Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin

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[1] Water column depth profiles along the North Pacific margin from Point Conception to the tip of Baja California indicate elevation of nitrate (NO$_3^-$) $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O associated with denitrification in the oxygen-deficient thermocline waters of the eastern tropical North Pacific. The increase in $\delta^{18}$O is up to 3%o greater than in $\delta^{15}$N, whereas our experiments with denitrifier cultures in seawater medium indicate a 1:1 increase in NO$_3^-$ $\delta^{18}$O and $\delta^{15}$N during NO$_3^-$ consumption. Moreover, the maximum in NO$_3^-$ $\delta^{18}$O is somewhat shallower than the maximum in NO$_3^-$ $\delta^{15}$N. These two observations can be summarized as an “anomaly” from the 1:1 $\delta^{18}$O-to-$\delta^{15}$N relationship expected from culture results. Comparison among stations and with other data indicates that this anomaly is generated locally. The anomaly has two plausible interpretations: (1) the addition of low-$\delta^{15}$N NO$_3^-$ to the shallow thermocline by the remineralization of newly fixed nitrogen, or (2) active cycling between NO$_3^-$ and NO$_2^-$ (coupled NO$_3^-$ reduction and NO$_2^-$ oxidation) in the suboxic zone.


1. Introduction

[2] The oceanic budget of biologically available (or “fixed”) nitrogen is poorly understood. Estimates of the global rate of nitrogen (N) loss by denitrification would leave the ocean N budget far out of balance unless N$_2$ fixation rates are much higher than previously estimated [Brandes and Devol, 2002; Codispoti et al., 2001; Middelburg et al., 1996]. While such imbalances cannot be ruled out, the stability of atmospheric CO$_2$ and of the N isotopic composition of deep sea sediments over the last ~5 kyr argues against such extreme imbalances [Deutsch et al., 2004; Kienast, 2000].

[3] Direct measurements of N fluxes in the ocean (e.g., N$_2$ fixation, denitrification, NO$_3^-$ assimilation, and nitrification) cannot, by themselves, provide a reliable picture of the ocean N cycle. Temporal and spatial complexity, combined with the limitations of shipboard sampling of the ocean, lead to uncertainty in the extrapolation of these measurements to regional and global fluxes. Moreover, assays for N transformations can perturb the samples they are attempting to measure. For these reasons, biogeochemical parameters in ocean water have become important as more integrative measures of the rates of N fluxes.

[4] Deviations in the [NO$_3^-$]-to-[PO$_4^{3-}$] relationship from the “Redfield” relationship driven by algal assimilation and remineralization are used to study the rates and distributions of both N$_2$ fixation and denitrification. “N*”, defined as [NO$_3^-$] $-$ 16 $\times$ [PO$_4^{3-}$] + 2.9 (in $\mu$mol/kg) [Deutsch et al., 2001], quantifies excesses and deficits in NO$_3^-$ relative to the globally derived [NO$_3^-$]-to-[PO$_4^{3-}$] relationship, indicating regions of N$_2$ fixation and denitrification, respectively. When combined with some measure of ocean circulation, rates of these processes can be derived [Deutsch et al., 2001; Gruber and Sarmiento, 1997]. While this use of nutrient data is extremely powerful, it has limitations. First, deviations from the Redfield [NO$_3^-$]-to-[PO$_4^{3-}$] relationship may not always be due to N inputs or outputs, arising instead from variations in the stoichiometry of nutrient uptake and remineralization. Second and most relevant here, NO$_3^-$ inputs and losses partially erase one another if they occur in the same water or if their host waters are mixed in a way that cannot be reconstructed.

[5] The complementary measurement of NO$_3^-$ $^{15}$N/$^{14}$N can address the first limitation described above. The N isotopes provide an additional test as to whether positive or negative N* in a given region is indeed driven by N$_2$ fixation or denitrification. Most of the deep ocean (>2 km) is homogenous in NO$_3^-$ $\delta^{15}$N, at ~5%o relative to atmospheric N$_2$ [Liu and Kaplan, 1989; Sigman et al., 2000]
over a broad range of amplitudes for the isotope effect [Casciotti et al., 2002; Granger et al., 2004b]. That we observe the same $^{18}e:^{15}e$ for both denitrification and NO$_3^-$ assimilation is consistent with evidence that NO$_3^-$ reduction is the dominant cause of fractionation in both processes [Needoba et al., 2004; Shearer et al., 1991]. Thus, while we have much to learn about the N:O fractionation ratios, NO$_3^-$ assimilation and denitrification, the processes of NO$_3^-$ consumption with the greatest effects on oceanic NO$_3^-$ distributions, apparently cause similar isotope fractionation of N and O in NO$_3^-$.

Unlike the consumption of NO$_3^-$, NO$_3^-$ production appears to have very different effects on the N and O isotopes of NO$_3^-$ in the open ocean subsurface, at least in oxic waters, almost all of the ammonium generated from organic N is eventually oxidized to NO$_3^-$, so that the N isotope effects associated with ammonium production and nitrification do not impact the $^{15}N$ of NO$_3^-$ produced. In this case, the $^{15}N$ of newly produced NO$_3^-$ is primarily controlled by the $^{15}N$ of the organic matter being remineralized. The $^{18}O$ of newly produced NO$_3^-$ obviously does not depend on the isotopic composition of the organic matter being remineralized.

Biochemical studies have derived mechanisms for ammonium oxidation to nitrite (NO$_2^-$) in which one O atom is donated from O$_2$ and the other from water [Andersson et al., 1982]. NO$_2^-$ oxidation to NO$_3^-$ involves the donation of O only from water [Disprito and Hooper, 1986; Kumar et al., 1983]. On this basis, the traditional interpretation has been that two thirds of the O atoms in NO$_3^-$ should originate from water and one third should originate from O$_2$ [Böhlke et al., 1997; Durka et al., 1994; Kendall, 1998; Wassenaar, 1995]. However, the same biochemical studies also demonstrated a strong nitrifier-catalyzed nitrite-water exchange of O atoms [Andersson et al., 1982]. On the basis of these observations, it is likely that much less than one out of two O atoms in NO$_3^-$ comes from O$_2$ [Casciotti et al., 2002]. A culture experiment in which Nitrosomonas europaea produces NO$_2^-$ in the presence of $^{18}O$-labeled water indicates that at least 50% of the O atoms in NO$_3^-$ have undergone exchange with water [Casciotti et al., 2002], such that at least 5 out of the 6 O atoms in NO$_3^-$ originate from water (i.e., 1 or less out of the 6 comes from O$_2$). It is also possible that catalysis of exchange with water occurs during the oxidation of NO$_2^-$ to NO$_3^-$ [Disprito and Hooper, 1986], reducing further the effective contribution of O atoms from O$_2$ and increasing the contribution from H$_2$O.

Measurements to date from the ocean indicate that away from regions of known denitrification, subsurface NO$_3^-$ $^{18}O$ varies relatively little and is close to the ambient water (0 ± 1‰ or 3 ± 1‰ different from it; see auxiliary material, endnote i in Auxmat1.txt) the ambient water ($^{18}O_{sample} = ((^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{reference} - 1) \times 1000‰$, where the reference is Vienna Standard Mean Ocean Water (VSMOW); see section 2). Deep NO$_3^-$ $^{18}O$ is within ±1‰ among regions with very different deep O$_2$.

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Nitrogen atoms in marine nitrate:

- $N_2$ fixation
- $\delta^{15}N = -1\%$

Nitrification

- $^{15}N = 17\%$

Nitrate assimilation

- $^{15}N = 5\%$

Denitrification

- $^{15}N = 25\%$

Oxygen atoms in marine nitrate:

- $\delta^{18}O = 0\%$

Nitrification

- $^{18}O = 15\%$

Nitrate assimilation

- $^{18}O = 15\%$

Denitrification

- $^{18}O = 15\%$

Figure 1. The major N transformations in the ocean as seen from the perspective of the (a) N atoms and (b) O atoms of nitrate ($NO_3^-\)$. Italicized fluxes indicated absolute sources or sinks of $NO_3^-$-N or $NO_3^-$. Characteristic estimates for N isotope effects ($^{15}N$) and $\delta^{15}N$ (relative to air $N_2$) are given in Figure 1a [Sigman and Casciotti, 2001; Casciotti et al., 2003]. The estimates for O isotope effects ($^{18}O$) and $\delta^{18}O$ (relative to VSMOW) are based on the available marine field measurements and laboratory culture studies of algae and denitrifiers in seawater media [Casciotti et al., 2002; Granger et al., 2004a, 2004b].

concentration (the Bering Sea, the North Pacific, the Southern Ocean, and the North Atlantic), arguing against a strong influence from $O_2$ $\delta^{18}O$ [Casciotti et al., 2002; Lehmann et al., 2005] (A. Knapp, unpublished data, 2005). This is consistent with ambient water being the dominant source of the O atoms in $NO_3^-$, although much work remains to be done on this question.

[12] In general, the additional insight that $NO_3^-$ $\delta^{18}O$ brings to measurements of $NO_3^-$ $\delta^{15}N$ and $N^*$ involves the processes that are not captured by the O isotopes (Figure 1). As seen from the N atom in $NO_3^-$, NO3 assimilation and nitrification are part of an internal cycle within the ocean that should cause no net change in the mean $\delta^{18}O$ of ocean $NO_3^-$ over time. $N_2$ fixation and denitrification (plus additional smaller terms) comprise the input/output budget of fixed N and control the mean $\delta^{15}N$ of ocean $NO_3^-$ [Brandes and Devol, 2002; Deutsch et al., 2004]. In contrast, for the O atoms in $NO_3^-$, nitrification is an absolute input, while both $NO_3^-$ assimilation and denitrification are absolute sinks. The $\delta^{18}O$ of newly produced $NO_3^-$ does not depend on the origin of $NH_3$ being nitrified, be it from newly fixed N, from the biomass of phytoplankton growing in a $NO_3^-$-rich environment, or from biomass of phytoplankton in a $NO_3^-$-poor environment that assimilate all of the $NO_3^-$ supplied to them.

[13] This fundamental difference between the N and O isotopes of $NO_3^-$ allows their coupled measurement to separate processes that overprint one another when they are measured using $NO_3^-$ $\delta^{15}N$ alone. For instance, with the added constraint of $NO_3^-$ $\delta^{18}O$, it should be possible to separate and quantify the impacts of $N_2$ fixation and denitrification. The N and O isotopes of $NO_3^-$ are fractionated to the same extent by denitrification. Thus $NO_3^-$ becomes enriched in both $^{15}N$ and $^{18}O$ as denitrification proceeds. The difference between the two isotope systems arises with their different sensitivities to $N_2$ fixation. While the nitrification of newly fixed N will work to lower the $\delta^{15}N$ of subsurface $NO_3^-$, the $\delta^{18}O$ of $NO_3^-$ produced by nitrification is insensitive to the origin of the N being remineralized in the subsurface. Thus O isotopes may indicate when the impact of $N_2$ fixation has caused $NO_3^-$ $\delta^{15}N$ (and $N^*$) to underestimate the $NO_3^-$ lost to denitrification.

[14] At the same time, the O isotopes may record other gross fluxes of $NO_3^-$ that do not impact the N isotopes. For instance, if $NO_3^-$ is reduced to some other form (organic N, $NH_4^+$, or $NO_2^-$) and then oxidized back to $NO_3^-$ without any N loss, the $\delta^{15}N$ of $NO_3^-$ is constrained by mass balance to remain unchanged, whereas the $\delta^{18}O$ of $NO_3^-$ may change (Figure 1). The direction in which $\delta^{18}O$ changes will depend on whether the $\delta^{18}O$ of the $NO_3^-$ removed is higher or lower than the $\delta^{18}O$ of the $NO_3^-$ added back. If the $NO_3^-$ added back is higher in $\delta^{18}O$ than that removed, then the $NO_3^-$ $\delta^{18}O$ will drift upward. Because isotope discrimination during $NO_3^-$ reduction often causes the $\delta^{18}O$ of the consumed $NO_3^-$ to be less than $\delta^{18}O$ of newly produced $NO_3^-$, it will generally be the case that $NO_3^-$ $\delta^{18}O$ will increase relative to $\delta^{15}N$ with the rate of an internal cycle of $NO_3^-$ consumption and production.

[15] Here we use the coupled N and O isotopes of water column nitrate as complementary constraints on the N transformations at work in and nearby the eastern tropical North Pacific denitrification zone. Our central new observation is that the $\delta^{18}O$ of $NO_3^-$ is up to 3‰ more elevated than is its $\delta^{15}N$ relative to “background” (e.g., deep open ocean) $NO_3^-$, with the greatest deviation between the two isotope systems at ~100 m shallower than the previously described $\delta^{15}N$ maximum. Given that our culture experiments indicate a 1:1 $\delta^{18}O$:$\delta^{15}N$ elevation by denitrification, we attempt to identify and quantify the process responsible for the deviation of the O and N isotopes from denitrification-only behavior.

2. Materials and Methods

2.1. Sample Collection

[16] Water samples were collected through the water column by hydrocast off the California coast from Point Conception to the southern tip of Baja California during coring cruise OXMO1MV aboard the RV Melville in November of 1999 (Figure 2) [van Geen, 2001]. Samples
Measurements

2.2. Dissolved Oxygen and Nutrient Concentration

isotope analysis.

[NO2

Melville OXMZ01MV aboard the RV preserved by acidification to a ethylene bottles after two rinses with sample water and were collected in acid- and distilled water-rinsed polyeth-

ylene bottles after two rinses with sample water and were preserved by acidification to a pH of 2–3 with 50% reagent-grade hydrochloric acid. Upon arrival at the laboratory 4 months after collection, an aliquot of each sample was frozen, and these aliquots were used for NO3

concentration of dissolved O2 was measured by Winkler titration. In the hydrocast profiles from OXMZ01MV, [NO2

was less than 0.1 μM in all but one 50-m sample and was typically less than 0.05 μM. This is much lower than measured at lower latitudes along the eastern tropical Pacific margin [Codispoti et al., 1986; Lipschultz et al., 1990] but fits with previously reported distributions [Cline and Richards, 1972] (see endnote ii in Auxmat1.txt [Deutsch et al., 2001; Graber and Sarmiento, 1997]).

2.3. NO3 Isotopic Analysis

[18] The 15N/14N and 18O/16O of NO3

were determined using the denitrifier method [Casciotti et al., 2002; Sigman et al., 2001]. Briefly, NO3 and NO2 are converted quantitatively to N2O by a strain of bacterial denitrifier that lacks nitrous oxide reductase activity, and the product N2O is extracted, purified, and analyzed by continuous flow isotope ratio mass spectrometry. Individual analyses are referenced to injections of N2O from a pure gas cylinder and then standardized using international NO3 isotopic reference material IAEA-N3. The O isoerte data are corrected for exchange with oxygen atoms from water during reduction of NO3 to N2O [Casciotti et al., 2002], which is quantified by analysis of IAEA-N3 in 18O-enriched water and was 5% or less for the analyses reported here. Reproducibility of replicates (which were analyzed for ~75% of the water samples) was generally consistent with previously reported analysis standard deviations of 0.2‰ for δ15N and 0.5‰ for δ18O (see endnote iii in Auxmat1.txt [Anbar and Gutmann, 1961; Böhlike et al., 2003; Bunton et al., 1952]).

[19] As stated above, referencing of 15N/14N to atmospheric N2 and of 18O/16O to VSMOW was through comparison to the potassium nitrate (KNO3) reference material IAEA-N3, with an assigned δ15N of +4.7‰ [Gonfiantini et al., 1995] and reported δ18O of +22.7‰ to +25.6‰ [Böhlike et al., 2003; Lehmann et al., 2003; Revez et al., 1997; Silva et al., 2000]. We adopt here a δ18O of 22.7‰ [Lehmann et al., 2003; Revez et al., 1997; Silva et al., 2000], as we have used in previous publications. If we were to assume the most recent and highest estimate for the δ18O of IAEA-N3 (25.6‰, [Böhlike et al., 2003]), then the NO3 δ18O of all of our samples would increase by ~2.9‰. Indeed, we expect that the new, higher δ18O of IAEA-N3 will prove to be correct, but we wish to guard against using multiple different referencing schemes through time and thus will wait for corroboration of the results of Böhlike et al. [2003]. The O isotopic difference between NO3 reference IAEA-N3 (and indeed all NO3 references) and Vienna SMOW is not addressable with the denitrifier method, which can only measure isotopic differences among NO3 samples. The uncertainty in the isotopic difference between IAEA-N3 and VSMOW is an unfortunate source of uncertainty in our reported values. However, our focus here is on the variation of NO3 18O/16O in the ocean, not its relationship to the isotope ratios found in seawater or other O-bearing materials. Our interpretation is not affected by a uniform shift in the δ18O of all of our data sets relative to VSMOW, because all of the O isotope rules used in the calculations below are based on our own NO3 isotope data.

3. Results

[20] While N* is generally negative throughout the eastern North Pacific (ENP), there is a thermocline-depth N* minimum that indicates in situ denitrification or rapid

Figure 2. Station locations from coring cruise OXMZ01MV aboard the RV Melville in November of 1999. Stations 8 to 17 have mid-depth [O2] minima reaching below 3 μM, while station 7 reaches a minimum [O2] of ~5 μM; the empirical upper limit [O2] for active water column denitrification is ~4 μM [Lipschultz et al., 1990; Codispoti et al., 2001]. The color scheme is used in subsequent Figures 3, 4a, 4c, and 6. Station 3 samples waters in the Santa Barbara Basin (SBB), which has a sill depth of ~475 m. Station 17 samples waters in the Soledad Basin (SB), which has a sill depth of ~300 m. Station 9 samples waters of an unnamed basin (UB) with a sill depth of ~460 m [van Geen et al., 2003]. All stations were sampled to near the depth of the seafloor. Contours are every 750 m.

were collected in acid- and distilled water-rinsed polyethylene bottles after two rinses with sample water and were preserved by acidification to a pH of 2–3 with 50% reagent-grade hydrochloric acid. Upon arrival at the laboratory 4 months after collection, an aliquot of each sample was frozen, and these aliquots were used for NO3-N and O isotope analysis.

2.2. Dissolved Oxygen and Nutrient Concentration Measurements

[17] During OXMZ01MV, the concentrations of phosphate (PO43–), nitrate (NO3), and nitrite (NO2) were measured at sea by automated colorimetric methods, and the concentration of dissolved O2 was measured by Winkler titration. In the hydrocast profiles from OXMZ01MV, [NO2] was less than 0.1 μM in all but one 50-m sample and was typically less than 0.05 μM. This is much lower than measured at lower latitudes along the eastern tropical
exchange with a region of denitrification (Figure 3c). The N* minimum is associated with the [O2] minimum (Figure 3e), with both O2 depletion and the N* minimum becoming more pronounced toward the south among our station locations. This is consistent with a requirement of very low [O2] (<4 μM or so) for denitrification to proceed rapidly in the water column [Lipschultz et al., 1990, and references therein]. The expectation based on the [O2] data is that water column denitrification is only active in the stations south of 25°N (blue symbols in Figure 3e). The N* minimum and NO3 δ15N and δ18O maxima of the more northern stations result from the coastal undercurrent carrying northward these signals of denitrification [Altabet et al., 1999; Liu and Kaplan, 1989; Sigman et al., 2003b; Wooster and Jones, 1970].

Comparison of profiles shows qualitatively that NO3 δ15N/δ14N (Figure 3b) is strongly anti-correlated with N* (Figure 3c), as would be expected from N isotope discrimination during denitrification. For a range of models of NO3 supply and consumption, a N isotope effect (15 ε) for denitrification of 24 to 25% has been estimated [Sigman et al., 2003b], consistent with other studies referenced above. A much lower net isotope effect applies in the Santa Barbara Basin (station 3) because of denitrification in the sediments of that basin [Sigman et al., 2003b].

Within the sample set, the depth variations in the O and N isotopes of NO3 are strongly related (Figures 3a and 3b). A trend through the bulk of the data in a plot of NO3 δ18O versus NO3 δ15N has a slope of ~1.25 or higher (Figure 4). Our culture studies indicate that denitrifiers in seawater express an O:N isotope effect ratio (18 ε:15 ε) of ~1 [Granger et al., 2004a] (see endnote iv in Auxmat1.txt [Lehmann et al., 2003]). In addition, NO3 assimilation by marine phytoplankton also exhibits an 18 ε:15 ε of ~1 [Casciotti et al., 2002; Granger et al., 2004b].

The overall δ18O:δ15N trend of 1.25 in the ENP data actually hides systematic depth-variations in the relationship between δ18O and δ15N. At ~350 m, as N* begins its upward increase and NO3 δ15N begins to decrease, NO3 δ18O holds steady or continues to increase an additional 100 m toward the surface before decreasing again, resulting in a NO3 δ18O maximum that is ~100 m shallower than the δ15N maximum and the N* minimum. In our plots of NO3 δ18O versus NO3 δ15N (Figure 4), this leads to a "loop" (counterclockwise up) pattern: shoaling from the deepest samples, the isotopic composition of NO3 progresses upward and to the right along a slope of ~1.25 in δ18O/δ15N space, then shifts toward a more vertical path as δ18O continues to increase but δ15N remains unchanged or decreases, then returns downward and to the left, typically reaching a δ18O-to-δ15N relationship at ~100 m that is similar to that of deep waters.

4. Interpretation

4.1. Quantifying the Deviation Between NO3 O and N Isotopes in the Thermocline

We focus first on the ENP profiles from the southern tip of Baja (stations 7–16), where conditions are appropriate for water column denitrification. As described above, the relationship between the NO3 N and O isotopes within the
suboxic zone (200–800 m) cannot be explained solely by denitrification with an \( ^{18}\delta^{15}N \sim 1 \), especially in its shallow portion (e.g., at \( \sim 200 \) m). Graphically, the discrepancy from a 1:1 fractionation relationship expected for denitrification can be visualized as the horizontal distance in \( \delta^{15}N \)-versus-\( \delta^{18}O \) space between the data and a line with a slope (\( ^{18}\delta^{15}N / ^{18}e \)) of 1 appropriate for denitrification running through the mean \( \delta^{15}N \) and \( \delta^{18}O \) of ENP deep water (Figure 4a). We formalize this as “\( \Delta(15,18) \)”,

\[
\Delta(15,18) \equiv \left( \delta^{15}N_{m} - \delta^{15}N_{m} \right) - \left( \delta^{15}N_{m} \right) \times \left( \delta^{18}O_{m} - \delta^{18}O_{m} \right),
\]

where \( \delta^{15}N_{m} \) and \( \delta^{18}O_{m} \) are the mean \( \delta^{15}N \) and \( \delta^{18}O \) of eastern North Pacific deep water, which is taken to approximate the source of \( NO_3^{-} \) to the upper water column of the eastern North Pacific, and \( ^{18}\delta^{15}N \) is the N-to-O isotope effect ratio for denitrification, which our culture studies indicate to be 1 [Granger et al., 2004a]. We use here 5\% and −0.5\% for \( \delta^{15}N_{m} \) and \( \delta^{18}O_{m} \) (based on samples taken from 3500 m and below at HOT station ALOHA (D. M. Sigman and D. Karl, unpublished data, 2005)), such that the 800–1450 m data from stations 7–16 yield a \( \Delta(15,18) \) close to 0\% (+0.2\%, Table 1) (see endnote v in Auxmat1.txt).

[25] For stations 7–16, \( \Delta(15,18) \) varies coherently with depth (Figure 5c), being close to zero below 800 m (by definition) and decreasing upward to a minimum of ~2.5\% at 200 m, with a sharp increase to 100 m and above. The minimum in \( \Delta(15,18) \) is ≥100 m shallower than the \( \delta^{15}N \) maximum (Figure 5b) and the \( N^{*} \) minimum (Figure 5e). Given that the deviation is not proportional to \( \delta^{15}N \) or \( N^{*} \), it is not well explained by a uniform deviation in \( ^{18}\delta^{15}N \) or \( ^{18}e \) from the culture-derived estimate of 1. Moreover, this sense of deviation would require an \( ^{18}\delta^{15}N \sim 1 \), for which there is no support from previous work in seawater or freshwater. Finally, an \( ^{18}\delta^{15}N \) of 1 yields an excellent fit to the data from the Santa Barbara Basin (indicated red circles in Figure 4c), in which denitrification is progressively drawing down \( NO_3^{-} \) after a springtime flushing event [Sigman et al., 2003b].

### 4.2. Regional Extent of the \( \Delta(15,18) \) Minimum

[26] The ~200-m-centered minimum in \( \Delta(15,18) \) weakens as one moves north along the California margin and is not evident near Point Conception (Figure 6). The shallowest samples in the more northern profiles tend to reach positive values for \( \Delta(15,18) \), which can be explained as a result of the algal uptake/remineralization cycle (see below). The lack of a strong \( \Delta(15,18) \) minimum in the more northern profiles rules out the possibility that the minimum near the tip of Baja originates from advection from the north, for instance, because of a negative \( \Delta(15,18) \) in preformed \( NO_3^{-} \) from regions of ventilation to the north. Comparison with Hawaii Ocean Time series station ALOHA shows clearly that the \( \Delta(15,18) \) minimum in the ENP is also not transported into the eastern North Pacific margin from the west (D. M. Sigman and D. Karl, unpublished data, 2005). While it is still possible that the suboxic zone to the South represents a source for the \( \Delta(15,18) \) minimum in the ENP near the tip of Baja, the data in hand indicate no role for transport and suggest that the \( \Delta(15,18) \) minimum is generated locally.

Table 1. Water Column Parameters for Model Targets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stations 7–16, 200–800 m</th>
<th>Stations 7–16, 800–1450 m</th>
<th>Difference, Shallow-Deep</th>
</tr>
</thead>
<tbody>
<tr>
<td>([NO_3^{-}]), mM</td>
<td>33.79</td>
<td>44.29</td>
<td>−10.50</td>
</tr>
<tr>
<td>(N^{*}) mM</td>
<td>−12.59</td>
<td>−6.35</td>
<td>−6.24</td>
</tr>
<tr>
<td>(\delta^{15}N) % versus air</td>
<td>11.39</td>
<td>7.02</td>
<td>4.37</td>
</tr>
<tr>
<td>(\delta^{18}O) % versus VSMOW</td>
<td>7.04</td>
<td>1.32</td>
<td>5.72</td>
</tr>
<tr>
<td>(\Delta(15,18)) %</td>
<td>−1.15</td>
<td>0.20</td>
<td>−1.35</td>
</tr>
</tbody>
</table>
the 200 m minimum toward the surface; this is well explained by the NO$_3$ assimilation/remineralization cycle. The lack of significant surface NO$_3$ in the region indicates that upwelled NO$_3$ is consumed to completion by algal uptake. Thus the organic matter produced and exported into the subsurface will have the same $\delta^{15}$N as the upwelled NO$_3$. However, the nitrification of this organic N produces NO$_3$ with a $\delta^{18}$O of $\sim$0‰ (i.e., close to that of water), essentially “washing” the $^{18}$O enrichment from the NO$_3$ pool. This should tend to increase the $\Delta$(15,18) as one approaches the top of the thermocline. Indeed, $\Delta$(15,18) reaches positive values in many cases (Figures 5 and 6), most likely because of this effect. These samples are evident in $\delta^{18}$O-$\delta^{15}$N space as the points that reach below the 1:1 line in the lower left sector of the plot (Figures 4a and 4c).

4.3. Cause of the $\Delta$(15,18) Minimum

[28] Owing to space limitations, we restrict ourselves to describing our two candidate explanations for the observed $\Delta$(15,18) minimum, relegating a more complete discussion of other relevant processes to the Auxiliary Materials (see endnote vi in Auxmat1.txt [Altabet et al., 1991; Altabet and Francois, 2001; Bender, 1990; Böhlke et al., 2003; Casciotti et al., 2002, 2003; Fritz et al., 1989; Fry et al., 1991; Granger et al., 2004a, 2004b; Lehmann et al., 2004; Libes and Deuser, 1988; Lourey et al., 2003; Mariotti et al.,]...
1981; Ostrom et al., 2000; Thunell et al., 2004; van Geen et al., 2003; Voss et al., 1997; Wada et al., 1987; Waser et al., 1988).

4.3.1. N₂ Fixation
[29] In the subthermocline of the North Atlantic and North Pacific, there is evidence for the production of a sizable NO₃ excess relative to expectations based on PO₄₂⁻ concentration and Redfield ratios; this finding has been interpreted to indicate that newly fixed N is accumulating as NO₃ in the thermocline waters of these regions [Deutsch et al., 2001; Gruber and Sarmiento, 1997; Hansell et al., 2004; Michaels et al., 1996]. NO₃ in the subthermocline of both the Pacific and the North Atlantic has been observed to have a low δ¹⁵N, as low as 2‰ [Karlst et al., 2002; Knaff et al., 2005; Liu et al., 1996]. Given the low δ¹⁵N of newly fixed N, the low δ¹⁵N of subthermocline NO₃ is consistent with the N*-based interpretation of the accumulation of newly fixed N as thermocline NO₃ [Gruber and Sarmiento, 1997]. More work is needed to validate this interpretation, but it would seem difficult for it to be strictly incorrect.

[30] On the basis of similar logic, Brandes et al. [1998] explain the upward decrease in δ¹⁵N above denitrification zones in the Arabian Sea and eastern tropical North Pacific as the result of oxidation of low-δ¹⁵N, newly fixed N to NO₃. This explanation fits with the upward change in the δ¹⁸O/δ¹⁵N relationship reported here. That is, the shallower δ¹⁸O maximum suggests that the nitrification of newly fixed N is “eroding” the tops of the NO₃ δ¹⁵N maximum and the N* minimum. It is not clear whether nitrification would be limited within the suboxic zone of our study region [Lipschultz et al., 1990]. In any case, the suboxia does not extend far offshore at the latitudes of our stations [Conkright et al., 2002], so NO₃ could be produced from nitrification in the oxic waters just to the west and imported along isopycnals.

[31] As described above, the upward increase in Δ(15,18) above its minimum at 200 m is well explained by complete assimilation of upwelled NO₃ and subsequent remineralization of most of the exported organic N in the shallow subsurface. That the minimum in Δ(15,18) is strongest at 200 m and not deeper could be explained by (1) the lower [NO₃] at shallower depths, which requires a smaller amount of newly fixed N to cause the same decrease in Δ(15,18), (2) the tendency for nitrification at the upper margin of the suboxic zone [Lipschultz et al., 1990], and/or (3) the rapid decrease in the sinking N flux with depth in the water column.

4.3.2. Nitrate/Nitrite Redox Cycling
[32] Since the work of Anderson [Anderson, 1982; Anderson et al., 1982], it has been hypothesized that there is significant redox cycling between nitrate and nitrite in ocean suboxic zones, with NO₃ reduction to NO₂ in the core of the suboxic zones, mixing of the NO₂ to the margins of the suboxic zone, and reoxidation of the NO₂ once it reaches higher [O₂] waters. Anderson suggested that roughly half of the nitrate reduction in open ocean suboxic zones can be coupled to nitrite oxidation, the other half proceeding to denitrification. This exact process is not plausibly significant in our study region, as the measured [NO₂] rarely climbed above 0.05 µM (typically ~0.01 µM) in the subsurface samples. However, there might be exchange along isopycnals with waters to the south where that process could occur. Moreover, there might well be simultaneous NO₃ reduction and NO₂ oxidation in the same water parcel within our study region [Lipschultz et al., 1990].

[33] Such a cycle might explain the deviation of NO₃ δ¹⁸O and δ¹⁵N from 1:1 covariation. NO₃ reduction will consume NO₃ with the N and O isotope effects of denitrification. If the ambient NO₃ δ¹⁵N and δ¹⁸O are 14‰ and 10‰, respectively, an isotope effect of 2‰ (for both N and O) will make the consumed NO₃ approximately −6‰ and −10‰, respectively. When the NO₂ produced is reoxidized to NO₃, it will return NO₃ with roughly the same δ¹⁵N as the loss, so that the ambient NO₃ δ¹⁵N is, in net, unchanged. The δ¹⁸O of the reoxidized NO₃, however, would most likely be higher than the δ¹⁸O of the NO₃ consumed. The preferential extraction of ¹⁶O from the chain of N species (i.e., a “branching fractionation”) yields NO₂ with a δ¹⁸O higher than that of the NO₃ consumed [Casciotti et al., 2002], such that its recycling back into the NO₃ pool may cause a net increase in NO₃ δ¹⁸O. In addition, the reduction to NO₂ and reoxidation to NO₃ will work to incorporate O atoms from H₂O, such that the reoxidized NO₃ would likely be shifted toward a δ¹⁸O of 0‰. This shift might be complete if there is rapid O atom exchange with water in the enzyme active site of NO₂ oxidase [Dispirito and Hooper, 1986], as has been observed to occur in the presence of enzymes catalyzing ammonium oxidation to NO₂ [Andersson et al., 1982]. Alternatively, the only O atoms added from H₂O may be the single O associated with denitrification in the Santa Barbara Basin (Figure 4c), where we presume such a NO₃/NO₂ redox cycle should be equally active.

4.4. Steady State Model of the Candidate Processes
[34] We describe the results from the simplest possible quantitative model that we could conceive to estimate the fluxes of the two alternative processes that we have proposed to explain the Δ(15,18) minimum (Figure 7). This model represents the effects of five N cycle processes acting
simultaneously on the suboxic thermocline zone of the ENP: (1) mixing with the deeper ENP (M), (2) denitrification (D), (3) mixing with a biologically active and NO3-deplete surface ocean (S), (4) addition of NO3 from the nitrification of N from new N2 fixation (F), and (5) redox cycling between NO3 and NO2 (C; C1 is NO3 reduction, C2 is NO3 oxidation). The following rules apply to the fluxes.

1. Mixing with deeper eastern North Pacific water (M in Figure 7) introduces NO3 with a concentration, δ15N, and δ18O measured in the water below the suboxic zone by our study (Table 1), while it removes NO3 with whatever concentration and isotope composition occurs in the thermocline box.

2. Denitrification (D in Figure 7) consumes NO3 with a kinetic isotope effect that is equivalent for 15N/14N and 18O/16O. The amplitude of the isotope effect is adjusted to fit the data and is reported below.

3. Mixing with the surface ocean (S in Figure 7) has no effect on [NO3] or NO3 δ15N because all NO3 mixed upward into the surface is consumed in the surface and exported as organic N back into the reservoir, where it is completely remineralized to NO3. However, the nitrification of this organic N export produces NO3 with a δ15N of -1‰ and a δ18O of 0‰, essentially ‘washing’ the 18O enrichment from the NO3.

4. The NO3 added from newly fixed N (F in Figure 7) has a δ15N of -1‰ and a δ18O of 0‰.

5. In the NO3/NO2 redox cycle (C in Figure 7), NO3 reduction (C1) occurs with the same 15ε and 18ε as denitrification (D). For a given 15ε for denitrification, the δ15N of the NO3 reoxidized from NO2 depends on the relative amplitudes of 15ε for NO2 reduction and NO2 oxidation; we assume that these isotope effects are equal in the calculations but consider other cases in the text. The δ18O of the NO3 reoxidized from NO2 depends on the same factors as does its δ15N; however, the δ18O is also affected by two additional factors. First, 16O is preferentially lost from the nitrogen species in the denitrification pathway [Casciotti et al., 2002]. This “branching fractionation” during NO3 reduction (assumed here to be equivalent to the 18ε of nitrate consumption by denitrification) yields NO3 with a δ18O ~ 18ε‰ higher than that of the NO3 consumed, such that its recycling back into the NO3 pool may cause a net NO3 δ18O increase. Second, incorporation of O from H2O during (1) NO2/H2O exchange and (2) NO2 oxidation drives the δ18O of the reoxidized NO3 toward 0‰. While Figure 7 shows only the case for complete O exchange between NO2 and H2O, the cases of complete O exchange and no exchange are both considered in the calculations below. For lack of better information, we assume that the 18ε/15ε ratio is the same for NO2 reduction as for NO2 oxidation, regardless of what that ratio might be.

Here we consider only the model steady state. Varying D, F, and C, we fit [NO3], NO3 δ15N, and (15,18) for the means for the 200–800 m depth zone from stations 7–16, using the 800–1450 m data from the same stations to estimate the values for background ENP conditions (Table 1). The nitrate isotopes and N* of the 800–1450 m water indicate that it is impacted by denitrification, by exchange with the eastern tropical Pacific suboxic zones and by sedimentary denitrification (P. DiFiore, unpublished results, 2005), and is thus far from reflecting the mean conditions of the global ocean or even the whole North Pacific. We address here only the fluxes that drive the isotopic and concentration differences between the suboxic thermocline box and the 800–1450 m water below it.

We opted here to use mixing with the deeper water from the same stations, as opposed to lateral exchange, as the mechanism for refreshing the 200–800 m suboxic thermocline box. This allowed the current study to be self-contained with respect to measurements. Efforts to use other mixing end-members (e.g., the thermocline from the open subtropical Pacific as measured at station ALOHA (D. M. Sigman and D. Karl, unpublished data, 2005) or the thermocline from our more northern stations (Figures 3 and 6)), yielded similar results that nevertheless require the consideration of additional factors (calculations not shown).
4.4.1. Quantifying the Needed N2 Fixation

separately in the sections below. However, they may both be
consistent with previous studies \([45]\). The results from the model are largely intuitive.

\[\Delta(15,18)\] decreases as N2 fixation increases, almost regardless of D (Figure 8, blue contours). Further visualization of model results are in the Auxiliary Materials (see endnote viii in Auxmat1.txt).

\[46\] In order to fit the 200–800 m data from stations 7–16 (Table 1), we find that the needed N2 fixation/denitrification ratio \((F/D)\) is roughly 0.65 (black circle in Figure 8).

This suggests that 65% of the denitrification occurring in the 200–800 m suboxic zone is countered by the nitrification of newly fixed N. N* in the suboxic zone (200–800 m) is 6.2 \(\mu\text{M}\) lower than in the deeper waters between 800 and 1450 m (−12.6 \(\mu\text{M}\) and −6.4 \(\mu\text{M}\), respectively; Table 1). Thus our results would require that N2 fixation is erasing a NO3 deficit of \((0.65/(1−0.65)) \times 6.2 \mu\text{M}, or −11.6 \mu\text{M}.

Added to the observed N* of −12.6 \mu\text{M}, this would yield a N* in the suboxic zone of −24.2 \mu\text{M}, were it not for N2 fixation, that is, a total N* minimum of roughly twice the observed amplitude.

\[47\] The isotope effect for denitrification that is required to simultaneously fit the N*, NO3 \(\delta^{15}\text{N},\) and NO3 \(\delta^{18}\text{O}\) (or \(\Delta(15,18)\)) data is 18.9\%, \(\sim 5–10\%\) lower than derived previously from regression of NO3 \(\delta^{15}\text{N}\) against N* in field data [AItabat et al., 1999; Brandes et al., 1998; Sigman et al., 2003b]. The need for a lower isotope effect than previous field studies at least partially arises from our isotope-derived inference that N2 fixation causes the N*-derived NO3 deficit to be less than the actual amount of NO3 consumed by denitrification. While the true biological isotope effect amplitude for denitrifiers in the ENP is not known, the value required by the model may be lower than that value, in which case it may indicate that a fraction of the NO3 consumption occurring within the suboxic zone is driven by sedimentary denitrification along the margin [Sigman et al., 2003b]. However, the isotope effect amplitude required by the model would also increase modestly if spatial heterogeneity were included in the model [Deutsch et al., 2004].

4.4.2. Quantifying the Needed Nitrate/Nitrite Redox Cycling

\[48\] The assimilation/remineralization cycle (S in Figure 7), in the case of complete NO3 consumption in the surface, decreases NO3 \(\delta^{18}\text{O}\) toward its nitrification production value \((\sim 0\%)\) while not affecting NO3 \(\delta^{15}\text{N};\) in net, the effect is to increase \(\Delta(15,18)\), that is, erode the \(\Delta(15,18)\) minimum (see above). We neglect this term in the calculation shown in Figure 8, setting S to 0. Including this term would yield an even higher N2 fixation/denitrification ratio (see endnote ix in Auxmat1.txt), but estimating the amplitude of S is difficult.

4.4.3. Quantifying the Required N2 Fixation Ratio

\[49\] Because there is essentially no NO3 in this region of the ENP, if the signal is generated locally, a putative NO3/NO2 redox cycle must occur within a given water sample (i.e., without NO3 transport). Thus we can meaningfully compare the model results to the peak amplitude of the \(\Delta(15,18)\) minimum \((-2.5\%)\) at 200 m as well as to the mean \(\Delta(15,18)\) of the 200–800 m interval \((-1.1\%)\) (Figure 9, dotted and solid gray bars, respectively). For the cases considered here, NO3 oxidation must be \(-0.7–0.95\) and \(-0.35–0.45\) times the rate of NO3 reduction to fit the observations at 200 m and over the 200–800 m...
added from water to form NO₃⁻ exchange (solid versus dashed line in Figure 9). For the same model, Δ(15,18) plotted versus C/D (the NO₂ oxidation/NO₂ reduction ratio) for the scenario where the Δ(15,18) minimum is caused by a NO₃⁻/NO₂⁻ redox cycle. The model results are compared to the peak amplitude of the Δ(15,18) minimum (~2.51‰) at 200 m and to the mean Δ(15,18) of the 200–800 m interval (~1.15‰; dotted and solid gray bars, respectively). In these simulations, D was set to match the target [NO₃]B – [NO₃]M (Table 1) and δ¹⁵N⁰ was matched by adjusting the denitrification ¹⁵ε = ¹⁸ε to 30.7‰. The δ¹⁸O of NO₂ produced is made ¹⁸ε‰ higher than the NO₃⁻ reduced to take into account the preferential ¹⁶O loss during NO₃⁻ reduction (see text). Then the reoxidized NO₃⁻ is nudged toward a δ¹⁸O of 0‰, (1) by 33% in the case of one O atom added from water to form NO₅ but no O atom exchange of NO₃⁻ with water (solid line) or (2) by 100% in the case of complete O atom exchange (dashed line). Lacking better information, we have assumed that (1) the ¹⁵ε for NO₂ oxidation is the same as the ¹⁵ε for NO₃⁻ reduction and (2) that the ¹⁸ε/¹⁵ε ratio is the same for NO₃⁻ reduction as for NO₂ oxidation (the value of that ratio having no effect in this case).

interval, respectively (Figure 9). The preference for ¹⁶O-O-NO₃ during NO₃⁻ reduction and the preferential extraction of ¹⁸O from the NO₃⁻ produced (the “branching fractionation”), which we assume here to have the same ¹⁸ε, is offset one another to yield NO₂ with a δ¹⁸O close to that of the NO₃⁻ in the water and thus <10‰ greater than the δ¹⁸O of the water. Therefore, for a given amount of NO₃⁻/NO₂, cycling, the case of no NO₂/H₂O exchange yields only slightly greater Δ(15,18) than the case with complete exchange (solid versus dashed line in Figure 9).

The unknown isotope systematics of NO₂ represent a major weakness in this modeling exercise (see endnote x in Auxmar1.txt [Bryan et al., 1983; Casciotti, 2002]). Nevertheless, the ratios given above for NO₂ oxidation to reduction are generally within the range of those originally proposed as part of a transport cycle (0.65–1.50) [Anderson, 1982; Anderson et al., 1982] or measured within individual water samples [Lipschultz et al., 1990]. However, we again note that those rates involved waters with 5–10 μM NO₃⁻, whereas there is essentially no NO₂⁻ in our profiles. Thus, if the isotopic signal of this process is important, it may be through exchange with NO₂⁻-bearing waters to the south.

5. Summary and Conclusions

[51] Here we report coupled N and O isotope measurements of NO₃⁻ from a set of hydrocast stations collected along the continental margin from Point Conception to the southern tip of Baja California. The isotope data from the California margin show a distinct anomaly in the δ¹⁸O vs δ¹⁵N relationship from expectations for denitrification alone, with δ¹⁵N being lower than expected from δ¹⁸O. This isotope anomaly (described as a negative value for “Δ(15,18)” is present from 200 to 800 m but peaks at 200 m, above the maximum in NO₃⁻ δ¹⁵N. Comparison of the data from the tip of Baja with the stations from further north and with data from near Hawaii (D. M. Sigman and D. Karl, unpublished data, 2005) indicates that the anomaly originates in or near the region of denitrification.

[52] One plausible explanation for the Δ(15,18) minimum is the addition of low-δ¹⁵N NO₃⁻ to the shallow thermocline in the same region where denitrification occurs, which “erodes” the tops of the denitrification-driven maximum in NO₃⁻ δ¹⁵N and minimum in N*. The most likely origin of this low-δ¹⁵N NO₃⁻ is N₂ fixation in the surface ocean, the rain of this newly fixed N out of the surface ocean, and the subsequent nitrification of its products to NO₃⁻ in the thermocline. This is consistent with a previous interpretation of NO₃⁻ δ¹⁵N data alone from the eastern tropical North Pacific and Arabian Sea that N₂ fixation was adding significant amounts of low-δ¹⁵N NO₃⁻ to the shallow thermocline in these regions [Brandes et al., 1998]. We use the coupled N and O isotope data, in the context of a simple model, to estimate that the rate of this putative N₂ fixation is roughly 0.65 of the rate of water column denitrification in the same region.

[53] Were the N₂ fixation input found to be the correct explanation for the Δ(15,18) minimum, it would indicate that a significant fraction of the NO₃⁻ loss to denitrification is subsequently compensated by N₂ fixation in the surface waters overlying or adjacent to the zone of denitrification. This would explain why PO₄³⁻-bearing waters are not observed penetrating far into the eastern ranges of the Pacific subtropical gyres: N₂ fixers strip out this P in the waters proximal to the upwelling zones. Moreover, it would bolster the view that oceanic N₂ fixation is strongly controlled by N/P variations in the waters supplied to the surface, with diazotrophs succeeding under N-poor, P-bearing conditions [Broecker and Peng, 1982; Redfield, 1958; Tyrrell, 1999], a situation that has been demonstrated in lakes [Schindler, 1977; Smith, 1983].

[54] An alternative plausible mechanism for the development of the Δ(15,18) minimum is the redox cycling of NO₃⁻ and NO₂ within suboxic zones. The logic is that NO₃⁻ δ¹⁸O can be gradually increased if the NO₃⁻ reduced to NO₂⁻ is lower in δ¹⁸O than the NO₃⁻ produced from the reoxidation of NO₂⁻. However, the isotope dynamics of NO₂⁻ are poorly understood and essentially unknown in the case of the O
isotopes. For reasonable assumptions, the mechanism can explain the $\Delta(15,18)$ minimum with a ratio of NO$_2$ oxidation to NO$_3$ reduction of as little as 0.7.

[55] Looking forward, several routes can be imagined that should allow for these two plausible explanations to be tested. First, work on the isotope systematics of NO$_2$ (especially the O isotope systematics) is clearly needed and would provide an immediate test of the premises behind the NO$_3$/NO$_2$ redox cycling scenario. Second, studies of other ocean regions, including model systems such as well-described isolated basins, would provide critical constraints on the coupled N and O isotopic effects of both N$_2$ fixation and NO$_3$ cycling through other oxidation states. For instance, it is not difficult to identify regions where N$_2$ fixation is occurring without denitrification, and vice versa.

[56] The isotopic impact of redox cycling of NO$_3$ and NO$_2$ represents something of a liability in the current study because of the uncertainties in its isotope systematics, especially with regard to the O isotopes. However, one can imagine circumstances where the rate of cyclic consumption and production of NO$_3$ could be well constrained by the N and O isotopes. At the base of the euphotic zone, a cycle of NO$_3$ assimilation and remineralization back to NO$_3$ should cause NO$_3$ $^{18}O$ to rise to above the 1:1 $^{18}$O/$^{16}$N increase expected from NO$_3$ assimilation alone, because the $^{18}$O of the NO$_3$ being consumed by assimilation is lower than the $^{18}$O of NO$_3$ being produced by remineralization/nitrification [Granger et al., 2004b]. In this case, the N and O isotopes should allow for the NO$_3$ recycling to be more accurately quantified.

[57] Acknowledgments. We thank M. Bender, K. Casciotti, C. Deutsch, N. Gruber, and B. Ward for discussions. This work was supported by U.S. NSF OCE-0136449 and Biocomplexity grants OCE-9981479 (to D. M. S., through the MANTRA project) and DEB-0838566 (to Simon Levin), and by British Petroleum and Ford Motor Company through the Carbon Mitigation Initiative at Princeton University. M. F. L. acknowledges support from the DFG through grant LE 1326/1-1. Cruise OXMZ01MV was supported by grant NSF OCE-9809026.

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Correction to “Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin”

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[1] In the paper “Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin” (Global Biogeochemical Cycles, 19, GB4022, doi:10.1029/2005GB002458, 2005), the fourth author name was erroneously listed as Moritz M. Lehmann rather than the correct Moritz F. Lehmann.

[2] In paragraph [6], in the third sentence the values $^{15}k/^{14}k$ should be reversed, so that the parenthetical phrase reads as follows: “(the N isotope effect, $^{15}\varepsilon$, is defined here as $(^{14}k/^{15}k - 1)\times1000\%$, where $^{14}k$ and $^{15}k$ are the rate coefficients of the reactions for the $^{14}N$- and $^{15}N$-bearing forms of NO$_3$, respectively).”

[3] In paragraph [11], in the first sentence the redundant phrase “the ambient water” should be removed so that the sentence reads as follows: “Measurements to date from the ocean indicate that away from regions of known denitrification, subsurface NO$_3$ $^{18}O$ varies relatively little and is close to the ambient water (0 ± 1% or 3 ± 1% different from it; see auxiliary material, endnote i in Auxmat1.txt) $^{18}O_{sample} = ((^{18}O/^{16}O)_{sample}/(^{18}O/^{16}O)_{reference} - 1)\times1000\%$, where the reference is Vienna Standard Mean Ocean Water (VSMOW); see section 2).”

[4] In paragraph [18], in the fourth sentence the value 5% should be changed to 3% so that the sentence reads as follows: “The O isotope data are corrected for exchange with oxygen atoms from water during reduction of NO$_3$ to N$_2$O [Casciotti et al., 2002], which is quantified by analysis of IAEA-N3 in $^{18}O$-enriched water and was 3% or less for the analyses reported here.”

[5] The above errors and additional ones were left uncorrected owing to editorial oversight. The authors have decided to allow several additional typographical errors to stand, to shorten the published correction.
Auxiliary Material for 2005GB002458
Coupled nitrogen and oxygen isotope measurements of nitrate along the eastern North Pacific margin
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Auxiliary Materials: Endnotes to main text
i Analyses of KNO3 reference material IAEA-N3 are used in our method to reference samples to the VSMOW scale. As described in section 2.3, our current uncertainty in the true d18O of deep water NO3- (~0 or ~3 permil) is due to a recent ~3 permil change in the reported d18O difference between IAEA-N3 and VSMOW.

ii We make use of the derived parameter N*, which quantifies the deviation of the [NO3-]:[PO43-] ratio from the "Redfield" N:P stoichiometry of 16:1. Generally, negative N* values indicate a net loss of NO3-, typically due to denitrification, whereas positive N* values suggest a net addition of new NO3-, typically due to the nitrification of newly fixed N. N* is defined here as [NO3-] - 16*[PO43-] + 2.9, in micromolar (µM), as opposed to µmol/kg used by [Deutsch et al., 2001; Gruber and Sarmiento, 1997]. Both ammonium (NH4+) and NO2- were exceedingly scarce in all of our samples and thus did not require inclusion in our N* expression.

iii During OXMX01MV, an additional set of samples were collected with the intention that they be frozen but not acidified; however, due to miscommunication, they were instead stored refrigerated (for three months) until they arrived at Princeton (where they were then frozen), requiring that we make use of the acidified samples. This raised the concern of a possible effect of sample acidification on the d18O of NO3-. The exchange of O atoms with water is accelerated under acidic conditions [Bohlke et al., 2003; Bunton et al., 1952]. According the rate expression derived by Anbar and Gutmann [1961], the residence time of O atoms in NO3- with respect to exchange with water is roughly 60 years at a pH of 2 but effectively infinite at a pH of 8. Given the
uncertainty in these experimentally derived estimates, we performed several tests to address whether our samples from OXMZ01MV had been compromised by acidification. The first was to carry out repeat analyses that had been analyzed as much as 3 years earlier. No coherent differences arose that indicated NO3- d18O change over time. A more stringent test of NO3- d18O stability was comparison of aliquots of samples that were acidified upon collection with those that were refrigerated upon collection. Direct comparison between these two preservation protocols for individual samples indicated no measurable d18O difference after four years of storage. For a full profile (station 10), the mean d18O difference between the acidified/frozen aliquots and the refrigerated aliquots was 0.05 permil, the acidified aliquots being slightly higher. The mean d18O difference between the aliquots with different preservation histories was 0.4 permil.

iv Relative to our field and culture data, previous freshwater studies indicate a much lower 18e:15e (~0.4-0.6 [Lehmann et al., 2003/ and references therein]). This remarkable difference between observations from marine and fresh waters will be discussed elsewhere in the context of our denitrifier culture experiments (Granger et al., in preparation).

v Rounded numbers for d15Nm and d18Om, rather than calculated mean values in Table 1, are used in the definition of D(15,18) to maintain some aspect of generality for this parameter and to discourage over-interpretation of its absolute value.

vi Our current understanding of nitrification is that it produces NO3- with a d18O close to ambient seawater, such that it should work to decrease the d18O of NO3- in the shallow ETNP subsurface, not raise it [Casciotti et al., 2002; Lehmann et al., 2004]. In this regard, remineralization/nitrification alone is not a suitable explanation for the D(15,18) minimum. Salinity-predicted variation in H2O d18O among the subsurface samples is typically less than 0.1 permil across the full gradient in D(15,18) and thus can be ruled out as the driver of the D(15,18) minimum.

Nitrification in the presence of elevated O2 d18O is an initially plausible cause for NO3- 18O enrichment such as would generate a minimum in D(15,18), and high O2 d18O is observed at low [O2] such as occurs at the margins of suboxic zones. However, several factors work against this explanation for the observed minimum in D(15,18). First, NO3- production from nitrification in the ocean subsurface would occur over the entire progress of the drawdown of O2 from its preformed concentration. Thus, even if all O atoms in NO3- originated from O2, the integrated pool of NO3- from nitrification would vary much less in d18O than would O2 in the ocean subsurface. This is analogous to the much smaller amount of isotopic variation in the "integrated product" compared to the "substrate pool" in the Rayleigh model [Marriott et al., 1981]. Second, observed variations in subsurface O2 d18O are less
than 20 permil [e.g., Ostrom et al., 2000]. Given the isotope effect of 
O2 consumption during respiration (~18 permil), this requires that 
mixing in the ocean works to reduce O2 d18O variation from, for 
instance, what would be expected from a "pipe flow" (i.e. Rayleigh) 
model [Bender, 1990]. Third, the evidence in hand suggests that 1 or 
less out of 6 oxygen atoms in NO3- comes from O2 (see section 1), so 
that O2 d18O has very little leverage on the d18O of NO3-. Taking these 
factors into account, a geochemical box model of the global ocean 
predicts a very modest isotopic imprint of O2 on NO3- d18O of <1 permil 
across the ocean interior (D.M. Sigman, unpublished results), much less 
than appropriate to explain the ~3 permil amplitude of variation 
associated with the observed D(15,18) minimum.

Other aspects of our measurements support the above arguments 
against a central role for O2. Near the tip of Baja, the most O2-
deficient waters are centered around ~600 m, and [O2] is equally low at 
800 m as at 400 m (Figure 3; see van Geen et al. [2003] for better 
visualization). Thus, the depth of the D(15,18) minimum, which peaks in 
amplitude at ~200 m and is absent by ~800, does not appear consistent 
with the expected depth variation in O2 d18O. The same conclusion 
arises if one considers the relatively modest N-S gradient in apparent 
O2 utilization at the depth of the [O2] minimum. Based on this 
parameter, there should be very little N-S gradient in O2 ?18O, while 
the D(15,18) minimum weakens rapidly to the north (Figure 6).

Oxygen isotope exchange between water and NO3- appears to have an 
equilibrium 18e of 20 permil [Bohlke et al., 2003], so as to yield NO3- 
with a higher d18O than the ambient water. However, the rate of 
exchange at a pH above 4 is >109 years [Anbar and Gutmann, 1961], so 
this process should be irrelevant in the ocean. Catalysis of NO3-/water 
O exchange by NO3- reductase in the denitrification pathway could cause 
NO3- d18O to increase (by analogy with sulfate O isotopes [Fritz et 
al., 1989]). However, this process has not been observed in our culture 
studies to date [Granger et al., 2004a; Granger et al., 2004b]. More 
generally, any candidate process would need to be preferentially 
associated with the oxic/suboxic boundary (as opposed to the core of 
the suboxic zone) as well as asymmetric around the suboxic zone 
greater at the top than the base). These are stringent constraints, 
which rule out many imagined possibilities.

Given this situation, we turn to possible processes affecting the 
N isotopes of NO3-, specifically, those that might significantly lower 
the d15N of thermocline NO3-. One might postulate that 14N-ammonium is 
preferentially released from sinking particles. However, there is no 
evidence for an increase in the d15N of sinking N with increasing 
depth, which would be expected if there were a strong preference for 
release of 14N during solubilization of sinking N; indeed, the typical 
observation is of decreasing sinking N d15N with depth [Altabet et al., 
1991; Altabet and Francois, 2001; Fry et al., 1991; Libes and Deuser,
N isotope discrimination during ammonium oxidation is significant [Casciotti et al., 2003; Mariotti et al., 1981]. However, if the ammonium produced in the oxic subsurface is eventually consumed by ammonium oxidation (i.e. ammonium does not accumulate or have another significant loss term), the isotope effect of ammonium oxidation is not relevant to the d15N of NO2- or NO3- produced. Other plausible sinks for ammonium in the shallow subsurface include microbial ammonium assimilation and perhaps anammox-like reactions. Ammonium assimilation as measured in phytoplankton cultures has an isotope effect of similar magnitude to that measured for ammonium oxidation [Casciotti et al., 2003; Waser et al., 1998]. Thus, we expect that any coincident ammonium loss by this route would not represent a shunt of 15N-ammonium away from NO2- production. Without any information on the isotope effect of anammox, we cannot say anything concrete about the effect of this process, but it seems safe to assume that it would have a significant isotope effect with regard to ammonium and NO2- consumption. If so, it too would fail to shunt 15N-rich N away from the NO3- pool. In oxic waters, NO2- will tend to be completely converted to NO3-, removing any fractionation associated with the conversion. In suboxic waters, a role for NO2- oxidation in producing the D(15,18) anomaly cannot be ruled out, as is described below.

One major simplification that was introduced above involves the isotope discriminations associated with oxidation and reduction of NO2-. Essentially, we have assumed above that the N isotope effect of NO2- oxidation (which has never been measured) is similar to the N isotope effect for NO2- reduction (which can vary between 5 permil and 25 permil, depending on the availabilities of NO2- and organic matter [Bryan et al., 1983; Casciotti, 2002]). If the isotope effect of NO2- oxidation is significantly smaller than that of NO2- reduction, the posited NO3-/NO2- redox cycle would cause N isotope enrichment of nitrate, and the efficacy of this cycle to explain the D(15,18) minimum would be reduced. The opposite would be true if the isotope effect of NO2- oxidase is greater than that for NO2- reductase.

See Auxiliary Materials Figure 1. Caption for AM Figure 1: (a) A plot showing the effects of variations of F and D on NO3- d15NB and d18OB and thus on D(15,18) (constant values of the latter shown as black dashed lines). As D is increased (solid red line), NO3- d15NB and d18OB increase equally (and [NO3-]B decreases). For a given D, as F increases (and [NO3-]B increases; blue line), d15NB decreases more than does d18OB, roughly along a trajectory toward (d15NF, d18OF). The endpoint of the increase in F ends at the observed mean D(15,18) for 200-800 m in stations 7-16, yielding a combination of F and D equivalent to that indicated by the black circle in Figure 8a. The same set of trajectories is also shown as dotted lines for the case in which the
The surface mixing term \( S \) is equivalent to the deep mixing term \( M \), as opposed to the case of \( S = 0 \) shown with the solid lines. The difference between the two cases is barely discernible. (b) \( D(15,18) \) plotted vs. \( F/D \) for the blue trajectories shown in (a), for the two cases of \( S=0 \) (solid blue line, as in (a)) and \( S=M \) (dotted blue line, as in (a)).

The effect of the upper ocean NO\textsubscript{3}\(-\) assimilation/remineralization cycle is apparent in Auxiliary Materials Figure 1, where the model calculations are performed for two cases for \( S \), the term of water mixing with NO\textsubscript{3}\(-\)-deplete surface ocean: \( S = 0 \) (solid red and blue lines in AM Figure 1) and \( S = M \) (dotted red and blue lines in AM Figure 1). NO\textsubscript{3}\(-\) d\textsuperscript{18}O\textsubscript{B} decreases as \( S \) increases, increasing the \( D(15,18) \) for any given set of values for the other parameters. This is presumably why \( D(15,18) \) increases toward the surface layer above the \( D(15,18) \) minimum at \(~200 \) m in the individual water column profiles. If \( S \) is non-zero, a higher ratio of fixation to denitrification is required to reach the target deviation between d\textsuperscript{18}O and d\textsuperscript{15}N (compare solid and dotted line in AM Figure 1 b). However, to quantitatively address the effect of the NO\textsubscript{3}\(-\) assimilation/remineralization cycle on our estimate of \( F/D \) (N\textsubscript{2} fixation/denitrification ratio), we would need to consider the appropriate ratio of \( S \) to \( M \) in the eastern North Pacific, which we do not attempt here. If \( S \) is equivalent \( M \) or greater, our treatment in the main text would significantly underestimate the needed \( F/D \) ratio.

In these calculations, we have assumed that (1) the N isotope effect of NO\textsubscript{2}\(-\) oxidation (which has never been measured) is the same as the N isotope effect for NO\textsubscript{2}\(-\) reduction (which can apparently vary between 5 permil and 25 permil [Bryan et al., 1983; Casciotti, 2002]) and (2) that the 18e/15e ratio is the same for NO\textsubscript{2}\(-\) reduction as for NO\textsubscript{2}\(-\) oxidation. Considering the first of these assumptions, if the isotope effect of NO\textsubscript{2}\(-\) oxidation is significantly smaller than that of NO\textsubscript{2}\(-\) reduction, the posited NO\textsubscript{3}\/-NO\textsubscript{2}\(-\) redox cycle would cause N isotope enrichment of the recycled NO\textsubscript{2}\(-\), and the efficacy of this cycle to explain the \( D(15,18) \) minimum would be reduced. The opposite would be true if the isotope effect of NO\textsubscript{2}\(-\) oxidase is greater than that for NO\textsubscript{2}\(-\) reductase. It would appear that this latter scenario is unlikely, because our current simulation already requires a very high 15e for NO\textsubscript{3}\(-\) reduction ("denitrification", D) of 30.7 permil to fit the target NO\textsubscript{3}\(-\) d15N in the suboxic thermocline box (Figure 9 caption), and an even higher (and thus unrealistic) 15e would be required if we were to assume that the 15e of NO\textsubscript{2}\(-\) oxidase were greater than the 15e for NO\textsubscript{2}\(-\) reductase (calculations not shown). Thus, if NO\textsubscript{2}\(-\) oxidation were the sole explanation for the \( D(15,18) \) minimum, we consider our estimates of the needed ratio of NO\textsubscript{2}\(-\) oxidation to NO\textsubscript{2}\(-\) reduction to be conservative.

References for Auxiliary Materials Endnote File


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Text 1
Size: 21 KB  
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Caption: Endnotes to main text

Figure A1
Size: 58 KB  
Format: EPS  
Recommended for viewing: latest version of GhostView  
Caption: (a) A plot showing the effects of variations of F and D on NO3- d15NB and d18OB and thus on D(15,18) (constant values of the latter shown as black dashed lines). As D is increased (solid red line), NO3- d15NB and d18OB increase equally (and [NO3-]B decreases). For a given D, as F increases (and [NO3-]B increases; blue line), d15NB decreases more than does d18OB, roughly along a trajectory toward (d15NF, d18OF). The end-point of the increase in F ends at the observed mean D(15,18) for 200-800 m in stations 7-16, yielding a combination of F and D equivalent to that indicated by the black circle in Figure 8a. The same set of trajectories is also shown as dotted lines for the case in which the surface mixing term S is equivalent to the deep mixing term M, as opposed to the case of S = 0 shown with the solid lines. The difference between the two cases is barely discernible. (b) D(15,18) plotted vs. F/D for the blue trajectories shown in (a), for the two cases of S=0 (solid blue line, as in (a)) and S=M (dotted blue line, as in (a)).


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