accurate measurement of O₂, N₂, and Ar
24 gases in water and the solubility of N₂. *Mar. Chem.*, 64 (4), 337–347, 1999.
25
26 Eugster, O. and Gruber, N.: A probabilistic estimate of global marine N-fixation and
27 denitrification, *Global Biogeochemical Cycles*, 26, 1–15, 2012.
28
29 Falkowski, P. G., Evolution of the Nitrogen Cycle and Its Influence on the Biological
30 Sequestration of CO₂ in the Ocean. *Nature*, 387 (6630), 272–275, 1997.
31

1 Friedman, S. H., Massefski, W., and Hollocher, T. C.: Catalysis of intermolecular oxygen atom
2 transfer by nitrite dehydrogenase of *Nitrobacter agilis*. *J. Biol. Chem.*, 261 (23), 10538–10543,
3 1986.
4
5 Füssel, J., Lam, P., Lavik, G., Jensen, M. M., Holtappels, M., Günter, M., and Kuypers, M. M.
6 M.: Nitrite oxidation in the Namibian oxygen minimum zone. *ISME J.*, 6 (6), 1200–1209,
7 2012.
8
9 Granger, J. and Sigman, D. M.: Removal of nitrite with sulfamic acid for nitrate N and O
10 isotope analysis with the denitrifier method. *Rapid Commun. Mass Spectrom.*, 23 (23), 3753–
11 3762, 2009.
12
13 Granger, J., Sigman, D. M., Lehmann, M. F. and Tortell, P. D.: Nitrogen and oxygen isotope
14 fractionation during dissimilatory nitrate reduction by denitrifying bacteria. *Limnol. Oceanogr.*,
15 53 (6), 2533–2545, 2008.
16
17 Granger, J., Sigman, D. M., Needoba, J. A., and Harrison, P. J.: Coupled nitrogen and oxygen
18 isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton.
19 *Limnol. Oceanogr.*, 49 (5), 1763–1773, 2004.
20
21 Großkopf, T., Mohr, W., Baustian, T., Schunck, H., Gill, D., Kuypers, M. M. M., Lavik, G.,
22 Schmitz, R. A., Wallace, D. W. R., and LaRoche, J.: Doubling of marine dinitrogen-fixation
23 rates based on direct measurements. *Nature*, 488, 361–364, 2012.
24
25 Gruber, N.: The dynamics of the marine nitrogen cycle and its influence on atmospheric CO₂
26 variations, in: *The Ocean Carbon Cycle and Climate*, edited by M. Follows and T. Oguz, pp.
27 97–148, Kluwer Academic, Dordrecht, The Netherlands, 2004.
28
29 Gruber N.: The marine nitrogen cycle: Overview of distributions and processes, in *Nitrogen in*
30 *the Marine Environment*, 2nd ed., edited by Capone, D. G., Bronk, D. A., Mulholland, M. R.,
31 and Carpenter, E. J., Elsevier, Amsterdam, 1–50, 2008.
32

1 Hamersley, M. R., Lavik, G., Woebken, D., Rattray, J. E., Lam, P., Hopmans, E. C., Damsté, J.
2 S. S., Krüger, S., Graco, M., Gutiérrez, D., Kuypers, M. M. M.: Anaerobic ammonium
3 oxidation in the Peruvian oxygen minimum zone. *Limnol. Oceanogr.*, 52, 923-933, 2007.
4
5 Hamme, R. C.: Mechanisms Controlling the Global Oceanic Distribution of the Inert Gases
6 Argon, Nitrogen and Neon. *Geophys. Res. Lett.*, 29 (23), 35-1-35-4, 2002.
7
8 Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Loscher, C. R., Oschlies, A.,
9 Paulmier, A., Stramma, L., and Kuypers, M. M. M.: Nitrogen Cycling Driven by Organic
10 Matter Export in the South Pacific Oxygen Minimum Zone. *Nat. Geosci.*, 6 (3), 228–234, 2013.
11
12 Kalvelage, T., Jensen, M. M., Contreras, S., Revsbech, N. P., Lam, P., Günter, M., LaRoche,
13 J., Lavik, G., and Kuypers, M. M. M.: Oxygen sensitivity of anammox and coupled N-cycle
14 processes in oxygen minimum zones. *Plos ONE*, 6:e29299.
15
16 Kritee K., Sigman, D. M., Granger, J., Ward, B. B., Jayakumar, A., and Deutsch, C.: Reduced
17 isotope fractionation by denitrification under conditions relevant to the ocean. *Geochim.*
18 *Cosmochim. Acta*, 92, 243–259, 2012.
19
20 Kuypers M. M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B. M., Amann, R., Jørgensen, B.
21 B., and Jetten, M. S. M.: Massive nitrogen loss from the Benguela upwelling system through
22 anaerobic ammonium oxidation. *PNAS*, 102(18):6478–6483, 2005.
23 Kuypers, M. M. M., Sliekers, A. O., Lavik, G., Schmid, M., Jørgensen, B. B., Kuenen, J. G.,
24 Sinninghe Damsté, J. S., Strous, M., and Jetten, M. S. M.: Anaerobic ammonium oxidation by
25 anammox bacteria in the Black Sea. *Nature*, 422 (6932), 608–611, 2003.
26
27 Lam, P., and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum
28 zones. *Annual Review of Marine Science*, 3, 317-345, 2011.
29
30 Lam, P., Jensen, M. M., Kock, A., Lettmann, K. A., Plancherel, Y., Lavik, G., Bange, H. W.,
31 and Kuypers, M. M. M.: Origin and fate of the secondary nitrite maximum in the Arabian Sea.
32 *Biogeosciences*, 8 (6), 1565–1577, 2011.
33

1 Lam, P. Lavik, G., Jensen, M. M., van de Vossenberg, J., Schmid, M., Woebken, D., Gutiérrez,
2 D., Amann, R., Jetten, M. S. M., and Kuypers, M. M. M.: Revising the Nitrogen Cycle in the
3 Peruvian Oxygen Minimum Zone. *PNAS*, 106 (12): 4752–57, 2009.
4
5 Lehmann, M. F., Sigman, D. M., McCorkle, D. C., Granger, J., Hoffmann, S., Cane, G., and
6 Brunelle, B. G.: The Distribution of Nitrate $^{15}\text{N}/^{14}\text{N}$ in Marine Sediments and the Impact of
7 Benthic Nitrogen Loss on the Isotopic Composition of Oceanic Nitrate. *Geochim. Cosmochim.*
8 *Acta*, 71 (22): 5384–5404, 2007.
9
10 Lipschultz, F., Wofsy, S. C., Ward, B. B., Codispoti, L. A., Friedrich, G., and Elkins, J. W.:
11 Bacterial transformations of inorganic nitrogen in the oxygen-deficient waters of the eastern
12 tropical South Pacific Ocean. *Deep-Sea Res.* 37: 1513–1541.
13
14 Liu, K. K.: Geochemistry of inorganic nitrogen compounds in two marine environments: The
15 Santa Barbara Basin and the ocean off of Peru. Ph.D. thesis, University of California, Los
16 Angeles, 1979.
17
18 Lomas, M.W. and Lipschultz, F.: Forming the primary nitrite maximum: Nitrifiers or
19 phytoplankton? *Limnol. Oceanogr.*, 51 (5), pp.2453–2467, 2006.
20
21 Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P.:
22 Experimental determination of nitrogen kinetic isotope fractionation: Some principles;
23 illustration for the denitrification and nitrification processes. *Plant Soil*, 62 (3), 413–430, 1981.
24
25 McIlvin, M. R. and Altabet, M. A.: Chemical conversion of nitrate and nitrite to nitrous oxide
26 for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal. Chem.*, 77 (17),
27 5589–5595, 2005.
28
29 McIlvin, M. R. and Casciotti, K. L.: Method for the analysis of $\delta^{18}\text{O}$ in water. *Anal. Chem.*, 78
30 (7), 2377–2381, 2006.
31

1 Noffke, A., Hensen, C., Sommer, S., Scholz, F., Bohlen, L., Mosch, T., Graco, M., and
2 Wallman, K.: Benthic iron and phosphorus fluxes across the Peruvian oxygen minimum zone.
3 *Limnol. Oceanogr.*, 57, 851–867, 2012.
4
5 Penven, P., Echevin, V., Pasapera, J., and Tam, J.: Average circulation, seasonal cycle and
6 mesoscale dynamics in the Peru Current System: a modelling approach. *J. Geophys. Res.*,
7 110(C10), C1002110.1029/2005JC002945.
8
9 Richards, F. A., and Benson, B. B.: Nitrogen / argon and nitrogen isotope ratios in two
10 anaerobic environments, the Cariaco Trench in the Caribbean Sea and Drømsfjord, Norway.
11 *Deep-Sea Res.*, 7, 254–264, 1961.
12
13 Ryabenko, E., Kock, A., Bange, H. W., Altabet, M. A., and Wallace, D. W. R.: Contrasting
14 biogeochemistry of nitrogen in the Atlantic and Pacific Oxygen Minimum Zones.
15 *Biogeosciences*, 9, 203–215, 2012.
16
17 Sigman, D. M., Granger, J., DiFiore, P. J., Lehmann, M. M., Ho, R., Cane, G., and Van Geen,
18 A.: Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North
19 Pacific margin. *Global Biogeochem. Cy.*, 19 (4), GB4022 doi: 10.1029/2005GB002458, 2005.
20
21 Sigman, D. M., Robinson, R., Knapp, A. N., Van Geen, A., McCorkle, D. C., Brandes, J. A.,
22 and Thunell, R. C.: Distinguishing between water column and sedimentary denitrification in the
23 Santa Barbara Basin using the stable isotopes of nitrate. *Geochem., Geophys., Geosyst.*, 4,
24 1040, DOI: 10.1029/2002GC000384, 2003.
25
26 Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., and Bohlke, J. K.: A
27 Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater.
28 *Anal. Chem.*, 73 (17), 4145–4153, 2001.
29
30 Stramma, L., Bange, H. W., Czeschel, R., Lorenzo, A., Frank, M.: On the role of mesoscale
31 eddies for the biological productivity and biogeochemistry in the eastern tropical Pacific Ocean
32 off Peru. *Biogeosciences*, 10, 7293–7306, doi:10.5194/bgd-10-9179-2013, 2013.
33

1 Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M. W., and Horn, M.:
2 Deciphering the Evolution and Metabolism of an Anammox Bacterium from a Community
3 Genome. *Nature*, 440 (7085), 790–794, 2006.
4
5 Ulloa, O., Canfield, D. E., DeLong, E. F., Letelier, R. M., and Stewart, F. J.: Microbial
6 oceanography of anoxic oxygen minimum zones. *PNAS*, 109, 15996-16003, 2012.
7
8 Voss, M., Dippner, J.W., and Montoya, J. P.: Nitrogen Isotope Patterns in the Oxygen-
9 Deficient Waters of the Eastern Tropical North Pacific Ocean. *Deep Sea Res. Part I*, 48 (8),
10 1905-1921, 2001.
11
12 Wada, E., and Hattori, A.: Nitrogen isotope effects in the assimilation of inorganic compounds
13 by marine diatoms. *Geomicrobiol. J.*, 1 (1), 85-101, 1978.
14
15 Ward, B. B., Devol, A. H., Rich, J. J., Chang, B. X., Bulow, S. E., Naik, H., Pratihary, A., and
16 Jayakumar, A.: Denitrification as the Dominant Nitrogen Loss Process in the Arabian Sea.
17 *Nature*, 461 (7260), 78–81, 2009.
18

1 **Table 1.** ϵ for NO_3^- reduction and net N loss estimated from both DIN consumption and
 2 produced biogenic N_2 using Rayleigh closed system equations (eqs. 1-3). Results are calculated
 3 for f based on either $\text{Np}_{\text{expected}}$ (eqs. 7-9), biogenic N_2 (eqs. 12-14) and measured substrate
 4 divided by maximum (upwelled) substrate concentrations (see text, section 2.4). The standard
 5 error of the slope (ϵ) is shown.

	Basis for f	ϵ	y-intercept	r^2
$\delta^{15}\text{N-NO}_3^-$	$\text{Np}_{\text{expected}}$	13.9 ± 0.7	3.74	0.92
	N_2 Biogenic	14.3 ± 0.9	3.71	0.95
	$[\text{NO}_3^-]/[\text{NO}_3^-]_{\text{max}}$	14.7 ± 0.6	-0.55	0.95
$\delta^{15}\text{N-DIN}$	$\text{Np}_{\text{expected}}$	6.3 ± 0.3	7.20	0.92
	N_2 Biogenic	6.6 ± 0.4	6.71	0.94
	$\text{DIN}/\text{DIN}_{\text{max}}$	7.4 ± 0.6	10.90	0.91
$\delta^{15}\text{N-}$ Biogenic	$\text{Np}_{\text{expected}}$	10.5 ± 1.5	2.94	0.70
	N_2 Biogenic	10.6 ± 1.5	3.04	0.72

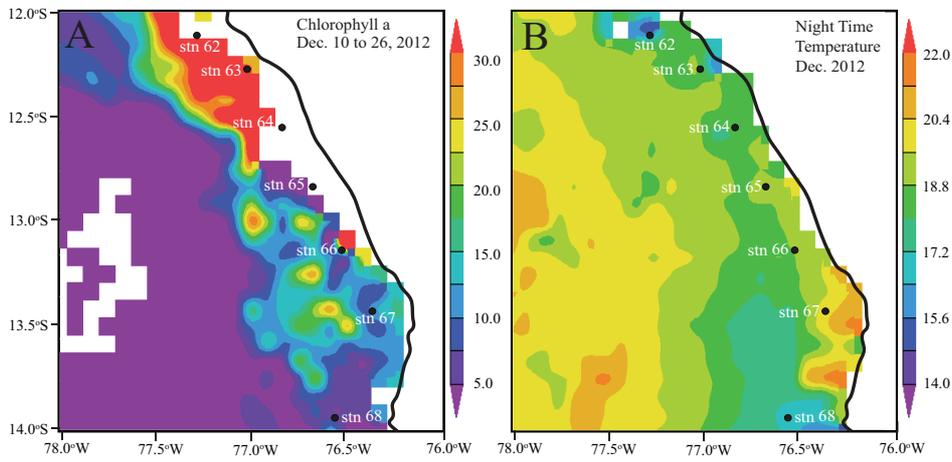
6
7

1 **Table 2.** ϵ for NO_3^- reduction, and net N loss estimated from both DIN consumption and
 2 produced biogenic N_2 using Rayleigh open system equations (eqs. 4-6). Results are calculated
 3 for f based on either $\text{Np}_{\text{expected}}$ (eqs. 7-9), biogenic N_2 (eqs. 12-14) and measured substrate
 4 divided by maximum (upwelled) substrate concentrations (see text, section 2.4). The standard
 5 error of the slope (ϵ) is shown.

	Basis for f	ϵ	y-intercept	r^2
$\delta^{15}\text{N-NO}_3^-$	$\text{Np}_{\text{expected}}$	63.0 ± 4.5	-18.42	0.86
	N_2 Biogenic	66.30 ± 6.2	-21.92	0.87
	$[\text{NO}_3^-]/[\text{NO}_3^-]_{\text{max}}$	38.9 ± 2.7	6.19	0.87
$\delta^{15}\text{N-DIN}$	$\text{Np}_{\text{expected}}$	17.4 ± 1.2	3.26	0.88
	N_2 Biogenic	20.0 ± 1.8	1.72	0.89
	$\text{DIN}/\text{DIN}_{\text{max}}$	13.2 ± 0.9	8.45	0.91
$\delta^{15}\text{N-}$ Biogenic N_2	$\text{Np}_{\text{expected}}$	12.3 ± 1.9	1.94	0.67
	N_2 Biogenic	14.15 ± 2.1	2.25	0.68

6
7

1 **Figures**



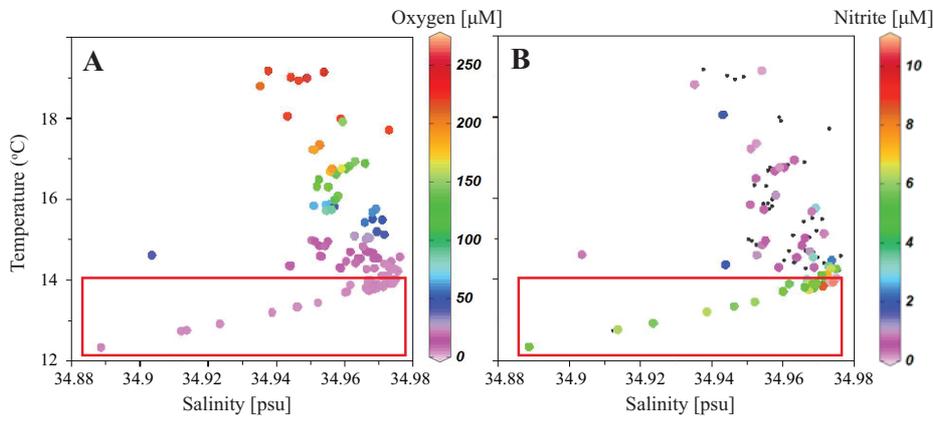
2

3 **Figure 1.** Station map with satellite data from <http://disc.sci.gsfc.nasa.gov/giovanni/>. A: sea
4 surface chlorophyll a concentrations (mg/m^3), B: night time sea surface temperature ($^{\circ}\text{C}$).

5

Annie Bourbonnais 1/14/2016 11:54 AM
Formatted: Font:Times New Roman, Italic

Annie Bourbonnais 1/14/2016 11:53 AM
Deleted: α



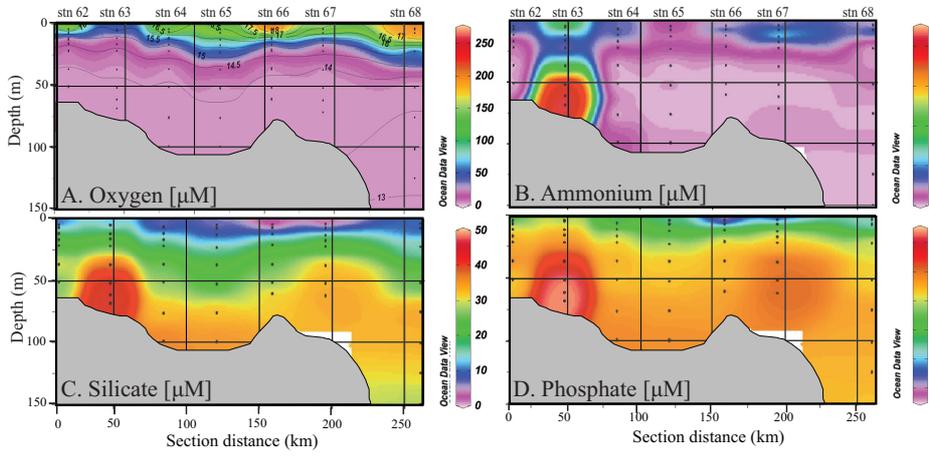
1
2
3
4
5
6

Figure 2. Temperature vs. salinity plots. In A, color indicates O₂ concentration (μM). In B, color indicates NO₂⁻ concentration (μM). Black dots in B mean no NO₂⁻ concentration data are available. Points in red rectangle at bottom of each plot belong to station 68 for depths greater than 150m.

LocalAdmin 1/13/2016 3:49 PM
Moved (insertion) [1]

LocalAdmin 1/13/2016 3:50 PM
Deleted: at bottom

LocalAdmin 1/13/2016 3:49 PM
Moved up [1]: Black dots in B mean no NO₂⁻ concentration data are available.



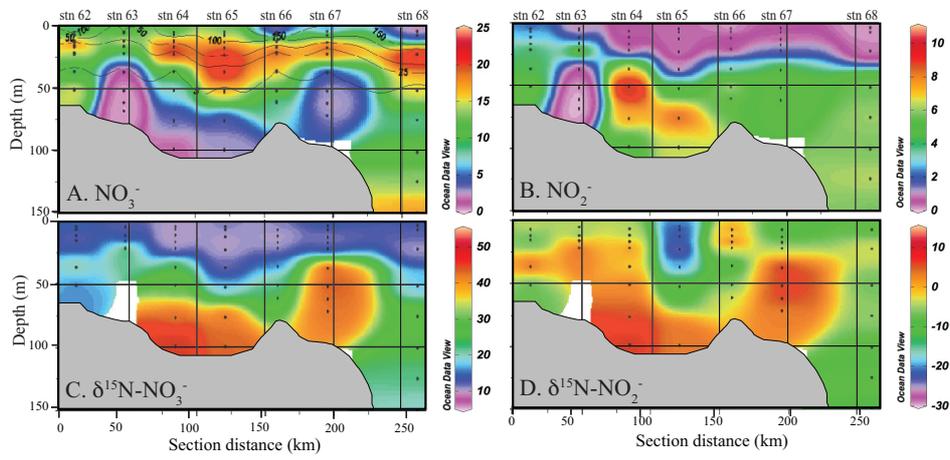
1

2 **Figure 3.** O₂ and nutrient distribution along the transect. A: O₂ concentration (μM) with
 3 isotherm overlay. B: NH₄⁺ concentration (μM), C: Si(OH)₄ concentration (μM) and D:
 4 PO₄³⁻ concentration (μM). Grey region represents bathymetry. The depth for station 68
 5 is 253m.

6

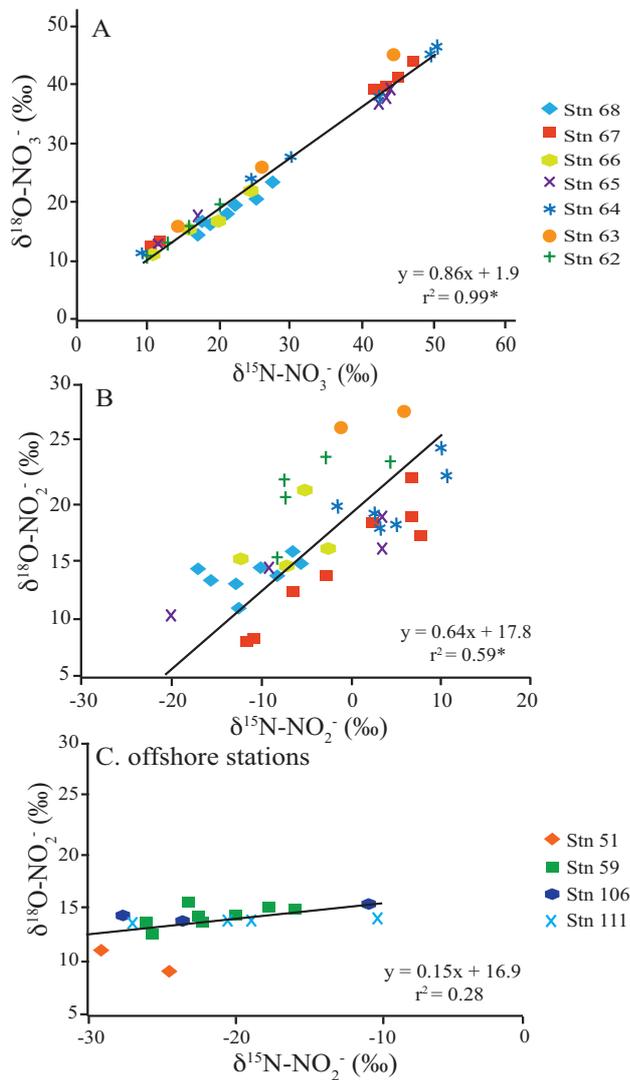
LocalAdmin 1/13/2016 3:50 PM

Deleted: and



1

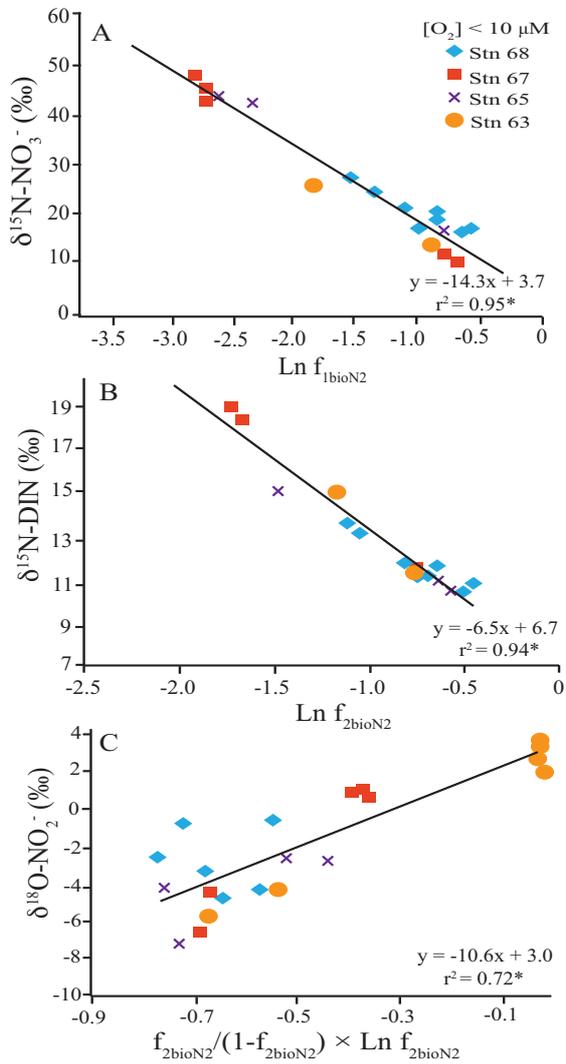
2 **Figure 4.** Transects off the Peru coast for A: NO_3^- concentration (μM) with O_2 overlay,
 3 B: NO_2^- concentration (μM), C: $\delta^{15}\text{N}\text{-NO}_3^-$ (‰) and D: $\delta^{15}\text{N}\text{-NO}_2^-$ (‰). Grey region
 4 represents approximate bathymetry. No isotopic data are available for the deeper
 5 samples collected at station 63, because NO_3^- and NO_2^- concentrations were below
 6 analytical limits ($<0.5\mu\text{M}$).
 7



1

2 **Figure 5.** Relationships between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for NO_3^- and NO_2^- , respectively, for
 3 $\text{O}_2 \leq 10 \mu\text{M}$. A: $\delta^{18}\text{O}-\text{NO}_3^-$ vs $\delta^{15}\text{N}-\text{NO}_3^-$ for station 62 to 68. B: $\delta^{18}\text{O}-\text{NO}_2^-$ vs $\delta^{15}\text{N}-\text{NO}_2^-$
 4 for station 62 to 68. C: $\delta^{18}\text{O}-\text{NO}_2^-$ vs $\delta^{15}\text{N}-\text{NO}_2^-$ for M90 offshore stations 51, 59, 106
 5 and 111 (see text, section 3.3). For each plot, overall linear regressions are shown.
 6 Significant correlation coefficients at a 0.05 significance level are denoted by *.

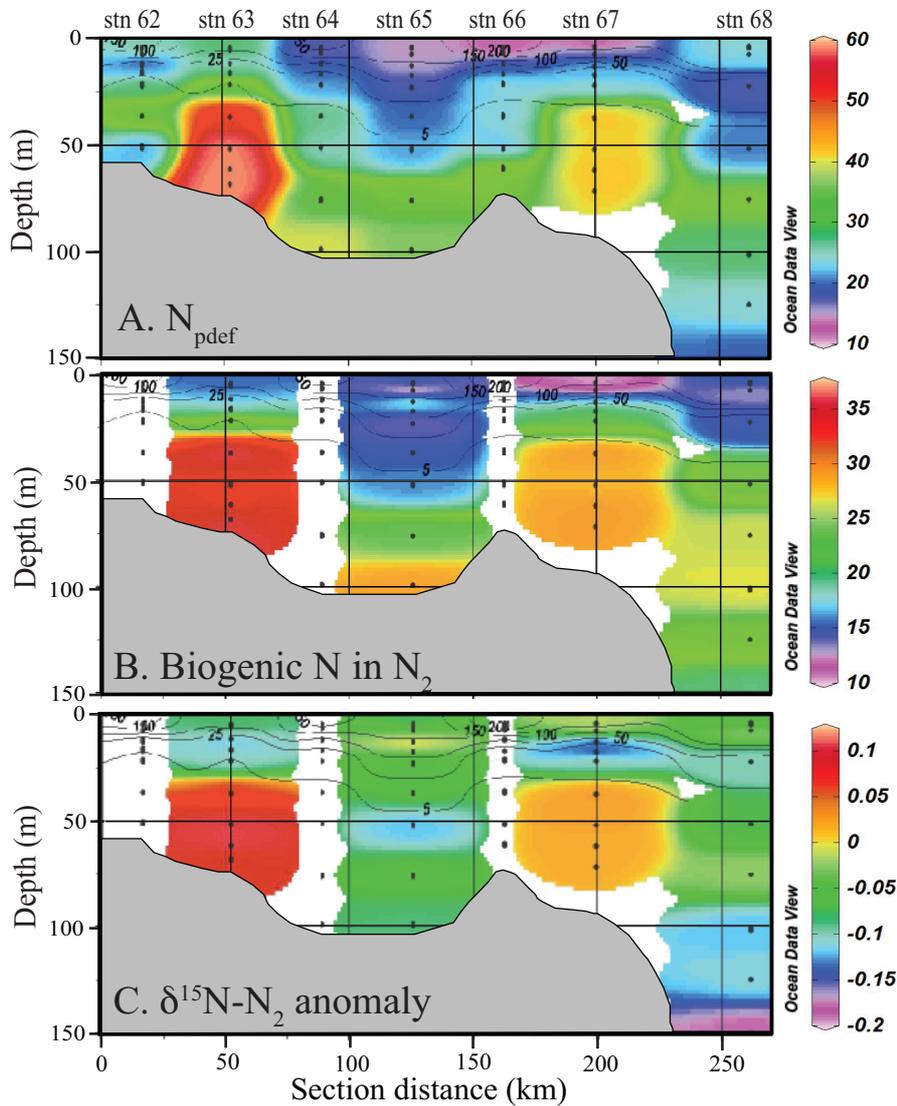
7



1

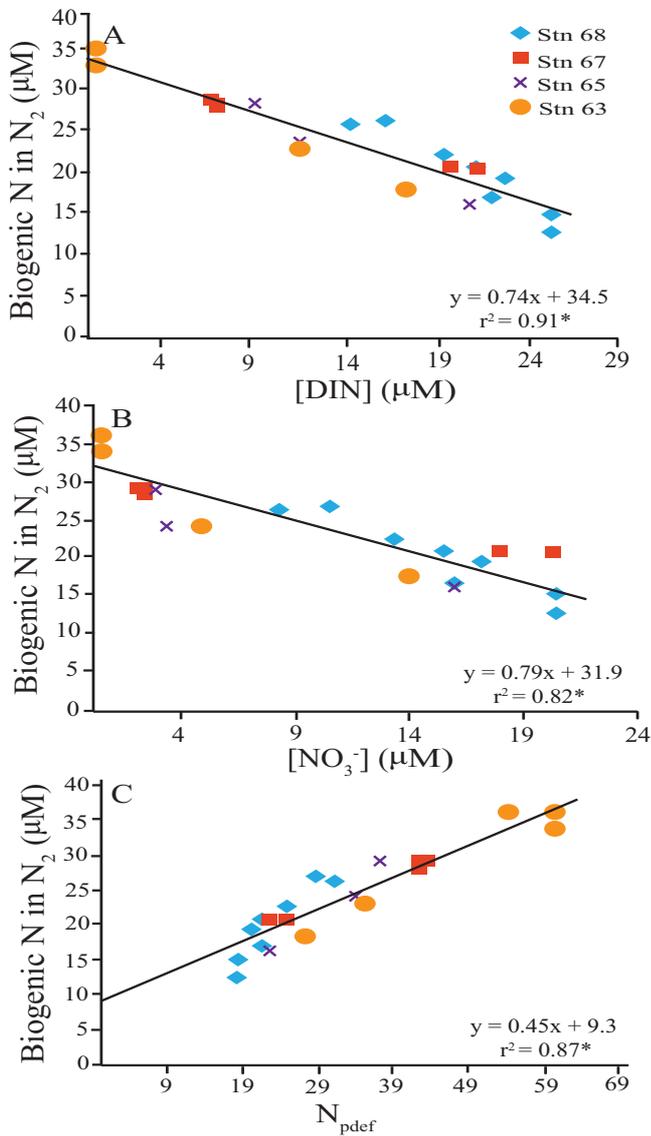
2 **Figure 6.** Raleigh relationships used to estimate ϵ (slope) and initial $\delta^{15}\text{N}$ -substrate (y-
 3 intercept) assuming a closed system. A: for NO_3^- reduction (Eq. 1 and text, section 2.4),
 4 B: for N-loss calculated from the substrate (DIN) consumption (Eq. 2 and text, section
 5 2.4) and C: for N-loss calculated from the $\delta^{15}\text{N}$ of biogenic N_2 (Eq. 3 and text, section
 6 2.4). In C, only samples with O_2 concentrations less than $10\ \mu\text{M}$ and biogenic N_2 values
 7 $>7.5\ \mu\text{M}$ were considered. Significant correlation coefficients at a 0.05 significance level
 8 are denoted by *.

9



1
 2 **Figure 7.** N deficit, biogenic N in N_2 and $\delta^{15}\text{N}-N_2$ anomaly with O_2 overlaid. A: N
 3 deficit calculated using PO_4^{3-} (μM) (N_{pdef}) and assuming Redfield stoichiometry (see
 4 Eqs. 9, 10 and 11, section 2.4). B: biogenic N in N_2 (μM). C: $\delta^{15}\text{N}-N_2$ anomaly relative
 5 to equilibrium with atmosphere (‰). Biogenic N_2 or $\delta^{15}\text{N}-N_2$ anomaly were not
 6 measured at stations 62, 64 and 66.

7



1
 2 **Figure 8.** Cross-plots of biogenic N in N₂ versus DIN (A), NO₃⁻ (B) and N_{pdef} (C), see Eqs. 9-
 3 11 in text). All plots have the overall linear regression overlaid. All the points are restricted to
 4 O₂ concentrations less than 10 µM. Biogenic N₂ was not measured for stations 62, 64 and 66.
 5 Significant correlation coefficients at a 0.05 significance level are denoted by *.

6

Annie Bourbonnais 1/14/2016 12:12 PM

Deleted:
 Unknown
 Formatted: Font:Bold