Author's response to reviewers

Anonymous Referee #1
Received and published: 30 June 2015

This was a generally well-written paper, describing the use of nitrate and nitrite N and O stable isotopes, as well as biogenic N_2 and $\delta^{15}N$-N_2 analyses to examine the cycling of N in oxygen-deficient Peruvian coastal waters. I particularly liked the variety of approaches used to estimate the isotope effect for N loss, including the measurement of the end product (N_2). It is a solid contribution to the literature and I had relatively minor comments for the authors, listed below.

Our response: We thank reviewer #1 for his/her helpful comments. We generally addressed all concerns below.

Specific comments:

p. 7259, lines 11-12: I know what you mean here, but found the wording to be a bit ambiguous, as neither NO_3^- or O_2 donate electrons in respiration. I think you could simply say "...in lieu of oxygen (O_2) for respiration."

Our response: Done.

p. 7261, lines 1-2: The wording here is also slightly ambiguous as the inverse isotope effect for NO_2^- oxidation is atypical of biochemical reactions, but is uniformly observed in nitrite oxidizing organisms.

Our response: We changed the sentence for: "… NO_2^- oxidation is associated with an inverse N isotope effect (Casciotti, 2009), atypical of biogeochemical reactions, …"

p. 7261, lines 4-6: On what basis is this "analogous relationship" expected?

Our response: We changed the sentence for: "Logically, NO_2^- reduction would be expected to produce a positive relationship between $\delta^{15}N$-NO_2^- and $\delta^{18}O$-NO_2^- though there are no quantitative observations in the literature. Analogous to NO_3^- reduction, it also involves enzymatic breakage of the N-O bond."

p. 7261, lines 10-12: I don't think that this is really still under discussion. Many more recent papers suggest a balanced budget. The citations used here are pretty outdated at this point. It's clear that there are still many questions regarding the mechanisms of N loss and cycling in oxygen deficient waters, but the budget seems pretty well understood, at least better than the cited 400 Tg/year imbalance.

Our response: We changed the text accordingly, citing more recent studies: "Current estimates from direct observations and models for N_2 fixation, considered the primary marine N source, range from 110-330 Tg N yr^{-1} (Brandes and Devol, 2002; Gruber, 2004;

Deutsch et al., 2007; Eugster and Gruber, 2012; Groβkopf et al., 2012). Estimates for major marine N-sinks, i.e., denitrification and anammox in the water-column of oxygen deficient zones and sediments account for 145-450 Tg N yr^{-1} (Gruber, 2004; Codispoti, 2007; DeVries et al., 2012; Eugster and Gruber, 2012)."

p. 7263, lines 12-13: It is my understanding that Casciotti and Bohlke have not distributed these primary NO_2^- isotope standards due to the possession of limited amounts. Perhaps the Altabet lab is an exception, but is it possible that you mean that your laboratory standards were calibrated against N23, N7373 and N10219?

Our response: We have these standards in our laboratory. We changed the text, to include the $\delta^{15}N/\delta^{18}O$ of these standards, as requested by reviewer #2: "In-house (i.e., MAA1, $\delta^{15}N$ = -60.6‰; MAA2, $\delta^{15}N$ = 3.9‰; Zh1, $\delta^{15}N$ = -16.4%) and other laboratory calibration standards (N23, $\delta^{15}N$ = 3.7‰ and $\delta^{18}O$ = 11.4‰; N7373, $\delta^{15}N$ = -79.6‰ and $\delta^{18}O$ = 4.5‰; and N10219; $\delta^{15}N$ = 2.8‰ and $\delta^{18}O$ = 88.5‰; see Casciotti and McIlvin, 2007) were used for NO^{2-} $\delta^{15}N$ and $\delta^{18}O$ analysis."

p. 7263, lines 14-16: These are not appear among the proportions tested by Granger and Sigman (2009). Do the authors have independent evidence for the removal of NO_2^- under these conditions?

Our response: We always use this method in Altabet's laboratory for NO_2^- removal immediately following sample collection. Of course, we previously thoroughly tested that all NO_2^- was removed under these conditions.

p. 7265, lines 6-7: This is an interesting finding, but it might be worth emphasizing here that it is very much an effective isotope effect since it involves the expression of fractionation at several steps between NO_3^-, NO_2^- and N_2.

Our response: Done.

p. 7265, lines 20-21: I think you mean that the maximum biogenic N_2 observed in this study was 20 µM, but found the wording here to be a bit ambiguous since it is given parenthetically after "small levels of biogenic N_2". I think you want to say that for biogenic N_2 levels less than 7.5 µM, the error associated with the calculation becomes too high?

Our response: We changed the sentence for: "greater than 7.5 µM because of increasing noise below this level due to the huge atmospheric dissolved N_2 background (typically up to ~500 µM)."

p. 7266, lines 22-23: I like this use of biogenic N_2 measurements.

p. 7267, lines 5-6: This third approach could use another sentence of clarification about the maximum $[NO_3^-]$ or [DIN] used. Is this drawn from an individual profile, or elsewhere along the isopycnal?

Our response: We changed the sentence for: "… by observed maximum [NO_3^-] or [DIN] for the source of the upwelled waters (see red rectangles in Fig. 2)."

p. 7271, line 18: I thought the background was closer to 500 µM, as stated on p. 7265.

Our response: We were referring here to µM N in N_2. We modified the text to express the background in µM N_2 (400 to 500 µM N_2) as previously, to avoid confusion.

p. 7272, lines 27-28: This sentence should also cite Buchwald and Casciotti, 2013 for T dependence of the equilibrium isotope effect.

Our response: We added this reference.

p. 7273, lines 18-19: The $\delta^{18}O$ source values as low as -8‰ as estimated by calculations in the cited papers do not appear to be representative of marine systems. Values between -1.5‰ and +1.3‰ appear to be more appropriate, as described in a more recent paper (Buchwald et al., 2012).

Our response: Noted. We changed the text accordingly.

p. 7274, lines 10-15: This is an interesting calculation of NO_2^- turnover time, but a few clarifications would be helpful. In particular, whether your estimates represent an upper or lower limit of turnover time. Figure 2 in Buchwald and Casciotti, 2013 results from a batch time course experiment and does not involve a steady state assumption. I would recommend calculating the rate constant for NO_2^- exchange at the appropriate T and pH, using equation 1 in Buchwald and Casciotti, 2013. You can then apply this to your steady-state model to calculate the NO_2^- turnover time. Since you don't know where you started in $\delta^{18}O$ space, the NO_2^- could be older than your estimate, giving you what I would consider a lower limit of turnover time (i.e., it could be longer). You state this more clearly in your conclusions (p. 7280, lines 6-7).

Our response: Using equation 1 in Buchwald and Caciotti (2013), we calculated a k, the specific rate of abiotic oxygen atom equilibration between nitrite and water, of 0.0765 day^{-1} and a reciprocal of 13.07 days. Considering that isotope equilibrium occurs as an exponential decay, ~100% equilibration is reached at 3 e-foldings (3/k), we can estimate an equilibration time of ~13.07 x 3 = 39 days using this method, which is equivalent to the ~40 days we estimated from Fig. 2.
We also modified the text to clarify that our estimate is a lower limit of turnover time: "… we estimated an equilibration time of at least ~40 days for pH near 7.8 (estimated from equation 1 and Fig. 2 in Buchwald and Casciotti, 2013). A turnover time as low as 40 days implies a flux of N through the NO_2^- pool of at least 0.21 µM d^{-1}, as estimated from the maximum NO_2^- concentration observed in this study divided by this estimated turnover time."

p. 7276, lines 1-2: I think the critical point here is that the $\delta^{18}O-NO_3^-$ added back is lower than the ambient $\delta^{18}O-NO_3^-$ at these high $\delta^{18}O$ levels, not that the $\delta^{15}N$ is high due to the inverse kinetic isotope effect. Even with the inverse fractionation, the low $\delta^{15}N$ of NO_2^- should produce NO_3^- with a low $\delta^{15}N$. You state this more clearly in your conclusions (p. 7280, line 15).

Our response: We agree and changed the sentence for: "Our observed deviation toward slopes < 1 can instead be explained by the addition of newly nitrified NO_3^- with a lower $\delta^{18}O-NO_3^-$, mostly derived from water (Andersson and Hooper, 1983), relative to the high ambient $\delta^{18}O-NO_3^-$ values."

p. 7276, lines 2-4: This was also argued in Casciotti et al., 2013.

Our response: We added this reference.

p. 7277, lines 8-10: Could you elaborate on how a contribution of NH_4^+ derived from organic matter would raise the calculated isotope effect? It's not clear that it should have this effect since the $\delta^{15}N$ of organic matter is relatively high in this area.

Our response: In fact, we measured relatively low $\delta^{15}N-NH_4^+$ values (between 3.8‰ and 6.1‰) in bottom waters at shallow stations located close to the studied area during the SFB754 M92 cruise that took place one month later, in January 2013 (unpublished results). The NH_4^+ concentrations at these stations ranged from 5.3 to 7.5 µM. Thus a contribution of NH_4^+ from organic material and consumption by anammox bacteria could theoretically raise the isotope effect derived from biogenic N_2. We added two sentences to clarify this point: "Supporting this hypothesis, NH_4^+ accumulation (5.3-7.5 µM) associated with a relatively low $\delta^{15}N-NH_4^+$ of 3.8‰ to 6.1‰ was observed at 125 and 200 m bottom water depths at shallow stations located in the studied area (~12.3°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 $\delta^{15}N$ to the biogenic N_2 pool, increasing ε that must be larger to account for the observed isotopic enrichment."

p. 7277, line 12: I think you mean when f=1, not when f=0, as ln(0) is undefined while ln(1) = 0.

Our response: We meant when f=1. We corrected this in the text.

p. 7278, lines 8-22: Can you say anything about the role of anammox based on your findings in comparing the isotope effects calculated in different ways?

Our response: As explained above, anammox could raise the isotope effect derived from biogenic N_2 only if the NH_4^+ consumed is originating from organic material with a relatively low $\delta^{15}N$ (see above). Otherwise, if the NH_4^+ is coming from dissimilatory nitrate reduction to ammonium (DNRA), we should still observe an isotopic balance between the DIN and biogenic N_2 pools. We already previously elaborated on this (see our response to your comment regarding p. 7277, lines 8-10), where it is more in context, and though it would be repetitive to repeat it again here.

p. 7278-7279: What would be the uncertainty on this estimate of % sedimentary denitrification from your analysis?

Our response: We added uncertainties for both ε_{obs} (6.77 ± 0.45‰) and ε_{wc} (13.75 ± 1.3‰). We also now include a range for our estimate of % sedimentary denitrification, i.e., 48 to 64%.

p. 7281, lines 15-16: This statement about most of the N loss occurring in the ETSP is not strongly supported by other studies. For example, Devries et al., 2012 infer similar denitrification rates in ETSP and ETNP.

Our response: We removed this statement.

p. 7281, lines 16-18: I don't disagree with this statement, but it I think it is worth reiterating that you advocate using a 14-15‰ isotope effect for the water column portion of the budget (as in Bourbonnais et al., 2015), and that the 6.5‰ isotope effect you measure here already includes a contribution from sedimentary denitrification.

Our response: We added the value, ~14‰, for the ε of N-loss estimated by Bourbonnais et al. (2015) in the previous sentence (p. 7281, line 14). We already discuss that the observed ε of 6.5‰ includes a contribution from sedimentary denitrification in the previous paragraph.

Figure 7: The significance of this figure, and the relationship between Δδ15N and fbioN2 was not clear. Please clarify or remove the figure.

Our response: We removed this figure.

Figure 10: I didn't think this figure was necessary as the information is more clearly presented in tables 1 and 2.

Our response: We also removed this figure.

Anonymous Referee #2
Received and published: 1 July 2015

General comments:
Hu et al. examine the N- and O isotopic signatures of nitrite, nitrate, and biogenic N_2 to asses the importance of various N-cycling processes in the Peruvian coastal OMZ. They provide a fairly comprehensive set of isotopic data, including measurements of ^{15}N on N_2, a relatively novel approach in deriving water column N-loss estimates. And, while I would like to see those data published, in its current state the manuscript does not clearly convey the main goals, outcomes, and implications of the study. Particularly the

introduction and the discussion section are not very concise and lack structure that guides the (non-expert) reader through the manuscript. Various parts of the introduction do not connect very well and contain unnecessary details that distract from the broader framework, in which the study should be viewed. In the discussion part, the authors' conclusions are not well integrated into the presentation of prior work. What I am particularly missing is a more thorough discussion of the here presented results in light of recent, extensive rate measurements of N-cycling processes in the Peruvian OMZ. Also, a number of statements/conclusions are not backed up by references or are highly speculative and not supported by the data shown.

Our response: We thank reviewer #2 for his/her helpful comments. We improved the introduction and discussion sections, making these more concise and structured. We also added a comparision with rate measurements of N-cycling processes in the Peru OMZ, from Lam et al. (2009) and Kalvelage et al. (2013). We generally addressed all other concerns below.

Specific comments:

Abstract

The abstract is missing any statement on the (novel) implications of your study.

Our response: We stress that we observed <u>for the first time</u>, a positive linear relationship between NO_2^- $\delta^{15}N$ and $\delta^{18}O$ at our coastal stations. This is a novel finding, implying fast NO_2^- cycling in coastal waters compared to offshore.

Page 7258 – line 4f. All oceanic OMZs subject to water column N-loss are coupled to regions of high productivity. Please rephrase.

Our response: We rephrased for: "OMZs are generally coupled to regions of high productivity leading to high rates of N-loss as found in the coastal upwelling region off Peru"

Page 7258 – line 13f. Do you mean nitrate assimilation (uptake)? Assimilatory reduction would refer to the cell internal reduction for N-incorporation into biomolecules following nitrate uptake.

Our response: We changed assimilatory reduction for NO_3^- uptake

Introduction

Page 7259 – line 2 Please change "Chemically combined nitrogen (N, e.g. NO_3^-)" to: "Chemically combined nitrogen (N), e.g. NO_3^-,"

Our response: Done.

Page 7259 – line 10ff. Please specify below which oxygen level nitrate respiration can be expected and provide some reference(s).

Our response: We changed the sentence for: "N-loss typically occurs under nearly anoxic conditions where the first step, dissimilatory NO_3^- reduction to NO_2^-, active at O_2 concentrations less than ~25µM (Kalvelage et al., 2011), is used by heterotrophic microbes in lieu of oxygen (O_2) for respiration."

Page 7259 – line 12ff. It currently reads: "successive reduction of NO_3^-, ... and finally N_2". Please rephrase.

Our response: We changed for: "successive reduction of NO_3^-, NO_2^-, nitric oxide (NO), and nitrous oxide (N_2O) to N_2"

Page 7259 – line 14ff. Maybe you could find a more suitable term for "considerable evidence", which suggests that anammox still awaits final proof to actually occur in the oceans. Same sentence: Which are those "other pathways for N-loss" besides anammox and denitrification? Further, Lam et al. (2009) is not an appropriate reference here. The N-loss rates presented in the former study were originally published in Hamersley et al. (2007).

Our response: We changed the sentence for: "However, in the early 2000s, anaerobic ammonium oxidation (anammox: $NO_2^- + NH_4^+ \rightarrow N_2$) was discovered to be found to be widespread in the ocean (Kuypers et al. 2003; 2005; Hamersley et al., 2007)."

Page 7259 – line 16ff. You should probably also refer to recent studies by Dalsgaard et al. (2012) and Kalvelage et al. (2013) examining the large-scale distribution of N-loss processes in the ETSP OMZ.

Our response: We added these references.

Page 7259 – line 18ff. These two sentences remain fairly vague. Please rephrase and provide references.

Our response: We rephrased for: "While it is still a matter of debate whether denitrification or anamox is the dominant pathways for N-loss in Oxygen Minimum Zones (OMZ's) (e.g., Lam et al., 2009; Ward et al., 2009), both N-loss processes have been shown to strongly vary spatially and temporally and are linked to organic matter export and composition (Kalvelage et al., 2013; Babbin et al., 2014)."

Page 7259 – line 23-26 Again, there are no references provided here.

Our response: We added the following references: "Marine N-loss to N_2 occurs predominately in reducing sediments and the O_2 deficient water columns of OMZ's as found in the Arabian Sea and Eastern Tropical North and South Pacific (Ulloa et al., 2012; Lam et al., 2011 and references therein). NO_2^- is an important intermediate during

N-loss and generally accumulates at concentrations up to ~10 µM in these regions (Codispoti et al., 1986; Casciotti et al., 2013)."

Page 7260 – line 4f. This is not well phrased. N- and O-isotopes are not useful because of their reaction rate but because they can provide information on the time-integrated activity of N-cycling processes.

Our response: We accordingly changed the text for: "NO_3^- and NO_2^- N and O isotopes represent a useful tool to study N cycle transformations as they respond to in situ processes and integrate over their characteristic time and space scales."

Page 7260 – line 17-28 This section needs some clarification. Particularly for the non-specialist reader, the underlying cause for the difference in 18ε:15ε between nitrate consumption and nitrification is not well explained (e.g. there is no mentioning of N- isotope fractionation during nitrification).

Our response: We added this sentence at the end of the paragraph (and modified the next paragraph accordingly): "NO_2^- oxidation is associated with an inverse N isotope effect (Casciotti, 2009), atypical of biogeochemical reactions, and can cause both lower and higher ratios for $^{18}\varepsilon$:$^{15}\varepsilon$ compared to pure NO_3^- assimilation or denitrification, depending on the initial isotopic compositions of the NO_2^- and NO_3^- and the ^{18}O added back (Casciotti et al., 2013)."

Page 7261 – line 4 Please add "during denitrification" after "NO_2^- reduction", as there is also NO_2^- reduction to NH_4^+."

Our response: Done.

Page 7261 – line 6 "NO_2^- O" looks odd. Maybe change to: "O-isotope exchange of NO_2^- with water"

Our response: Done.

Page 7261 – line 10ff. These numbers don't seem quite up to date. E.g. compare with Bianchi et al. (2009), who list a number of (more balanced) oceanic N-budget estimates.

Our response: This was also a concern raised by reviewer #1. We accordingly changed the text for: "Current estimates from direct observations and models for N_2 fixation, considered the primary marine N source, range from 110-330 Tg N yr^{-1} (Brandes and Devol, 2002; Gruber, 2004; Deutsch et al., 2007; Eugster and Gruber, 2012; Groβkopf et al., 2012). Estimates for major marine N-sinks, i.e., denitrification and anammox in the water-column of oxygen deficient zones and sediments account for 145-450 Tg N yr^{-1} (Gruber, 2004; Codispoti, 2007; DeVries et al., 2012; Eugster and Gruber, 2012)."

Page 7261 – line 16 Maybe change "N-loss" to "denitrification", which back then was considered the sole N-loss process.

Our response: Done

Page 7261 – line 17 Please provide some numbers for the accepted range of
ε during denitrification.

Our response: We now provide a range and changed the text for: “Liu (1979) was first to suggest a lower ε for denitrification in the Peru OMZ as compared to the subsequently accepted canonical range for NO_3^- reduction of 20‰ to 30‰ (Brandes et al., 1998; Voss et al., 2001; Granger et al., 2008).”

Page 7261 – line 24ff. Results and conclusions don’t belong here. Please remove.

Our response: We removed the results and conclusions from this section: “To investigate further, we present here N and O isotope data for NO_2^- and NO_3^- from shallow coastal waters near Callao, off the coast of Peru. These waters are highly productive as a consequence of active upwelling that is also responsible for shoaling of the oxycline. We determine the relationship between NO_2^- $\delta^{15}N$ and $\delta^{18}O$ and its implication for NO_2^- cycling in these shallow waters as compared to offshore stations. We finally derive isotope effects for N-loss and infer the likely influence of sedimentary N-loss, which incurs a highly suppressed isotope effect, at our relatively shallow sites.

Material and Methods

Page 7262 – line 10ff. Please delete unnecessary information that are anyways found in the acknowledgements.

Our response: Done. The sentence now reads: “The R/V Meteor 91 research cruise (M91) to the eastern tropical South Pacific Ocean off Peru in December 2012 was part of the SOPRAN program and the German SFB 754 project.”

Page 7262 – line 16ff. Please move to results section.

Our response: We moved this sentence to the results section 3.1: “During the study period, there was active coastal upwelling as seen by relatively low satellite sea surface temperature and higher chlorophyll α concentrations along the shore (Fig. 1).”

Page 7263 – line 11f. Please delete “resulting in a final concentration...”. That is unnecessary information.

Our response: Done.

Page 7263 – line 12f. & line 20 Please provide $\delta^{15}N/\delta^{18}O$ values for nitrite and nitrate isotope standards.

Our response: We changed the text accordingly. Line 12: "In-house (i.e., MAA1, δ^{15}N = -60.6‰; MAA2, δ^{15}N = 3.9‰; Zh1, δ^{15}N = -16.4%) and other laboratory calibration standards (N23, δ^{15}N = 3.7‰ and δ^{18}O = 11.4‰; N7373, δ^{15}N = -79.6‰ and δ^{18}O = 4.5‰; and N10219; δ^{15}N = 2.8‰ and δ^{18}O = 88.5‰; see Casciotti and McIlvin, 2007) were used for NO^{2-} δ^{15}N and δ^{18}O analysis."
Line 20: "Standards for NO_3^- isotope analysis were N3 (δ^{15}N = 4.7‰ and δ^{18}O = 25.6‰), USGS34 (δ^{15}N = -1.8‰ and δ^{18}O = -27.9‰) and USGS35 (δ^{15}N = 2.7‰ and δ^{18}O = 57.5‰) (Casciotti et al., 2007)."

Page 7263 – line 25ff. I don't think those details on the purge and trap system, e.g. sample run time, are necessary. Please shorten.

Our response: We shortened to: "N_2O produced by the azide reaction was purged with He from the septum sealed 20 ml vials and trapped, cryofocused and purified prior to transfer to the IRMS."

Page 7264 – line 11 Please add "samples" after "warming".

Our response: Done.

Page 7264 – line 23f. Some words seem to be missing here. Please check.

Our response: We changed the sentence for: "The following equations are used for a closed system (Mariotti et al., 1981):"

Page 7265 – line 4f. There is a large number of abbreviations, symbols, and formulas, which, particularly for the non-expert reader, is challenging enough to remember. Unnecessary abbreviations, such as "[NO_3^-]" instead of "NO3- concentrations", should thus be avoided. It also assists readability. Please check throughout the manuscript.

Our response: We significantly reduced the number of abbreviations in the text, especially the uses of brackets for concentrations, as suggested. When we use abbreviations, we made sure that they were defined at first use in the abstract and main text.

Page 7266 – line 5f. Please move the sentence to the results/discussion section.

Our response: We moved this sentence to the results section (section 3.4).

Page 7266 – line 10 Please define "Npdef/expected".

Our response: We defined $Np_{expected}$ after equation (7): "where $Np_{expected}$ is the concentration expected based on Redfield stoichiometry."
We also defined Ndef before equation 8: "This has also been a traditional approach to quantify N-loss in OMZ's (N deficit, Np_{def}) by comparing observed DIN concentrations ($N_{observed}$) to $Np_{expected}$:"

Page 7266 – line 22 Noffke et al. (2012), who quantified benthic iron and phosphate fluxes along the Peruvian margin, would be a more suitable reference here than the study by Reed et al. in the Baltic Sea.

Our response: We added Noffke et al. (2012) as a reference and removed Reed et al. (2011).

Results

Page 7276 – line 9ff. From your T/S plots it is not evident that the sampled waters originate from further offshore (there are no offshore data for comparison). Further, you suggest that originally all waters are oxygen deplete and only become oxygenated once they reach the surface and there is exchange with the atmosphere. That contradicts your statement that surface currents dominate the shallow coastal waters (any references?). These waters are originally oxic and become oxygen deplete below the euphotic zone (which near the coast can be very shallow due to high phytoplankton densities) as a result of oxic microbial respiration of organic matter. Also, I don't quite agree with your interpretation of the observed north-south temperature increase. Is it not more likely, that sea surface temperatures increase towards st. 67 because of the indentation of the Peruvian coastline at ~14°S combined with the sheltering effect of the Paracas National Park peninsula near Pisco, impeding the advection of cold surface waters from the south? In my understanding, upwelling (vertical water mass transport) is very slow compared to lateral advection of (surface) water masses.

Our response: Upwelling along the Peru Coastal Current, with a typical depth of origin of ~200 m is well documented (Penven et al., 2005). Accordingly, if we compare with data for offshore ODZ waters from Bourbonnais et al. (2015), we observe similar T/S signatures between 100 and 200 m depths for these waters, suggesting that they are most likely the source waters. We also believe that the explanation of more intense upwelling at our more northern station is consistent with all our relevant observations.

Accordingly, we added a reference (Penven et al, 2005) and changed the text to: "During the study period, there was active coastal upwelling especially at station 63 as seen by relatively low satellite sea surface temperatures, higher chlorophyll α concentrations, and a shallow oxycline (Fig. 1). A common relationship and narrow range for T and S were found, comparable to T/S signatures for offshore ODZ waters between ~100 and 200 m depths (Bourbonnais et al. (2015), indicating a common source of water upwelling at these inner shelf stations (Fig. 2). This is expected as in these coastal, shallow waters, upwelling of the Peru Coastal Current, with low O_2 and high nutrients play a dominant role (Penven et al., 2005)."

Page 7267 – line 19f. See previous comment

Our response: See our response above, Page 7276 – line 9.

Page 7267 – line 21ff. You also examine possible effects of nitrite oxidation, an aerobic process (at least an alternative electron acceptor has not been identified, yet, in OMZs), on the isotopic composition of nitrite and nitrate.

Our response: We added the following sentence: "In contrast, NO_2^- oxidation, an aerobic process, was shown to occur even at low to non-detectable O_2 (Füssel et al., 2012)."

Page 7268 – line 3 Please specify "CTD deployed O_2 sensors". A STOX sensor, which can resolve nanomolar changes in oxygen concentration, can be mounted to a CTD rosette system, too.

Our response: We specified that we used a Seabird sensor. We also added information about the O_2 sensor, calibration and detection limit in the material and methods (section 2.1): "O_2 concentrations were determined using a Seabird sensor, calibrated using the Winkler method (precision of 0.45 µmol L^{-1}) with a lower detection limit of 2 µmol L^{-1}."

Page 7268 – line 8f. I do not think "intense local upwelling" is a likely reason for elevated phosphate and/or silicate levels in the shelf bottom waters. The very high concentrations of ammonium, which typically does not accumulate at such high levels in the OMZ, clearly indicate benthic release.

Our response: We changed the sentence for: "Station 63 had the highest near-bottom concentrations, a likely result of release from the sediments, which is further supported by high near-bottom NH_4^+ concentrations (up to ~4µM) as compared to the other stations (Fig. 3. B, C, and D)."

Page 7268 – line 13ff. I agree that nitrate depletion indicates N-loss, but you did not measure actual N-removal. Please rephrase accordingly.

Our response: We changed the sentence for: "Across most of our stations, NO_3^- concentration was 22 µM at 20 to 40 m depth but decreased to near zero deeper within the O_2-depleted zone due to microbially mediated NO_3^- reduction (Fig. 4.A)."

Page 7268 – line 24 According to Fig. 4c, $\delta^{15}N\text{-}NO_3^-$ was ~40 ‰ at stations 65 + 67. Please check those numbers.

Our response: According to our data, we observed a maximum $\delta^{15}N\text{-}NO_3^-$ of 50‰ at 99 m depth at station 64 and 47‰ at 37 m depth at station 67. In Fig. 4c, the maximum $\delta^{15}N\text{-}NO_3^-$ is clearly higher than 40‰.

Page 7268 – line 25f. Maybe move this to methods section (e.g., "samples down to xx µM N were analyzed for their isotopic composition").

Our response: We moved this sentence to the method section (section 2.1): "The lowest concentration of NO_2^- or NO_3^- analyzed for isotopic composition was 0.5 µM, thus $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_2^-$ could not be measured below 37 m at station 63."

Page 7269 – line 1 Maybe substitute “N-loss” with “NO_3^- reduction“, to be more specific.

Our response: Done.

Page 7270 – line 4f. Please provide reference.

Our response: We added a reference here (Bourbonnais et al., 2015).

Page 7270 – line 18f. How does this observation align with actual rate measurements of nitrite oxidation in the Peruvian coastal OMZ?

Our response: We now discuss this better in the discussion (section 4.1). See your comment below, page 7275, line 11.

Page 7271 – line 1ff. In my understanding, N deficit and excess P express exactly the same and are mutually interchangeable. Hence, does “excess PO_4^{3-}“ refer to benthic P release here? That is only obvious at station 63, but not at the remaining sampling sites. Please clarify.

Our response: We changed the sentence for: “However, the slope of 0.45 for the linear relationship shows biogenic N in N_2 to be only half that expected from Np_{def}, as a possible consequence <u>of benthic PO_4^{3-} release</u>.”

Discussion
Page 7271 to 7273 – line 19 This is largely a summary of previous studies on the effects of microbial activity on DIN isotopic compositions and is not well tied in with the discussion of the here presented data.

Our response: We think it is essential to first summarize the background information on what affect the isotopic composition of NO_2^- in this section. We nonetheless reorganized and condensed the text to have a better flow of ideas, and eliminated unnecessary information and repetitions (see five first paragraphs, section 4.1). See also comments below.

Page 7271 – line 21f. I disagree that micromolar levels of nitrite as found in OMZs are “low concentrations”. Also, later in the same paragraph you write: “Accordingly, relatively high [NO_2^-] was observed ...”.

Our response: We changed the sentence for: “can accumulate at relatively high concentrations through the ocean.”

Page 7272 – line 3 There are more fitting references here, e.g. Lipschultz et al. (1990), Lam et al. (2009), and Kalvelage et al. (2013), all of which provide actual rate measurements of nitrate reduction in the Peruvian OMZ.

Our response: We removed Codispoti et al. (1986) and added the suggested references.

Page 7272 – line 3ff. Are you suggesting that the observed nitrite is actually not formed in the shallow shelf waters but originates in deeper OMZ waters and is simply advected (upwelled)? Previous studies have shown that nitrite is produced at those shallow, oxygen depleted depths (see also above).

Our response: We removed this sentence.

Page 7272 – line 22ff. This needs better explanation and should be discussed in the context of the preceding sections. By describing observations made in previous studies and your own ones in separate sections, the non-expert reader has a hard time to follow your line of argumentation here.

Our response: We accordingly reorganized the text in this section, see five first paragraphs, section 4.1.

Page 7273 – line 25ff. Although high rates of N-loss have been measured on the Peruvian shelf that alone does not explain rapid nitrite turnover. The nitrite pool in these waters is affected by aerobic ammonium and nitrite oxidation, nitrate reduction to nitrite, as well as further reduction to either ammonium or N_2 (see Lam et al. (2009) and Kalvelage et al. (2013)).

Our response: We changed this sentence for: "Higher rates for aerobic NH_4^+ and NO_2^- oxidation, as well as anaerobic NO_3^- reduction to NO_2^-, and further reduction to NH_4^+ (DNRA) or N_2, have been reported in shallow waters off Peru presumably due to increased coastal primary production and organic matter supply to the in-shore OMZ (e.g. Codispoti et al., 1986; Lam et al., 2011; Kalvelage et al., 2013). However as our observations are restricted to anoxic waters, only high rates of N-loss could explain this more rapid NO_2^- turnover."

Page 7274 – line 14ff. Are these rates calculated based on your own data or do you refer to previously results?

Our response: We refer to the maximum $[NO_2^-]$ observed in our study (i.e., our own data). We clarified this in the text.

Page 7275 – line 11ff. I would like to see a more thorough discussion of how the estimates of nitrite oxidation vs. nitrate reduction compare to previous rate measurements of these processes. Anammox bacteria (in culture) only oxidize a minor fraction of nitrite to nitrate. At the same time, rates of nitrite oxidation mostly exceed those of N-loss via anammox several fold on the Peruvian shelf (Kalvelage et al. (2013)), clearly indicating non-anammox related nitrite oxidation.

Our response: We agree and now added this discussion following line 19 (Page 7275): "Furthermore, anammox bacteria only oxidize a minor fraction of NO_2^- to NO_3^- in culture. At the same time, estimates of NO_2^- oxidation (8.48 to 928 nmol N l^{-1} d^{-1}) are significantly higher than N-loss rates by anammox (2.84 to 227 nmol N l^{-1} d^{-1}) on the Peruvian shelf (Kalvelage et al., 2013), clearly indicating non-anammox related nitrite oxidation."

Page 7275 – line 21 see comment Page 7258 – line 13f.

Our response: We changed for "NO_3^- uptake".

Page 7276 – line 2ff. This sentence is not very clear and needs some rewording.

Our response: We changed the sentence for: "Our observed deviation toward slopes < 1 can instead be explained by the nitrified NO_3^- with a lower $\delta^{18}O\text{-}NO_3^-$, mostly derived from water (Andersson and Hooper, 1983), relative to the high ambient $\delta^{18}O\text{-}NO_3^-$ values."

Page 7276 – line 6f. Is this your own observation or an observation made in the cited study?

Our response: This is an observation made in the cited study. We clarified this, changing the sentence for: "… as observed in Casciotti et al. (2013) and Bourbonnais et al. (2015)."

Page 7276 – line 16ff. This paragraph is again very hard to digest for any non-expert reader. Maybe you could expand here a bit on the different approaches used to calculate ε.

Our response: We changed the first sentence of this paragraph for: "Linear regression coefficients for ε calculated using the different approaches presented in section 2.4 are listed in Tables 1 and 2."
The manuscript is already long and we think it is preferable to refer the reader to section 2.4, where the different approaches used to calculate ε are well explained, rather then repeating this information again here.

Page 7277 – line 4ff. Please specify those "mass balance considerations".

Our response: We changed the sentence to: "The latter two, using DIN or biogenic N_2 as the basis to calculate ε, are more representative of N-loss."

Page 7277 – line 25ff. As mentioned before, your T/S data merely indicate a relatively homogeneous water mass over the shelf, but that does not necessarily exclude any mixing with waters further offshore. Could you provide data from neighboring, offshore stations that show a significantly different T/S signature for those waters?

Our response: Again, if we compare with data for offshore waters from Bourbonnais et al. (2015), we observe a similar T/S signature for the "upwelled" source waters, which make sense in this context. However, given the narrow range in T and S, further mixing between different water masses on the shelf is unlikely, favoring a closed system.
We changed the sentence to clarify: "Closed system estimates of ε are likely more reliable in our setting because of low likelihood of mixing between water masses of contrasting characteristics on the shelf. Temperature and salinity in the OMZ at our stations narrowly ranged from 13.5 to 15 ^{o}C and 34.88 to 34.98 (Fig. 2), similar to T/S signatures from offshore source waters (Bourbonnais et al., 2015), and suggestive of a single water mass."

Page 7278 – line 21f. Please specify how potential effects of contributions from organic N to N_2 formation were taken into account.

Our response: We already explain this in the above text (Page 7277, lines 6 to 10): "Calculations based on changes in $\delta^{15}N$-NO_3^- are affected by NO_2^- accumulation and isotope effects of NO_2^- oxidation (see above). The 4‰ difference in ε calculated from changes in $\delta^{15}N$ of biogenic N_2 vs. $\delta^{15}N$ of DIN may arise from the contribution of NH_4^+ derived from organic matter to biogenic N_2 via the anammox process."
We think that it would be repetitive to state this again.

Page 7279- line 12ff. This is too simple a conclusion. One could also argue that N-flux measurements over a relatively short time span and at relatively few locations overestimate benthic N-loss. And, there are hot spots of water column N-loss, too.

Our response: We revised the sentence for: "However, our comparison to direct measurements of fluxes should be considered tentative as they are made at single locations over relatively short time periods are thus subject to considerable spatial and temporal heterogeneity."

Page 7279 – line 21f. Not only N-loss processes, but, as you have demonstrated in previous sections, also nitrification and incomplete denitrification determine the isotopic compositions of nitrite and nitrite. Please revise.

Our response: We accordingly changed the sentence for: "We found that N-loss representing the net effect of partial denitrification, anammox and nitrification produced in sum large variations in isotopic composition."

Page 7280 – line 6f. How does your estimate of nitrite turnover time compare to recently published N-fluxes and N-inventories for the Peruvian costal OMZ by Kalvelage et al. (2013)?

Our response: We added the following discussion, Page 7275, line 14: "This estimate is higher than ratios of NO_2^- oxidation/NO_3^- reduction of up to 54% for the Peruvian coastal ODZ derived from direct rate measurements (Lam et al., 2009; Kalvelage et al., 2013), and should thus be considered as an upper limit."

Page 7281 – line 4f. Please include some reference for benthic N-loss on the Peruvian shelf, e.g. Bohlen et al. (2011).

Our response: We added this reference.

Tables and Figures

Table 1 and 2 For non-expert readers it may not be obvious that ε corresponds to the slope of the linear regression. Hence, instead of listing ε and the error of the slope of the linear regression separately, I suggest to delete the "Error on slope" column and include the error in the ε column (e.g. N_2 biogenic = 14.27 ± 0.86).

Our response: Done.

Figure 1 Panel A is not labeled as such. Further, the inserted ODV map is very small and provides little extra information. I suggest removing it.

Our response: We labeled panel A and removed the inserted ODV map.

Figures 3, 4 and 8 A cosmetic detail: ODV allows plotting bathymetry using station bottom depths. Those grey sediment boxes look somewhat arbitrarily drawn.

Our response: We improved all ODV figures based on more accurate bathymetry using station bottom depths.

Figures 5, 6 and 9 Please increase figure size.

Our response: We will ask copyediting to increase figure size for these figures, as requested.

Figure 7 The axis label looks somewhat skewed (aspect ratio not locked during increase/decrease of plot size). Please correct.

Our response: We removed this figure, as suggested by reviewer #1.

Figure 10 The figure does not add any information, all values are given in Table 1 and 2. Please remove.

Our response: We also removed this Figure, as suggested by reviewer #1.

Technical corrections:

Page 7258 – line 11 Please add comma after "10 µM".
Page 7259 – line 12 Please add comma after "nutrient".
Page 7259 – line 17 Please change to: "dominant pathway for N-loss"
Page 7262 – line 17 "temperatures" instead of "temperature".

Page 7265 – line 4 “parcels” instead of “parcel”.
Page 7267 – line 9 Please add comma after “found”.
Page 7268 – line 20 Please add comma after “[NO_2^-]”.
Page 7269 – line 20 Please add comma after “N_2
Page 7269 – line 24 Please add “ones” after “canonical”.
Page 7270 – line 2f This should probably read: “The Rayleigh equations’ y-intercepts, where f = 1 represents the initial $\delta^{15}N$ of NO_3^- or DIN, varied from ...”
Page 7274 – line 1 Change to: “Kalvelage et al., 2013”
Page 7275 – line 1 “average” instead of “avering”
Page 7277 – line 4 Please add comma after “two”.

Our response: We applied all these technical corrections.

Anonymous Referee #3
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General impression
This manuscript presents a suite of what appears to be high quality N-isotopic data from the Peru margin OMZ. From these data they draw conclusions that seem relatively sound. However, right now the discussion is rather unfocused and sometimes redundant and their data is not put into the context of the larger global data set on N-isotopes in OMZs. For example. Although they note the difference between the epsilon values calculated from their data and Bourbonnais et al. and briefly mention values from the ETNP and Arabian Sea, there is no thoughtful discussion of these as a whole. Elaboration of these points follows below. Consequently, my opinion is that the manuscript needs revision before publication.

Our response: We thank the reviewer for its helpful comments. We generally addressed all concerns below. We tried to improve the discussion section and better use background information from previous studies to support our results.

Scientific

Page 7265. On this page they give the equations for open and closed system calculation of epsilon. They say “The fraction of remaining DIN is a better estimation of the overall isotope effect for N-loss (Bourbonnais et al., 2015), while using NO_3^- as the basis to calculate ε specifically targets NO_3^- reduction.” I agree DIN is better. OK, so on line 2 they give the equation for $\delta^{15}N$-NO_3^- which has no equation number and then on line 3 for $\delta^{15}N$-DIN which is equation (1) and they use the corresponding values for f for each equation. If I have this correct, the $\delta^{15}N$-NO_3^- equation is the one they say is specifically for NO_3^- reduction. It seems to me that almost all of their samples have NO_3^- and NO_2^- and some N-deficit. In that case then this equation is not NO_3^- reduction to NO_2^- because some went to N-deficit and it’s not denitrification because some remains as NO_2^-. Why do this calculation? What does it mean?? The same comment applies to the open system equation (line 15).

Our response: We fixed the equations numbering, the equation on line 2 is now equation 1 and the equation on line 3, equation 2, etc…
Equation 1 is to determine ε associated with NO_3^- reduction, regardless of whether the produced NO_2^- accumulates or is further reduced to N_2. This equations has been widely used in other studies for this purpose, for example, see Granger et al. (2009) (Limnol. Oceanogr.). Note that Granger et al. (2009) specifically removed NO_2^- before determining ε for NO_3^- reduction using equation 1, as we also did.
Equation 2 (δ^{15}N-DIN) is appropriate to estimate ε for total N-loss, as it considers both δ^{15}N-NO_3^- and δ^{15}N-NO_2^- (weighted average). Another way to estimate ε for global N-loss is to use δ^{15}N-biogenic N_2 (previously equations 2 and 4). In practice, if the source of biogenic N_2 is solely from NO_3^- and NO_2^-, then the two estimates should converge, but it is also possible to have generally small contributions from organic matter remineralization to NH_4^+ and conversion to N_2 through anammox, as discussed in our manuscript, p. 7277, lines 8 to 10.

Page 7267 line11-13. Why do they say the upwelled water appears to be a single water mass originating from the offshore OMZ? Why can't it be a coastal undercurrent? Do they have evidence for stronger wind forcing at station 63?

Our response: We accordingly modified this section: "During the study period, there was active coastal upwelling as seen by relatively low satellite sea surface temperatures, higher chlorophyll α concentrations, and a shallow oxycline along the shore, and especially at station 63 (Fig. 1). A common relationship and narrow range for T and S were found, comparable to T/S signatures for offshore ODZ waters between ~100 and 200 m depths (Bourbonnais et al. (2015), indicating a common source of water upwelling at these inner shelf stations (Fig. 2). This is expected as in these coastal, shallow waters, upwelling of the Peru Coastal Current, with low O_2, high nutrients and a typical depth of ~200 m, play a dominant role (Penven et al., 2005)."

Page 7271 line15. δ^{15}N-N_2 anomaly..... ranged from -0.2 to +0.1." Figure 8c shows that most anomalies are negative and only highest biogenic N_2s have positive anomalies. What would cause a negative N_2 anomaly? I don't think this is ever discussed.

Our response: We added the following sentence after line 15 (page 7271) to better discuss this point: "Negative δ^{15}N-N_2 anomaly (i.e., lower δ^{15}N-biogenic N_2) is produced at the onset of N-loss, because extremely depleted ^{15}N-N_2 is first produced. At a more advanced N-loss stage, we expect δ^{15}N-N_2 anomaly and δ^{15}N-biogenic N_2 to increase, as we observed in this study, as heavier ^{15}N is added to the biogenic N_2 pool."
We think that only referring to δ^{15}N-N_2 anomaly here, which is the difference between the δ^{15}N-N_2 observed and at equilibrium, might be confusing because we later only refer to δ^{15}N of biogenic N_2. We thus also added the corresponding δ^{15}N-biogenic N_2 range after line 15 (page 7271):
"The corresponding range in δ^{15}N biogenic N_2, calculated from the δ^{15}N-N_2 anomaly as in Bourbonnais et al. (2015), was from -9.0 to 3.2‰."

Specific.

Page 7259 line 27, DIN=NO_3^-, NO_2^- and NH_4^+ should be DIN=NO_3^-+NO_2^-+NH_4^+

Our response: Corrected!

Page 7260 line 11. The sentence starting with "Cannonical" says epsilon associated with NO_3^- reduction. NO_3^- reduction is the reduction of NO_3^- to NO_2^-. Do they mean NO_3^- reduction or canonical denitrification, which is NO_3^- to N_2? The studies by Brandies et al and Voss et al and Granger et al that they cite are actually equivalent to their DIN because they measured NO_3^-+NO_2^-.

Our response: We meant NO_3^- reduction, as in other cited studies. See our response to your comment for page 7265.
Regarding your comment about these other cited studies. In older studies, e.g., Brandes et al. (1998) and Voss et al. (2001), the authors always used NO_3^- concentrations only when calculating their isotope effects and although they claim also measuring NO_2^- concentrations in their method sections, there is no further mention of NO_2^- anywhere in their papers afterward. I agree that since they did not removed NO_2^- before using the alkaline Devardas alloy method for the conversion of NO_3^- (and NO_2^-) to NH_4^+, their measured δ^{15}N-NO_3^- must also include δ^{15}N-NO_2^-. The fact that they then calculated their isotope effects using only NO_3^- concentrations (and assuming that they only measured the δ^{15}N of NO_3^-) is thus a bit problematic. However, I assume that the contribution from NO_2^- should have been be minimal since their isotopes effects are comparable with Granger et al. (2009).
In a most recent study, using the denitirifer method for analysis of δ^{15}N-NO_3^- (Granger et al., 2009), the authors specifically removed NO_2^- before δ^{15}N-NO_3^- analysis, as we also did. I am citing from their paper here: "Consequently, we proceeded to remove nitrite from samples within a few weeks of their collection. Isotope ratios measured for experiments that had been stored for approximately 6 months or more prior to nitrite removal showed sporadic and haphazard isotope behavior at lower nitrate concentrations when the proportion of nitrite was relatively high. Data generated from these experiments were discarded."

Page 7260 line 13 "are ranging" should be "range"

Our response: Corrected.

Page 7260 line 15 "...sedimentary denitrification is highly suppressed in the water column." This is confusing (although I think I know what they are trying to say). Delete "in the water column".

Our response: We changed the sentence for: "In contrast, the expression of the isotope effect of sedimentary denitrification is highly suppressed as compared to the water-column…"

Page 7261 line 22 Ryabenko et al. not in References

Our response: We added this reference.

Page 7262 Line 20 name of the manufacturer of the CTD/Rosette and O_2 sensor and type? This is important because we are talking about processes that take place at the limit of detection of O_2 sensors. How were the O_2 sensors calibrated?

Our response: We added the following sentence (page 7261, after line 21): “O_2 concentrations were determined using a Seabird sensor, calibrated using the Winkler method (precision of 0.45 μmol L^{-1}) with a lower detection limit of 2 μmol L^{-1}.”

Page 7262 line 1. They say “NO_2^- samples were collected and stored in ...HDPE bottles” but on the previous page they say the samples were collected in Niskin bottles. Delete the word “collected”.

Our response: Done.

Page 7262 line 14. Same for NO_3^- samples change collected to stored.

Our response: Done.

Page 7263 line 21. I assume for nutrient analysis that DIN=NO_3^- +NO_2^- was done by Cd reduction and NO_2^- was done colorometrically and NO_3^- was determined by difference. How do their concentrations measured by their methods compare with the hydrographic ones?

Our response: Yes, this is the method that was used to measure nutrient concentrations (NO_3^- and NO_2^-). Concentrations were measured onboard during the M91 cruise (SFB 754 Project), as described in Stramma et al. (2013). We did not independently measured nutrient concentrations in our laboratory.

Page 7265 Line 20-22. “..... increasing noise with small levels of biogenic N_2 (up to 20 μM in this study)” This makes it seem like 20 is the small level with increased noise. Why not just say something like “..... greater than 7.5 μM because of increasing noise below this level”

Our response: We changed the sentence for: “… greater than 7.5 μM because of increasing noise below this level due to the huge atmospheric dissolved N_2 background (typically up to ~500 μM).”

Line 7268 line1. “.... below this value.” What value? Does this refer to undetectable or 10 μM? And then on line 4 ”...such low concentrations..” Again, what are such low concentrations. Any good O_2 sensor should be able to go somewhat below 10. Then on line 14 “O_2-depleted zone”. Is there a difference between OMZ and O_2-depleted zone? What oxygen values define the OMZ and O_2-depleted zone?

Our response:
Line 1: We changed “below this value” for “10 µM”.
Line 4: The full sentence reads: “Whereas a recent study indicates that denitrification and anammox are reversibly suppressed at nanomolar O_2 levels (Dalsgaard et al., 2014), CTD deployed Seabird O_2 sensors are not sufficiently sensitive to detect such low concentrations and hence our choice of a 10 µM threshold.” “Such low concentrations” is thus referring to nanomolar O_2 levels.
Line 14: The current accepted view in the community is that OMZs are regions where oxygen saturation in the water columns is at its lowest, whereas ODZs are where oxygen concentrations are zero, within O_2 sensor errors. In our case, we meant ODZ. We accordingly changed OMZ for ODZ throughout the text.

Page 7269 Line 3 is the slope of 0.86 statistically different from 1.0?

Our response: Yes, the slope was statistically different than 1.0 ($p\text{-value} < 0.05$, confidence intervals for the slope = 0.84 to 0.89). We clarified this in the text.

Page 7269 Line 20. They are using the biogenic N_2 data before they present it. Shouldn’t they present the data first. Also in this section that present results of epsilon calculation for changes in $\delta^{15}N$-DIN and $\delta^{15}N\text{-}NO_3^-$ using equations 1-4. However the equations for $\delta^{15}N\text{-}NO_3^-$ have no equation numbers. Shouldn’t they have numbers?

Our response: We changed the sub-section order, sections 3.5 and 3.6 now come <u>before</u> section 3.4. We present biogenic N_2 data in section 3.6 (now 3.5). We also renumbered the equations, such that the equation for $\delta^{15}N\text{-}NO_3^-$ is now equation 1.

Page 7220. Lines 8-11. Again, they say for “NO_3^- reduction alone” but Brandes et al., Voss et al., Granger et al and Cline and Kaplan did their studies with N+N not nitrate alone.

Our response: See our response to your comment above (Page 7260, Line 11).

Page 7220. Line 21. What are $\delta^{15}N\text{-}N_2$ anomalies. I think this means the deviation from atmospheric equilibrium but I’m not sure. If that is indeed what they are, how do they compare to those given by Brandes et al., and Chang et al.?

Our response: We clarified this in the text: “The $\delta^{15}N\text{-}N_2$ anomaly, i.e., the difference between the $\delta^{15}N\text{-}N_2$ observed and at equilibrium and derived as in Charoenpong et al. (2014)…”.
We cannot compare our values to Brandes et al., and Chang et al., as they do not report $\delta^{15}N\text{-}N_2$ anomalies.

Page 7272 Paragraph starting on line 6. Much of this is a repeat of a previous paragraph. Condense this into a single paragraph.

Our response: We reorganized this section according to reviewer #2 comments. We tried to condense and remove repetitive information. We however think it is important to remind the reader about background information here, providing a framework to explain our results.

Page 7272 line 14. "have" should be "has"

Our response: Corrected.

Page 7273. Paragraph starting on line 4. There is a lot of background here but it is generally not summed up as to how it might explain their data. One is left with the general feeling that we don't really understand much more than we did before. Is there a conclusion they can draw?

Our response: Again, we reorganized this section according to reviewer #2 comments. The background information is now more in context with our results.

Page 7273 line 3. "M90" In the methods you say this paper is from M91. Is this just a typo. If not you need a reference for this.

Our response: The data we present in Fig. 5 C are new data from the M90 cruise. We added the following sentence in the method section, Page 7262, Line 18: "We additionally sampled deep offshore stations during the M90 cruise in November 2012."

Page 7273 line 9. Sentence starting with NO_2^- oxidation. First, use the word Nitrite at the beginning of a sentence. Second, is this sentence really necessary, all this has been explained before?

Our response: We now start the sentence with "Nitrite". This is briefly mentioned in the introduction, but we think it is important to remind the reader about this background information in the discussion, as it is important to explain our results.

Page 7273 line 26. Delete the word "presumably".

Our response: Done.

Page 7275 Lines 16-18. "our data suggests (sic) NO_2^- oxidation up to only up to 80% of total NO_3^- reduction." On the bottom of page 7272 they said "the dominance of NO_2^- reduction over oxidation. 80% to 100% doesn't seem like dominance to me. Also, it should be "our data SUGGEST".

Our response: We corrected for "suggest". We think 80% to 100% implies a dominant process.

Page 7276 lines 13-15. I'm not sure how this tests the assumptions in the balance.

What is the result of this test, and what do they think is correct. They then go on on line 19 of this page to say that relationships are not sensitive to the method of calculating epsilon. This seems that it's not much of a test.

Our response: We are referring to two different things. Lines 13-15, we say that by calculating the different ε using either δ^{15}N of DIN or δ^{15}N of biogenic N_2, we can test whether there is isotopic mass balance between the substrate (δ^{15}N-DIN) and the product (δ^{15}N-biogenic N_2). If there is perfect isotopic mass balance, the ε calculated either ways should be equal. Differences can be explained by the contribution from other source(s) than DIN to the δ^{15}N biogenic N_2 pool. We discuss this point on Page 7277, Lines 8 to 10.
On Line 19, we say that ε values are not sensitive to choice of method for calculating f (see Page 7267, Lines 3 to 6 for the different methods employed to calculate f).

Page 7279 line 25. Concentrations of what were "relatively low? Concentrations of oxygen or concentrations of the different N species?

Our response: N species. We clarified this in the text.

Page 7280 lines 23-25. Again in the T/S plot I see a surface mixed layer (above 14 degrees C) and a deeper mixing line pointing at some unresolved water mass (points in the box). So I would like to see their choice of epsilon of 7 better supported.

Our response: Reviewer #2 also raised this concern. If we compare with data for offshore waters from Bourbonnais et al. (2015), we observe a similar T/S signature for the source of the upwelled waters. However, given the narrow range in T and S, further mixing between different water masses on the shelf is unlikely, favoring a closed system.
We discuss this on Page 7277, Lines 25 to 28: "Closed system estimates of ε are likely more reliable in our setting because of low likelihood of mixing between water masses of contrasting characteristics on the shelf. Temperature and salinity in the OMZ at our stations narrowly ranged from 13.5 to 15 ^{o}C and 34.88 to 34.98 (Fig. 2), similar to T/S signatures from offshore source waters (Bourbonnais et al., 2015), and suggestive of a single water mass."

Table 2. What does "error on slope" mean? Is it S.D. or confidence limits on slope or at what level of significance?

Our response: We meant standard error of the slope. We added this information in the Table 1 and 2 legends.

Figure 5. The x-axis in panel C should be smaller, i.e. from -30 to zero, so we can see the scatter better. Also, for this figure and others, are all regressions significant at the 0.05 level?

Our response: We changed the x-axis in Figure 5, as suggested. We also added a sentence in Figure legends (Figures 5, 6 and 9): “Significant correlation coefficients at a 0.05 significance level are denoted by *.”

Figure 7. is the regression line for the >30 m data only or for all data?

Our response: We removed this figure, as suggested by reviewer #1.

Again, I think it is important that all the data be available as supplementary information to this proposal.

Our response: Regarding data availability: we uploaded these data on the Data Management Portal for Kiel Marine Sciences hosted at GEOMAR: https://portal.geomar.de/. The data are also available upon request to the corresponding author. We added this information in the acknowledgement section.

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

Happy Hu[a,1], Annie Bourbonnais[a,1], Jennifer Larkum[1], Hermann W. Bange[2], Mark A. Altabet[1]

[a] These authors contributed equally to this work.

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

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

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

Abstract

O_2 deficient zones (ODZs) of the world's oceans are important locations for microbial dissimilatory nitrate (NO_3^-) reduction and subsequent loss of combined nitrogen (N) to biogenic N_2 gas. ODZs are generally coupled to regions of high productivity leading to high rates of N-loss as found in the coastal upwelling region off Peru. Stable N and O isotope ratios can be used as natural tracers of ODZ N-cycling because of distinct kinetic isotope effects associated with microbially-mediated N-cycle transformations. Here we present NO_3^- and nitrite (NO_2^-) stable isotope data from the nearshore upwelling region off Callao, Peru. Subsurface oxygen was generally depleted below about 30 m depth with concentrations less than 10 µM, while NO_2^- concentrations were high, ranging from 6 to 10 µM, and NO_3^- was in places strongly depleted to near 0 µM. We observed for the first time, a positive linear relationship between NO_2^- $\delta^{15}N$ and $\delta^{18}O$ at our coastal stations, analogous to that of NO_3^- N and O isotopes during NO_3^- uptake and dissimilatory reduction. This relationship is likely the result of rapid NO_2^- turnover due to higher organic matter flux in these coastal upwelling waters. No such relationship was observed at offshore stations where slower turnover of NO_2^- facilitates dominance of isotope exchange with water. We also evaluate the overall isotope fractionation effect for N-loss in this system using several approaches that vary in their underlying

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

1 Introduction

Chemically combined nitrogen (N) e.g., nitrate (NO_3^-) is an important phytoplankton nutrient limiting primary productivity and carbon export throughout much of the ocean (e.g. Gruber, 2008). The marine nitrogen cycle involves a series of microbial processes, which transfer N between a number of chemical forms. These include N_2 fixation, nitrification (ammonium (NH_4^+) and nitrite (NO_2^-) oxidation), and loss of combined N to N_2 via denitrification and anaerobic ammonium oxidation (anammox). Of particular importance is the global balance between sources of combined N (N_2 fixation) and N-loss processes which ultimately control the combined N content of the ocean and thus its productivity and strength of the biological carbon pump. N-loss typically occurs under nearly anoxic conditions where the first step, dissimilatory NO_3^- reduction to NO_2^-, active at oxygen (O_2) concentrations less than ~25 µM (Kalvelage et al., 2011), is used by heterotrophic microbes in lieu of O_2 for respiration. Canonically, the denitrification pathway of successive reduction of NO_3^-, NO_2^-, nitric oxide (NO), and nitrous oxide (N_2O) to N_2 was considered as the dominant pathway for N-loss. However, since the early 2000s, anammox ($NO_2^- + NH_4^+ \rightarrow N_2$) was found to be widespread in the ocean (Kuypers et al. 2003; 2005; Hamersley et al., 2007; Dalsgaard et al., 2012; Kalvelage et al., 2013). While it is still a matter of debate whether denitrification or anamox is the dominant pathways for N-loss in Oxygen Minimum Zones (OMZ's) (e.g., Lam et al., 2009; Ward et al., 2009), both N-loss processes have been shown to strongly vary spatially and temporally and are linked to organic matter export and composition (Kalvelage et al., 2013; Babbin et al., 2014). It follows that there is still considerable uncertainty as to the controls on N-loss as well as the role for other linking processes such as DNRA ($NO_3^-$to NH_4^+) and NO_2^- oxidation in the absence of O_2.

Marine N-loss to N_2 occurs predominately in reducing sediments and the O_2 deficient water columns of OMZ's as found in the Arabian Sea and Eastern Tropical North and South Pacific (Ulloa et al., 2012; Lam et al., 2011 and references therein). NO_2^- is an important intermediate during N-loss and generally accumulates at concentrations up to ~10 µM in these regions

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(Codispoti et al., 1986; Casciotti et al., 2013). The depletion of NO_3^- is typically quantified as a dissolved inorganic N (DIN=NO_3^- + NO_2^- + NH_4^+) deficit relative to phosphate (PO_4^{-3}) assuming Redfield stoichiometry and the accumulation of biogenic N_2 (when measured) is detected as anomalies in N_2/Ar relative to saturation with atmosphere (Richards and Benson, 1961; Chang et al., 2010; Bourbonnais et al., 2015).

NO_3^- and NO_2^- N and O isotopes represent a useful tool to study N cycle transformations as they respond to in situ processes and integrate over their charasteristic time and space scales. Biologically mediated reactions are generally faster for lighter isotopes. For instance, both NO_3^- uptake and dissimilatory NO_3^- reduction produce a strong enrichment in both ^{15}N ($\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard})–1]\times1000$) and ^{18}O ($\delta^{18}O = [(^{18}O/^{16}O_{sample})/(^{18}O/^{16}O_{standard})–1]\times1000$) in the residual NO_3^- (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001; Granger et al., 2004; 2008; Sigman et al., 2005).

Canonical values for the N isotope effect ($\varepsilon \approx \delta^{15}N_{substrate} – \delta^{15}N_{product}$, at no significant substrate depletion) associated with microbial NO_3^- reduction during water-column denitrification range from 20 to 30‰ (Brandes et al., 1998; Voss et al., 2001; Granger et al. 2008). In contrast, the expression of the isotope effect of sedimentary denitrification is highly suppressed as compared to the water-column (generally <3‰) mostly due to near complete consumption of the porewater NO_3^- and diffusion limitation (Brandes and Devol, 1997; Lehmann et al., 2007; Alkhatib et al., 2012). The $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- are affected in fundamentally different ways during NO_3^- consumption and production processes. The ratio of the ^{15}N and ^{18}O fractionation factors ($^{18}\varepsilon$:$^{15}\varepsilon$) during NO_3^- consumption during denitrification or assimilation by phytoplankton in surface waters is close to 1:1 (Casciotti et al., 2002; Granger et al., 2004; 2008). While the $\delta^{15}N$ of the newly nitrified NO_3^- depends on the $\delta^{15}N$ of the precursor molecule being nitrified, the O atom is mostly derived from water (with a $\delta^{18}O$ of ~0 ‰), with significant isotopic fractionation associated with O incorporation during NO_2^- and NH_4^+ oxidation (Casciotti, 2002; Buchwald and Casciotti, 2010; Casciotti et al., 2010). Therefore, any deviation from this 1:1 ratio in the field has been interpreted as evidences that NO_3^- regeneration is co-occurring with NO_3^- consumption (Sigman et al., 2005; Casciotti and McIlvin, 2007; Bourbonnais et al., 2009). NO_2^- oxidation is associated with an inverse N isotope effect (Casciotti, 2009), atypical of biogeochemical reactions, and can cause both lower and higher ratios for $^{18}\varepsilon$:$^{15}\varepsilon$ compared to pure NO_3^- assimilation or denitrification, depending on

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

Additional information on N-cycling processes can be obtained from the isotopic composition of NO_2^-. For example, because of its inverse N isotope effect, NO_2^- oxidation results in a lower NO_2^- $\delta^{15}N$ than initially produced by NH_4^+ oxidation and NO_3^- reduction (Casciotti and Buchwald 2012). Logically, NO_2^- reduction would be expected to produce a positive relationship between $\delta^{15}N$-NO_2^- and $\delta^{18}O$-NO_2^- though there are no quantitative observations in the literature. Analogous to NO_3^- reduction, it also involves enzymatic breakage of the N-O bond. However, O-isotope exchange of NO_2^- with water (as a function of pH and temperature) would reduce the slope of a NO_2^- $\delta^{18}O$ vs. $\delta^{15}N$ relationship toward zero. NO_2^- turnover time can therefore be assessed from this observed relationship and in situ pH and temperature (Buchwald and Casciotti, 2013)

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

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

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

2 Material and Methods

2.1 Sampling

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

2.2 NO_2^- and NO_3^- isotope analysis

NO_2^- samples were stored in 125 ml HDPE bottles preloaded with 2.25 ml 6 M NaOH to prevent microbial activity as well as alteration of $\delta^{18}O$-NO_2^- by isotope exchange with water (Casciotti et al., 2007). Bottles were kept frozen after sample collection, though we have subsequently determined in the laboratory that seawater samples preserved in this way can be kept at room temperature for at least a year without alteration of NO_2^- $\delta^{15}N$ or $\delta^{18}O$ (unpublished data). Samples were analyzed by continuous He flow isotope-ratio mass spectrometry (CF-IRMS; see below) after chemical conversion to N_2O using acetic acid buffered sodium azide (McIlvin & Altabet 2005). Because of high sample pH, the reagent was modified for NO_2^- isotope analysis by increasing the acetic acid concentration to 7.84 M. In-house (i.e., MAA1, $\delta^{15}N$ = -60.6‰; MAA2, $\delta^{15}N$ = 3.9‰; Zh1, $\delta^{15}N$ = -16.4%) and other

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laboratory calibration standards (N23, δ^{15}N = 3.7‰ and δ^{18}O = 11.4‰; N7373, δ^{15}N = -79.6‰ and δ^{18}O = 4.5‰; and N10219; δ^{15}N = 2.8‰ and δ^{18}O = 88.5‰; see Casciotti and McIlvin, 2007) were used for NO^{2-} δ^{15}N and δ^{18}O analysis.

NO_3^- samples were stored in 125 ml HDPE bottles preloaded with 1 ml of 2.5 mM sulfamic acid in 25 % HCl to both act as a preservative and to remove NO_2^- (Granger and Sigman, 2009). Samples were also kept at room temperature and we have found that they can be stored in this way for many years without alteration of NO_3^- δ^{15}N or δ^{18}O. Cadmium reduction was used to convert NO_3^- to NO_2^- prior to conversion to N_2O and IRMS analysis also using the "azide method" (McIlvin & Altabet 2005). Standards for NO_3^- isotope analysis were N3 (δ^{15}N = 4.7‰ and δ^{18}O = 25.6‰), USGS34 (δ^{15}N = -1.8‰ and δ^{18}O = -27.9‰) and USGS35 (δ^{15}N = 2.7‰ and δ^{18}O = 57.5‰) (Casciotti et al., 2007). The lowest concentration of NO_2^- or NO_3^- analyzed for isotopic composition was 0.5 µM, thus δ^{15}N-NO_3^- and δ^{15}N-NO_2^- could not be measured below 37 m at station 63.

A GV Instruments IsoPrime Isotope Ratio Mass Spectrometer (IRMS) coupled to an on-line He continuous-flow purge/trap preparation system was used for isotope analysis (Sigman et al., 2001; Casciotti et al., 2002; McIlvin & Altabet 2005). N_2O produced by the azide reaction was purged with He from the septum sealed 20 ml vials and trapped, cryofocused and purified prior to transfer to the IRMS. Total run time was 700 s/sample (McIlvin & Altabet 2005). Isotopic values are referenced against atmospheric N_2 for δ^{15}N and VSMOW for δ^{18}O. Reproducibility was 0.2‰ and 0.5‰, respectively.

2.3 N_2/Ar IRMS analysis and calculation of biogenic N_2

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

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produced biogenic N_2 was obtained by subtracting excess N_2 at the corresponding density surface for waters outside of the ODZ (O_2 >10 µM) not affected by N-loss (Chang et al., 2010; Bourbonnais et al., 2015). Reproducibility was better than 0.7 µM for excess N_2 and 0.03 ‰ for δ^{15}N-N_2. δ^{15}N of biogenic N_2 was calculated by mass balance as in Bourbonnais et al. (2015).

2.4 Isotope effect (ε) calculations

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

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

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

where f_1 is the fraction of remaining NO_3^- and f_2 is the fraction of remaining DIN (NO_3^- + NO_2^- concentrations). δ^{15}N-DIN is the average δ^{15}N for NO_3^- and NO_2^- weighted by their concentrations. The fraction of remaining DIN is a better estimation of the overall effective isotope effect for N-loss (Bourbonnais et al., 2015), while using NO_3^- as the basis to calculate ε specifically targets NO_3^- reduction. See below for details of f value calculation.

The overall isotope effect for N-loss can also be estimated from the δ^{15}N of biogenic N_2 produced:

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

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

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

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

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

For all equations, the slope represents ε and the y-intercept is the initial δ^{15}N prior to N-loss. For calculations using equations 3 and 6 we only used δ^{15}N values associated with biogenic N_2 greater than 7.5 µM because of increasing noise below this level due to the huge atmospheric dissolved N_2 background (typically up to ~500 µM).

Since the closed system equations assume no loss or resupply of substrate or production in a water parcel, they are appropriate where there is little mixing and/or advection is dominant over mixing. The open system equations take into account supply from or loss to surrounding water

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parcels, e.g. mixing dominance. Both cases represent extreme situations. In the next section, we will estimate and compare ε using both sets of equations.

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

$$f_{1p} = [NO_3^-] / Np_{expected} \qquad (7)$$

$$\text{or } f_{2p} = ([NO_3^-] + [NO_2^-]) / Np_{expected} \qquad (8)$$

$$Np_{expected} = 15.8*([PO_4^{3-}] - 0.3) \qquad (9)$$

$$N_{observed} = [NO_3^-] + [NO_2^-] + [NH_4^+] \qquad (10)$$

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

$$Np_{def} = Np_{expected} - N_{observed} \qquad (11)$$

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

$$N_{expected}\text{ bio } N_2 = [NO_3^-] + [NO_2^-] + 2 \times [\text{Biogenic } N_2] \qquad (12)$$

$$f_{1bioN2} = [NO_3^-] / N_{expected}\text{ bio } N_2 \qquad (13)$$

$$\text{or } f_{2bioN2} = [NO_3^- + NO_2^-] / N_{expected}\text{ bio } N_2 \qquad (14)$$

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

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3 Results

3.1 Hydrographic characterization

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

3.2 Dissolved O_2 and nutrient concentrations

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

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

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

3.3 NO_2^- and NO_3^- isotope compositions

As a consequence of kinetic isotope fractionation during N-loss, the N and O isotope composition of NO_3^- and NO_2^- varied inversely with NO_3^- and NO_2^- concentrations, with maximum $\delta^{15}N$ and $\delta^{18}O$ values near the bottom at each station. $\delta^{15}N$-NO_3^- increased from about 10‰ in surface waters to up to 50‰ in the O_2-depleted zone (Fig. 4 C), with near bottom values at station 64 significantly higher (50‰) than at the other stations which ranged from 20 to 30‰. $\delta^{15}N$-NO_2^- varied from –25 to about 10‰ (Fig. 4 D) with maximum values also in deeper waters at station 64.

As expected for NO_3^- reduction, $\delta^{18}O$-NO_3^- positively co-varied with $\delta^{15}N$-NO_3^- and ranged from 12 to 46‰. We observed an overall linear relationship between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- with a slope of 0.86, which was significantly different than 1 (p-value<0.05), and a y-intercept of 1.90 (r^2= 0.996, see Fig. 5 A). NO_3^- $\delta^{15}N$ and $\delta^{18}O$ have been shown to increase equally (ratio 1:1) during assimilatory and dissimilatory NO_3^- reduction (Casciotti et al. 2002; Sigman et al. 2003). However, deviations from this trend have been observed in the ocean and interpreted as evidence for co-occurring NO_3^- production processes (Sigman et al. 2005; Casciotti and McIlvin, 2007 ; Bourbonnais et al., 2009; 2015). In this study, we observed a NO_3^- $\delta^{18}O$ vs $\delta^{15}N$ relationship less than 1, likely originating from NO_2^- re-oxidation to NO_3^- in our environmental setting as in Casciotti and McIlvin (2007). We also observed, for the first time, a significant correlation between $\delta^{15}N$-NO_2^- and $\delta^{18}O$-NO_2^- in the ODZ for our in-shore water stations (Fig. 5 B). As in prior studies (Casciotti and McIlvin 2007; Casciotti et al., 2013), no such relationship was observed by us for a nearby set of offshore stations (see Fig. 5 C) where longer NO_2^- turnover times likely facilitated O isotope exchange with water. We will discuss implications of this unique finding in the next section.

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3.4 The δ^{15}N difference between NO_3^- and NO_2^-

The difference in δ^{15}N between NO_3^- and NO_2^- ($\Delta\delta^{15}$N) reflect the combined isotope effects of simultaneous NO_3^- reduction, NO_2^- reduction, and NO_2^- oxidation. For NO_3^- reduction alone, highest $\Delta\delta^{15}$N values would be around 25‰ at steady-state (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001; Granger et al., 2004; 2008; Sigman et al., 2005). The effect of NO_2^- reduction would be to increase the δ^{15}N of the residual NO_2^-, decreasing $\Delta\delta^{15}$N. In contrast, NO_2^- oxidation is associated with an inverse kinetic isotope effect (Casciotti 2009) and would act to decrease the residual δ^{15}N of NO_2^-, and hence overall increases the $\Delta\delta^{15}$N. Therefore, following NO_2^- oxidation, $\Delta\delta^{15}$N may be larger than expected from NO_3^- and NO_2^- reduction alone, especially if the system is not at steady-state (Casciotti et al., 2013). $\Delta\delta^{15}$N ranged from 15‰ to 40‰ (average = 29.78‰ and median = 32.5‰) for samples with O_2<10 µM. These results confirm the presence of NO_2^- oxidation for at least some of our depth intervals.

3.5 N deficit, biogenic N_2 and δ^{15}N-N_2

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

The δ^{15}N-N$_2$ anomaly, i.e., the difference between the δ^{15}N-N$_2$ observed and at equilibrium and derived as in Charoenpong et al. (2014), ranged from –0.2 to 0.1‰ (Fig. 7 C). The corresponding range in δ^{15}N biogenic N$_2$ at O$_2$ < 10 µM, calculated from the δ^{15}N-N$_2$ anomaly as in Bourbonnais et al. (2015), was from -9.0 to 3.2‰. Negative δ^{15}N-N$_2$ anomaly (i.e., lower δ^{15}N-biogenic N$_2$) is produced at the onset of N-loss, because extremely depleted ^{15}N-N$_2$ is first produced. At a more advanced N-loss stage, we expect δ^{15}N-N$_2$ anomaly and δ^{15}N-biogenic N$_2$ to increase, as we observed in this study, as heavier ^{15}N is added to the biogenic N$_2$ pool. The δ^{15}N-N$_2$ anomaly signal appears small as compared to the isotopic composition of NO$_3^-$ and NO$_2^-$ but is 1) analytically significant and 2) the result of dilution by the large background of atmospheric N$_2$ (400 to 500 µM N$_2$).

3.6 Isotope effect (ε)

Isotope effects were calculated using eqs. 1 to 6 to compare closed vs. open system assumptions as well as different approaches to estimating f. Examples of plots of the closed system equations, with f calculated using biogenic N$_2$, are shown in Fig. 6. Comparison of results using all 3 approaches for calculating f (i.e. Redfield stoichiometry, biogenic N$_2$ and observed substrate divided by maximum “upwelled” concentration, (see section 2.4) are shown in Table 1 (closed system) and 2 (open system). In the case of the closed system, ε values were in all cases lower than canonical ones, ranging narrowly from about 6‰ for changes in the δ^{15}N of DIN to about 14‰ for changes in δ^{15}N-NO$_3^-$ (Table 1). For the open system equations, estimated ε was higher and covered a large and unrealistic range from about 12‰ for changes in the biogenic N$_2$ to about 63‰, respectively for changes in the δ^{15}N of NO$_3^-$. For our inshore water stations, where we observed a single water mass (Fig. 2), a closed system should be a more realistic approximation of ε. The Rayleigh equations' y-intercepts, where f=1, represent the initial δ^{15}N of NO$_3^-$ or DIN, and varied from -0.5 to 3.7‰ and –18.4 to 6.2‰ for the closed and open systems, respectively. The higher end of this range is more realistic based on prior isotopic measurements for source waters (e.g., see Bourbonnais et al., 2015).

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4 Discussion

4.1 Behavior of NO_2^-

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

To assess the influence of the various N cycle processes that have NO_2^- as either a substrate or product, we first examined the relationship between the $\delta^{15}N$ and $\delta^{18}O$ of NO_2^-. Several processes can influence the isotopic composition of NO_2^-. NO_3^- reduction to NO_2^- is associated with a ε of 20 to 30‰ (Cline and Kaplan, 1975; Brandes et al., 1998; Voss et al., 2001; Granger et al., 2004; 2008; Sigman et al., 2005) and acts to produce NO_2^- depleted in ^{15}N and ^{18}O. In contrast, NO_2^- reduction as part of either anammox, denitrification or DNRA increases both the $\delta^{15}N$ and $\delta^{18}O$ of residual NO_2^-, with laboratory and field estimates for ε clustering around 12‰ to 16‰ (Bryan et al., 1983; Brunner et al., 2013; Bourbonnais et al., 2015). However, NO_2^- oxidation to NO_3^- at low or non-detectable O_2 has been shown to be an important sink for NO_2^- in ODZs (e.g. Füssel et al., 2012). Anammox bacteria can also use NO_2^- as an electron donor during CO_2 fixation under anaerobic conditions (Strous et al., 2006).

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

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Past studies have found NO_2^- $\delta^{18}O$ values in ODZ's in isotope equilibrium with water as a likely consequence of relatively long turnover time (e.g., Buchwald and Casciotti, 2013; Bourbonnais et al., 2015). O isotope exchange involves the protonated form, HNO_2, but because of its high pKa as compared to NO_3^-, this process can occur even at neutral to alkaline ocean pH on a time scale of 2 to 3 months at environmentally relevant temperatures (Casciotti et al., 2007). NO_2^- $\delta^{18}O$ isotopic composition at equilibrium with water is a function of the $\delta^{18}O$ of water and temperature (+14‰ for seawater at 22 °C) (Casciotti et al., 2007; Buchwald and Casciotti, 2013) and independent of its $\delta^{15}N$ value such that plots of NO_2^- $\delta^{18}O$ vs $\delta^{15}N$ usually have a slope of near zero. This is seen in our NO_2^- data from offshore stations occupied during M90 (Fig. 5).

We observed, for the first time, a significant linear relationship for NO_2^- $\delta^{18}O$ vs. $\delta^{15}N$ at our inshore stations (slope = 0.64 ± 0.07, $r^2 = 0.59$, p-value=3×10^{-6}) where $O_2 < 10$ µM (Fig. 5 B). Coupled $\delta^{15}N$ and $\delta^{18}O$ effects for NO_2^- have not been as well studied as for NO_3^-. Nevertheless, if NO_2^- turnover was faster than equilibration time with water, NO_3^- and NO_2^- reduction whether as part of the denitrification, anammox or DNRA pathways, should also produce a positive relationship between NO_2^- $\delta^{15}N$ and $\delta^{18}O$, but the corresponding slopes are not yet known. In contrast to our offshore stations (Fig. 5 C), this positive relationship thus demonstrates that the oxygen isotopic composition of NO_2^- is not in equilibrium with water due to both rapid NO_2^- turnover and the dominance of NO_2^- reduction over oxidation in Peru coastal waters as compared to offshore. Higher rates for aerobic NH_4^+ and NO_2^- oxidation, as well as anaerobic NO_3^- reduction to NO_2^-, and further reduction to NH_4^+ (DNRA) or N_2, have been reported in shallow waters off Peru presumably due to increased coastal primary production and organic matter supply to the in-shore OMZ (e.g. Codispoti et al., 1986; Lam et al., 2011; Kalvelage et al., 2013). However as our observations are restricted to anoxic waters, only high rates of N-loss could explain this more rapid NO_2^- turnover."

In principal, we can estimate NO_2^- turnover time from knowledge of rates for exchange with water and assumptions of the $\delta^{18}O$ vs $\delta^{15}N$ slope expected in the absence of exchange. Unfortunately, the slope of the relationship between NO_2^- $\delta^{18}O$ vs $\delta^{15}N$ expected in the absence of equilibration with water is not yet known. However, an upper limit for turnover time for NO_2^- can be estimated based on equilibration time as a function of *in situ* pH and temperature (Buchwald and Casciotti, 2013). During the M91 cruise in December, subsurface temperature was 13 to 15°C along our transect and corresponding pH was near 7.8 (Michelle Graco,

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

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

The inverse kinetic isotope effect (Casciotti, 2009; Brunner et al., 2013) associated with NO_2^- oxidation is likely responsible for these high $\Delta\delta^{15}N$ values (e.g. Casciotti and Buchwald, 2012; Casciotti et al., 2013). Taking all isotope effects into account, the following equation can be derived to estimate $\Delta\delta^{15}N$ at steady-state:

$$\Delta\delta^{15}N\text{ (steady state)} = \varepsilon_{NO3\text{-}red} - (1\text{-}\gamma)\ x\ \varepsilon_{NO2\text{-}red} - \gamma\ x\ \varepsilon_{NO2\text{-}oxid} \qquad (15)$$

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

Given that $\varepsilon_{NO2\text{-}\ oxid}$ has been reported to be -13‰ for aerobic NO_2^- oxidation and using the literature ranges for $\varepsilon_{NO3\text{-}red}$ and $\varepsilon_{NO2\text{-}red}$ above, our observed $\Delta\delta^{15}N$ implies that up to 100 % of NO_2^- produced by NO_3^- reduction could be oxidized back to NO_3^-. This estimate is higher than ratios of NO_2^- oxidation/NO_3^- reduction of up to 54% for the Peruvian coastal ODZ derived from direct rate measurements (Lam et al., 2009; Kalvelage et al., 2013), and should thus be considered as an upper limit. Alternatively, NO_2^- oxidation also occurs as part of the overall

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metabolism of anammox bacteria (Strous et al., 2006) which can be the dominant N_2 producers in the Peru ODZ (Kalvelage et al., 2013). A large inverse kinetic ε for NO_2^- oxidation of ~-30‰ has been observed for anammox bacteria in culture (Brunner et al., 2013). If the sole pathway for NO_2^- oxidation, our data suggest NO_2^- oxidation up to only ~80% of total NO_3^- reduction. However, anammox bacteria only oxidize a minor fraction of NO_2^- to NO_3^- in culture. At the same time, estimates of NO_2^- oxidation (8.48 to 928 nM d^{-1}) are significantly higher than N-loss rates by anammox (2.84 to 227 nmol N l^{-1} d^{-1}) on the Peruvian shelf (Kalvelage et al., 2013), clearly indicating non-anammox related nitrite oxidation.

The deviations from a 1:1 relationship for NO_3^- $\delta^{18}O$ and $\delta^{15}N$ can also be indicative of NO_2^- oxidation. During NO_3^- uptake or dissimilative NO_3^- reduction, NO_3^- $\delta^{15}N$ and $\delta^{18}O$ increase equally with a ratio of 1:1 (Granger et al., 2004; 2008). We observed a slope of about 0.86 (Fig. 5 A) for the relationship for NO_3^- $\delta^{18}O$ versus $\delta^{15}N$ in the in-shore Peru ODZ, similar to recent off-shore observations (Bourbonnais et al., 2015). Prior reports of deviations toward higher values for the slope were indicative of addition of newly nitrified NO_3^- from a relatively low $\delta^{15}N$ source (e.g. see Sigman et al., 2005; Bourbonnais et al., 2009). Our observed deviation toward slopes < 1 can instead be explained by the addition of newly nitrified NO_3^- with a lower $\delta^{18}O$-NO_3^-, mostly derived from water (Andersson and Hooper, 1983), relative to the high ambient $\delta^{18}O$-NO_3^- values. In fact, a slope for $\delta^{18}O$:$\delta^{15}N$ of either greater or less than 1 can be observed, depending on initial environmental NO_3^- isotopic composition relative to any in-situ sources (Casciotti et al., 2013). Casciotti and Buchwald (2012) showed model results where NO_2^- oxidation generally produces a slope <1 for the NO_3^- $\delta^{18}O$ versus $\delta^{15}N$ relationship, when the NO_3^- $\delta^{15}N$ and $\delta^{18}O$ are higher than about 15‰ as observed in Casciotti et al. (2013) and Bourbonnais et al. (2015).

4.2 Isotope effects for N-loss

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

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Linear regression coefficients for ε calculated using the different approaches presented in section 2.4 are listed in Tables 1 and 2. For illustration, example Rayleigh closed system plots for δ^{15}N-NO$_3^-$, δ^{15}N-DIN, or δ^{15}N biogenic N$_2$ as a function of f_{2bioN2} are shown in Fig. 6. Surprisingly, ε values estimated from the slope of these relationships are not sensitive to choice of method for calculating f despite the lack of 1:1 correspondence between different bases (Np$_{expected}$, biogenic N$_2$, or [NO$_3^-$]/[NO$_3^-$]$_{max}$). In the case of ε calculated from changes in δ^{15}N-DIN, ε ranged narrowly with choice of f from 6.3 to 7.4 with standard errors on the slope of <0.6 (Table 1). As there was no significant difference between bases for calculating f, it appears that all three of our approaches are valid for this purpose.

However, ε for N-loss (closed system) does vary significantly between calculations using changes in δ^{15}N-NO$_3^-$, δ^{15}N-DIN, or δ^{15}N biogenic N$_2$. ε is largest for changes in δ^{15}N-NO$_3^-$ (~14 ‰) and smallest for changes in δ^{15}N-DIN (~7‰). ε based on δ^{15}N biogenic N$_2$ is intermediate (~11‰). The latter two, using DIN or biogenic N$_2$ as the basis to calculate ε, are more representative of N-loss. Calculations based on changes in δ^{15}N-NO$_3^-$ are affected by NO$_2^-$ accumulation and isotope effects of NO$_2^-$ oxidation (see above). The 4‰ difference in ε calculated from changes in δ^{15}N of biogenic N$_2$ vs. δ^{15}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 δ^{15}N-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 (~12.3°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 δ^{15}N to the biogenic N$_2$ pool, increasing ε that must be larger to account for the observed isotopic enrichment."

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

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

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

Closed system estimates of ε are likely more reliable in our setting because of low likelihood of mixing between water masses of contrasting characteristics on the shelf. Temperature and salinity in the ODZ at our stations narrowly ranged from 13.5 to 15 °C and 34.88 to 34.98 (Fig. 2), similar to T/S signatures from offshore source waters (Bourbonnais et al., 2015), and suggestive of a single water mass. Accordingly, as in Bourbonnais et al. (2015), we view the closed system equations as most reliable with a value of ~ 6.5‰ for ε based on changes in δ^{15}N DIN as the likely best estimate. However, given the overlap with the results of open system equations for changes in δ^{15}N of biogenic N_2, an upper bound of ~11‰ appears appropriate. This range in ε for N-loss falls below the results of Bourbonnais et al. (2015) for a near-coastal eddy in the same region and time period (~14‰) and is much less than the canonical range of 20 to 30‰ (Brandes et al., 1998; Voss et al., 2001; Granger et al., 2008).

There are several reasonable explanations for these relatively low ε values. These include higher microbial growth rates associated with higher productivity, which would shift biochemical rate limitation away from enzyme reactions to membrane transport with low fractionation potential (e.g. Wada and Hattori, 1978). Another is greater influence from benthic N cycling processes in our relatively shallow inshore system as compared to deeper waters. Sediment N-loss has been shown to incur low ε due to, in analogous fashion to the affect of microbial growth rate, dominance of substrate transport limitation through the sediment (Brandes and Devol, 1997). This possibility will be explored further in the next section. Unlikely explanations for our relatively low ε values for N-loss include the effects of decreasing NO_3^- concentration (Kritee et al., 2012) and contributions from organic N via anammox to biogenic N_2. Lack of curvature in the Rayleigh plots demonstrates a lack of dependence of substrate concentration (Fig. 6 A & B) as the range in f corresponds to a large range in NO_3^- or DIN concentrations. The possible effects of contributions from organic N to biogenic N_2 has already been taken into account in calculations based on changes in the δ^{15}N of biogenic N_2, as discussed above.

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4.3 Using ε values for estimating sediment N-loss

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

$$\varepsilon_{obs} = \varepsilon_{wc} \times (1 - P_{sed}) + \varepsilon_{sed} \times P_{sed} \qquad (13)$$

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

5 Conclusions

The inshore Peru ODZ is distinguished from offshore by its high productivity as a consequence of coastal upwelling as well as possible greater influence from benthic processes. To examine impact on N-loss processes and their isotope effects, we investigated the dynamics of N and O isotope of NO_2^- and NO_3^- at 6 coastal stations off Peru.

We found that N-loss representing the net effect of partial denitrification, anammox and nitrification produced in sum large variations in isotopic composition. NO_2^- $\delta^{15}N$ ranged from –20 to 10‰ and NO_3^- $\delta^{15}N$ ranged from 10 to 50‰. Generally, NO_3^- and NO_2^- isotope values varied inversely with their concentrations as expected for Rayleigh-like fractionation effects. Isotope values were usually higher in low-O_2 near bottom waters where N species concentrations were also relatively low.

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We observed, for the first time, a positive linear relationship between NO_2^- $\delta^{15}N$ and $\delta^{18}O$ at our inshore stations. In offshore ODZ waters, such a relationship has never previously been observed as NO_2^- $\delta^{18}O$ reflected equilibration with water in these regions (Buchwald and Casciotti, 2013). Our results suggest a turnover time for NO_2^- faster than the equilibration time with water and the dominance of NO_2^- reduction over NO_2^- oxidation in these highly productive coastal waters. We estimated a NO_2^- turnover time of at least ~40 days from our data.

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The difference in $\delta^{15}N$ between NO_3^- and NO_2^- $\Delta(\delta^{15}N)$ was high, reaching up to 40‰ in deeper waters and greater than expected from NO_3^- and NO_2^- reduction only. The influence of NO_2^- oxidation is consistent with this observation due to its inverse fractionation effect (Casciotti, 2009). Additional evidence for NO_2^- oxidation is found in the relationship between NO_3^- $\delta^{15}N$ and $\delta^{18}O$. NO_3^- reduction alone is expected to produce a 1:1 relationship (Granger et al., 2008). While we observed a linear relationship between NO_3^- $\delta^{15}N$ and $\delta^{18}O$, the slope of 0.86 is indicative of simultaneous addition of NO_3^- with relatively low $\delta^{18}O$, also consistent with a role for NO_2^- oxidation at our coastal sites. However, a favorable thermodynamic couple for NO_2^- oxidation in the absence of O_2 in these waters remains unknown.

A number of different approaches for estimating ε for N-loss were compared including choice of N form for changes in $\delta^{15}N$ (NO_3^-, DIN, or biogenic N_2), closed vs open system Rayleigh equations, and the basis for calculating the denominator in f ($Np_{expected}$, biogenic N_2, or maximum NO_3^-). For the latter, there was little difference in estimated ε despite discrepancies between the removal of NO_3^- and appearance of N_2 estimated from them. Observation of a single water mass (T-S plot) in our coastal region as well as more realistic ranges for derived ε and initial $\delta^{15}N$ indicated that closed system assumptions were more realistic. Using closed system equations, relatively low ε values were calculated; ~7 ‰ for changes in the $\delta^{15}N$ of DIN and ~11 ‰ for changes in the $\delta^{15}N$ of biogenic N_2. As in Bourbonnais et al. (2015), ε calculated from changes in the $\delta^{15}N$ of NO_3^- alone was not representative of the ε for overall N-loss in consideration of the build up of NO_2^- with distinct $\delta^{15}N$. These estimates for ε for net N-loss are lower than recently reported for a nearby offshore eddy with intense N-loss (~14‰; Bourbonnais et al., 2015). This lower ε may be attributed to the influence of sedimentary N-loss on the Peruvian shelf (e.g., Bohlen et al., 2011), with a highly suppressed ε, on the overlying water column at our shallow stations. Given this assumption, we estimate that

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sedimentary N-loss (by both denitrification and anammox) could account for up to 60% of the total N-loss in in shore Peru ODZ waters.

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

Acknowledgements

Data for this paper are available on the Data Management Portal for Kiel Marine Sciences hosted at GEOMAR: https://portal.geomar.de/, or upon request to the corresponding author. This research was supported by the Deutsche Forschungsgemeinschaft- project SFB-754 (Climate-Biogeochemistry Interactions in the Tropical Ocean: www.sfb754.de), SOPRAN_II (grant # FKZ_03F0611A; Surface Ocean Processes in the Anthropocene: www.sopran.pangaea.de), the Nitrogen Isotope and N_2/Ar Biogeochemistry of the Peru Suboxic Zone project (National Science Foundation award OCE-0851092), the University of Massachusetts Intercampus Marine Science graduate program support to H. Hu and a NSERC Postdoctoral Fellowship to A.B. We would like to thank the captain and crew of R/V Meteor during the M91 cruise and Daniel Kiefhaber, Avi Bernales and Violeta Leon for their help during sampling and/or sample analysis. We thank the authorities of Peru for the permission to work in their territorial waters.

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

	Basis for f	ε	y-intercept	r^2
$\delta^{15}N\text{-}NO_3^-$	$Np_{expected}$	13.9 ± 0.7	3.74	0.92
	N_2 Biogenic	14.3 ± 0.9	3.71	0.95
	$[NO_3^-]/[NO_3]_{max}$	14.7 ± 0.6	-0.55	0.95
$\delta^{15}N\text{-}DIN$	$Np_{expected}$	6.3 ± 0.3	7.20	0.92
	N_2 Biogenic	6.6 ± 0.4	6.71	0.94
	DIN/DIN_{max}	7.4 ± 0.6	10.90	0.91
$\delta^{15}N$-Biogenic N_2	$Np_{expected}$	10.5 ± 1.5	2.94	0.70
	N_2 Biogenic	10.6 ± 1.5	3.04	0.72

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

	Basis for f	ε	y-intercept	r^2
$\delta^{15}N$-NO_3^-	$Np_{expected}$	63.0 ± 4.5	-18.42	0.86
	N_2 Biogenic	66.30± 6.2	-21.92	0.87
	$[NO_3^-]/[NO_3^-]_{max}$	38.9 ± 2.7	6.19	0.87
$\delta^{15}N$-DIN	$Np_{expected}$	17.4 ± 1.2	3.26	0.88
	N_2 Biogenic	20.0 ± 1.8	1.72	0.89
	DIN/DIN_{max}	13.2 ± 0.9	8.45	0.91
$\delta^{15}N$-Biogenic N_2	$Np_{expected}$	12.3 ± 1.9	1.94	0.67
	N_2 Biogenic	14.15 ± 2.1	2.25	0.68

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Figure legends

Figure 1. Station map with satellite data from http://disc.sci.gsfc.nasa.gov/giovanni/. A: sea surface chlorophyll α concentrations (mg/m^3), B: night time sea surface temperature (°C).

Figure 2. Temperature vs. salinity plots. In A, color indicates O_2 concentration (μM). In B, color indicates NO_2^- concentration (μM). Points at bottom in red rectangle of each plot belong to station 68 for depths greater than 150m. Black dots in B mean no NO_2^- concentration data are available.

Figure 3. O_2 and nutrient distribution along the transect. A: O_2 concentration (μM) with isotherm overlay and B: NH_4^+ concentration (μM), C: $Si(OH)_4$ concentration (μM) and D: PO_4^{3-} concentration (μM). Grey region represents bathymetry. The depth for station 68 is 253m.

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

Figure 5. Relationships between $\delta^{15}N$ and $\delta^{18}O$ for NO_3^- and NO_2^-, respectively, for $O_2 \leq 10$μM. A: $\delta^{18}O\text{-}NO_3^-$ vs $\delta^{15}N\text{-}NO_3^-$ for station 62 to 68. B: $\delta^{18}O\text{-}NO_2^-$ vs $\delta^{15}N\text{-}NO_2^-$ for station 62 to 68. C: $\delta^{18}O\text{-}NO_2^-$ vs $\delta^{15}N\text{-}NO_2^-$ for M90 offshore stations 51, 59, 106 and 111 (see text, section 3.3). For each plot, overall linear regressions are shown. Significant correlation coefficients at a 0.05 significance level are denoted by *.

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

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

Figure 8. Cross-plots of biogenic N in N_2 versus DIN (A), NO_3^- (B) and N_{pdef} (C), see Eqs. 9-11 in text). All plots have the overall linear regression overlaid. All the points are restricted to O_2 concentrations less than 10 μM. Biogenic N_2 was not measured for stations 62, 64 and 66. Significant correlation coefficients at a 0.05 significance level are denoted by *.

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