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Meytal B. Higgins

Rebecca S. Robinson
University of Rhode Island, rebecca_r@uri.edu

See next page for additional authors

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Citation/Publisher Attribution
Available at: http://dx.doi.org/10.1073/pnas.1104313109

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Dominant eukaryotic export production during ocean anoxic events reflects the importance of recycled NH$_4^+$

Meytal B. Higgins$^{a,b,1}$, Rebecca S. Robinson$,^c$, Jonathan M. Husson$^{a,b}$, Susan J. Carter$,^d$ and Ann Pearson$^{a,1}$

$^a$Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138; $^b$Department of Geosciences, Princeton University, Princeton, NJ 08544; and $^c$Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882

The Mesozoic is marked by several widespread occurrences of intense organic matter burial. Sediments from the largest of these events, the Cenomanian–Turonian Oceanic Anoxic Event (OAE 2) are characterized by lower nitrogen isotope ratios than are seen in modern marine settings. It has remained a challenge to describe a nitrogen cycle that could achieve such isotopic depletion. Here we use nitrogen-isotope ratios of porphyrins to show that eukaryotes contributed the quantitative majority of export production throughout OAE 2, whereas cyanobacteria contributed on average approximately 20%. Such data require that any explanation for the OAE nitrogen cycle and its isotopic values be consistent with a eukaryote-dominated ecosystem. Our results agree with models that suggest the OAEs were high-productivity events, supported by vigorous upwelling. Upwelling of anoxic deep waters would have supplied reduced N species (i.e., NH$_4^+$) to primary producers. We propose that new production during OAE 2 primarily was driven by direct NH$_4^+$-assimilation supplemented by diazotrophy, whereas chemocline denitrification and anammox quantitatively consumed NO$_3^-$ and NO$_2^-$. A marine nitrogen reservoir dominated by NH$_4^+$, in combination with known kinetic isotope effects, could lead to eukaryotic biomass depletion in $^{15}$N.

nitrogen fixation, stable isotopes, palaeoceanography

Mid-Cretaceous episodes of deposition of organic-rich sediments in the proto-Atlantic and Western Tethys basins known as Oceanic Anoxic Events (OAEs) (1) are attributed to high productivity and/or enhanced organic-matter preservation resulting from increases in nutrient supply and/or decreases in the ventilation of deep waters (2–6). Because OAEs are thought to be associated with enhanced CO$_2$ outgassing during emplacement of large igneous provinces (7, 8), understanding the feedbacks between CO$_2$, anoxia, and nutrient availability may help us understand better the effects of anthropogenic climate change on ocean circulation, oxygen balance, and marine ecology (9).

Basinal anoxia during OAEs would have promoted loss of fixed nitrogen through the processes of denitrification and anammox. The resulting nitrogen deficits in waters returning to the surface via upwelling would have been amended by nitrogen-fixing cyanobacteria, assuming iron and other micronutrients were adequately available (10). Indeed, enhancement of cyanobacterial production during many episodes of ocean anoxia has been proposed based on increased burial of 2-methylhopanoids (11–13), as these compounds are thought to be markers for cyanobacteria (14). Because such biomarker indices are only qualitative indicators of change and cannot provide quantitative estimates of export flux, complementary data generally include isotope ratios of total sedimentary nitrogen ($\delta^{15}$N$_{TN}$) (11, 15–17), as diazotrophy also affects the nitrogen isotopic budget of the ocean (18–20).

The modern ocean has several localized regions of anoxia, and in these regions, values of $\delta^{15}$N$_{TN}$ are generally higher than the present deep-water average $\delta^{15}$N$_{NO_3^-}$—value of +5‰—because of the isotopic fractionation of denitrification expressed in the water column (19, 21, 22). In contrast, sediments from OAE 2 record striking nitrogen isotopic depletion. They are characterized by values of $\delta^{15}$N$_{TN}$ consistently $<-1\%_o$, and often $<-3\%_o$ (11, 16, 17, 23). Expression of these negative values of $\delta^{15}$N$_{TN}$ varies consistently by depositional location, with the average value of $\delta^{15}$N$_{TN}$ for OAE 2 horizons of the Bonarelli section (Gubbio and Furlo, Italy) being $-3.3\%_o$ and the South Ferrily formation (England) being $-2.8\%_o$ (16); whereas the average value for the South Atlantic is $-1.9\%_o$ (23), for the proto-North Atlantic is $-1.8\%_o$ (11, 17, this work), and for the Tarfaya Basin, Morocco is $-1.7\%_o$ (between 45–60 m in section) (16). Such differences reflect regional heterogeneity of water masses, phototroph ecology, and/or nutrient biogeochemistry; and it has been suggested that patterns of intrabasinal upwelling intensity and nutrient concentrations correspond directly to regional patterns of sedimentation (8, 23, 24).

When viewed alongside the elevated 2-methylhopanoid ratios, negative values of $\delta^{15}$N have been interpreted as evidence for diazotrophic rebalancing of the nitrogen budget and cyanobacterial dominance of the nitrogen supply for new (export) primary production (6, 11, 15, 25, 26). However, the minimum value of $\delta^{15}$N for the biomass of marine diazotrophs ($\delta^{15}$N$_{Baxa}$) should be on average approximately $-1.3\%_o$, based on the fractionation associated with nitrogenase ($\epsilon_{\text{N2}} = 0-2\%_o$) and the $\delta^{15}$N value of dissolved N$_2$ in seawater (approximately +0.7‰). This number is supported by data that consistently show N-fixing cyanobacteria to have values of $\delta^{15}$N$_{Baxa}$ between 0.5‰ and $-2\%_o$ (average $-1.4\%_o$) (25, 27–35). Reports of values significantly $<-2\%_o$ are from a cultured Trichodesmium sp. ($-3.5\%_o$) that was more negative than field samples collected in situ by the same investigators (32) and from experiments on N$_2$-sparged Anabaena spp. ($-2.4\%_o$) grown in an artificial-seawater medium (ASP-2) that also contained NH$_4^+$ (31). Non-N$_2$-derived N in the culture media may explain these outliers. Given the likelihood that in situ values of $\delta^{15}$N$_{Baxa}$ would average approximately $-1\%_o$, the prevalence of sedimentary values lower than $-2\%_o$ in many OAE sections cannot be explained solely by N supplied via N fixation. These patterns require that additional N-cycling processes be invoked to explain the source of nitrogen driving primary production during OAEs.

**Nitrogen Isotopic Records of Sediments, Porphyrins, and Kerogen**

Chlorophyll-derived sedimentary porphyrins can be used to generate records of $\delta^{15}$N values of eukaryotic and prokaryotic phytoplankton that are unaffected by diageneis (36, 37), as well as to estimate the contribution of cyanobacteria to burial flux (38). We examined nitrogen cycling during OAE 2 using measurements of coeval bulk and porphyrin nitrogen isotopes in sediments from

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1104313109/DCSupplemental.

Author contributions: M.B.H., R.S.R., and A.P. designed research; M.B.H., R.S.R., J.M.H., S.J.C., and A.P. performed research; M.B.H., R.S.R., and A.P. analyzed data; and M.B.H. and A.P. wrote the paper.

The authors declare no conflict of interest.

Edited by Donald E. Canfield, University of Southern Denmark, Odense M., Denmark, and approved December 23, 2011 (received for review March 17, 2011)
A well-defined positive carbon isotope excursion in this section is contained within the high total organic carbon (TOC) interval commonly associated with the OAE (39). Values of $\delta^{15}$N_TN (Fig. 1B) from before the OAE through the first two-thirds of the OAE (428.5–423 m composite depth; mcd) decrease from approximately $-0.1\%$ to approximately $-1.4\%$ and fluctuate with greater variance than in the middle and top intervals ($p < 0.05$). The middle of the analyzed section (423 to 419.5 mcd), which spans the end of the OAE as defined by $\delta^{13}$C_carb, is characterized by stable $\delta^{15}$N_TN values of $-2.1 \pm 0.3\%$. The top section (419.5 to 415.5 mcd) is characterized by an increase in $\delta^{15}$N_TN values, returning to $-0.7\%$ in the uppermost samples. All of these values are lower than the $\delta^{15}$N minima that are observed in modern sediments, even in regions underlying zones of water-column anoxia or intense nitrogen fixation (40, 41). Because diagenesis and interstitial NH$_4^+$ in clays can shift values of $\delta^{15}$N_TN (18, 42), we also measured $\delta^{15}$N values of kerogen. They show an average offset of $0.4\%$ relative to bulk sediment (Fig. 1B), suggesting the original primary producers had even lower $\delta^{15}$N values than what remains recorded by values of $\delta^{15}$N_TN.

Porphyrin values of $\delta^{15}$N ($\delta^{15}$N$_{por}$) also show patterns that are similar to bulk N isotopes, although they exhibit more scatter than the $\delta^{15}$N_TN and $\delta^{15}$N_kerogen values. The large error ranges are due to full propagation of analytical uncertainty associated with preparation and analysis by the denitrifier method (43). To overcome the scatter, we plotted 3-, 5-, and 9-point moving averages (Fig. 1C). These different resolutions all show similar patterns, indicating that temporal trends in the results are not sensitive to the degree of smoothing and are not dependent on data density. The data are spaced relatively uniformly (0.5 m), although sampling resolution is higher in some horizons surrounding the top and bottom sections of the core. Hence the 9-point moving averages can shift values of $\delta^{15}$N_TN $\approx$ 2.1 $\pm$ 0.3\% relative to bulk sediment (Fig. 1B), suggesting the original primary producers had even lower $\delta^{15}$N values than what remains recorded by values of $\delta^{15}$N_TN.

The relative fraction of eukaryotic vs. cyanobacterial export production can be estimated from $\delta^{15}$N values of porphyrins and their associated sediments. In previous work we examined the biochemical and physiological basis for a fractionation of nitrogen isotopes between biomass and chloropigments (38). The offset, known as $\epsilon_{por}$ ($\epsilon_{por} = \delta^{15}$N$_{por} - \delta^{15}$N_{kerogen}), differentiates significantly between eukaryotes and cyanobacteria. Values of $\epsilon_{por}$ for eukaryotes are around $5 \pm 2\%$, i.e., chlorophyll $5\%$ more depleted in $^{15}$N than biomass (33, 38, 46). In contrast, cyanobacteria have values of $\epsilon_{por}$ between 0 and $-10\%$ (i.e., chlorophyll equal to or up to $10\%$ enriched in $^{15}$N relative to biomass). One example of a value of $\epsilon_{por}$ near $-10\%$ had been observed previously for Anabaena cylindrica (33). To expand on this finding, we recently reported data showing that among the seven species of cyanobacteria tested to date, freshwater ecotypes cluster around the $\epsilon_{por} = -10 \pm 2\%$ endmember, whereas marine ecotypes cluster around the $\epsilon_{por} = 0 \pm 2\%$ endmember (38).

Because $\epsilon_{por}$ reflects intracellular partitioning of N isotopes downstream of the amino acid glutamate, it is independent of the nitrogen substrate utilized by the organism (N$_2$, NO$_3^-$, or NH$_4^+$; ref. 38). Thus, we proposed that $\epsilon_{por}$ would be an excellent proxy for calculating the relative contributions of eukaryotes and cyanobacteria to marine export production. Measured values of $\epsilon_{por}$ would be $5 \pm 2\%$ in a 100% eukaryotic system and would be $0 \pm 2\%$ in a 100% marine cyanobacterial system, regardless of the proportion of diazotrophic species among the latter (not all marine cyanobacteria are diazotrophs). Moreover, influx of terrestrial biomass and/or cyanobacteria from fresh waters would lead to values of $\epsilon_{por} < 0\%$. Indeed, to date the only situ value of cyanobacterial $\epsilon_{por}$ from the environment is from a freshwater lake in Japan in which $\epsilon_{por}$ was determined to be $-13$ to $-16\%$ (47).

At Site 1258A the observed value of $\epsilon_{por}$ throughout the section averages $4.3 \pm 0.8\%$ (if calculated vs. $\delta^{15}$N$_{TN}$) or $1.0 \pm 1.1\%$ (if calculated vs. $\delta^{13}$C$_{CTOC}$).

**Fig. 1.** Elemental and isotopic data for site 1258A. The shaded bar represents the $\delta^{13}$C$_{org}$ excursion interval that defines the OAE. (A) %TOC (squares) and $\delta^{13}$C$_{org}$ (circles) from (39). (B) $\delta^{15}$N values of bulk sediment (triangles) and kerogen (diamonds), and their 1-m averaged trends. (C) Porphyrin $\delta^{15}$N values, and their 3- (green), 5- (tan), and 9- (blue) point averaged trends. (D) The isotopic offset $\epsilon_{por}$ between bulk sediment and porphyrins (triangles), and kerogen and porphyrins (diamonds), as well as the corresponding fraction of cyanobacterial export based on the endmember values described in the text. The solid vertical line represents a typical algal value of $\epsilon_{por}$, and the dotted vertical line represents a marine cyanobacterial value of $\epsilon_{por}$ (38). All error bars represent 1σ, and preparative and analytical errors are compounded when possible. Raw data are shown in Table S1.
4.0 ± 0.8‰ (if calculated vs. δ15N_ocean) (Fig. 1D). The values of ϵ_{pot} reach minima—reflecting maximum burial of cyanobacterial biomass—toward the end of the OAE. There appears to be a qualitative trend of decreasing magnitude of ϵ_{pot} from the beginning of the OAE until approximately 1 mcd below the termination. At this point, ϵ_{pot} increases and fluctuates repeatedly until approximately 4 mcd after termination of the OAE, after which ϵ_{pot} then returns to the starting value near 5‰. This again shows that changes in nitrogen cycle processes are out of phase with the changes in the carbon cycle that define the OAE. Such differences may be expected: in multiple OAE 2 sections the local primary productivity is known to be variable within the overall record of the OAE as defined by δ13C values (24, 48).

If we assume that only eukaryotic algae and marine cyanobacteria contribute significantly to the burial flux of photosynthetic pigments (i.e., eliminating the possibility of freshwater cyanobacteria), a value of ϵ_{pot} consistently >4‰ during the OAE indicates that the export flux remained on average ≥80% eukaryotic throughout the event. In contrast with previous interpretations invoking N fixers as the primary source of nutrient N (11, 15, 26), these results indicate that the abundance of cyanobacteria contributing directly to export production during OAE 2 was not large; and it suggests that another N source would have been required to sustain such high rates of eukaryotic export production. However, the data for ϵ_{pot} are consistent with a large relative change in the cyanobacterial population, as observed values of ϵ_{pot} approximately 4‰ within the OAE indicate approximately 20% cyanobacterial biomass, whereas pre- and post-OAE values of ϵ_{pot} nearer 5‰ indicate less burial of cyanobacterial biomass (certainly <5–10%). The ϵ_{pot} data thus indicate at minimum a doubling to quadrupling of cyanobacterial production, but within a system consistently and overwhelmingly dominated by eukaryotic primary producers. If Site 1258A is representative of OAE 2 in general, the widespread negative values of sedimentary δ15N_{TN} throughout OAE 2 deposits must be attributed to burial of eukaryotes having significant 15N-depletion in their biomass.

Nitrogen Cycle in Anoxic Oceans: A Paradox of Nitrification and Denitrification

A modest increase in cyanobacterial production is consistent with expected changes to the nitrogen cycle. During OAE 2, anoxic deep waters of the proto-Atlantic and Western Tethys would have contained nitrogen predominantly in the form of NH4+. Upwelling rates were high (8), and NH4+ upwelled into oxic surface waters either was assimilated by phytoplankton or oxidized to NO3− and/or NO2−. Reducing conditions (i.e., lower oxygen concentrations) in the photic zone likely meant that a greater fraction of this NO3− and NO2− subsequently was reduced to N2 via denitrification and anammox, causing a modestly greater fixed-nitrogen deficit. Such widespread N deficits suggest it is unlikely that negative values of δ15N_{TN} in sediments of OAE 2 could be due to the expression of isotopic discrimination during nutrient uptake, which occurs for eukaryotes only in nutrient-replete systems in which the nitrogen supply is in excess of biological demand (49, 50). The extent to which fixed N was used to completion in OAE 2 surface waters would have determined the ecological niche for N-fixing cyanobacteria, either as free-living cells or as symbionts; but the overall system was N limited, as generally is the case in the marine photic zone (10). Complete utilization of the available nutrient N implies that the total flux must have been isotopically negative.

A deficit in fixed N during OAEs is not surprising, as anoxia promotes denitrification. What may be surprising is that the deficit was not larger. We suggest that counterintuitively, rates of denitrification may decrease under conditions of extreme basin-wide anoxia. Denitrification and anammox depend on sufficient availability of NO3− and NO2−. Because these oxidized N species are produced aerobically, extreme oxygen limitation in the water column may decrease their rate of formation, leaving a greater fraction of remineralized organic nitrogen to cycle throughout these regionally isolated basins and reenter the photic zone as NH4+. This in turn would limit the need for compensating N fixation. Evidence for photic-zone sulfate oxidation during OAEs suggests that NO3− indeed was completely absent beneath the photic zone, at least episodically (5, 51), and that fixed N in these deep waters would have remained in reduced form. We propose that the values of δ15N_{TN} < −2‰ found in OAE sediments reflect severe diminishment of the deep-water NO3− component of the marine N cycle, implying that the deep ocean was a reservoir of NH4+. Upwelled NH4+ rather than newly fixed N, was the main N source for primary production. Chemocline impingement on the photic zone would have driven nitrification, denitrification, and anammox into competition with NH4+-assimilation. The balance between these processes—which varied regionally—would have set the loss rate of N from the ocean and the compensatory rates of N fixation.

To explain the observed values of δ15N_{TN}, isotopic mass balance would then require that the newly fixed N (δ15N_diazp = 0 to −2‰), plus the upwelled NH4+ supply, together can yield new production that has values of δ15N < −2‰ (e.g., Bonarelli and South Ferriby sections; ref. 16). This is different from a modern-ocean scenario, in which denitrification associated with the spreading of anoxic zones leads to progressively higher (positive) values of δ15N_{NO3} that are then propagated to δ15N_{TN} (21, 22). The modern-ocean endmembers are thus near-zero (diazotrophs) and more positive (nitrate assimilation and/or recycling), whereas the OAE endmembers must be near-zero (diazotrophs) and more negative (NH4+ assimilation and recycling). Although required to explain the data, such a scenario is far from intuitive: it requires that the fixed N lost from the ocean by the processes of denitrification plus anammox have a net positive value of δ15N. Below we explore how such a system might be possible.

NH4+–Upwelling Model

To yield a marine system in which the burial flux of δ15N_{TN} has a negative value, we assume that NO3− (and NO2−) are produced only in the aerobic photic zone and are reduced quantitatively to N2 in the chemocline by denitrification and/or anammox. This loss is analogous isotopically to sedimentary denitrification in the modern ocean, which is considered to impart zero fractionation because it proceeds to completion, and by mass balance, δ15N_{inputs} = δ15N_{outputs} (19).

The following additional conditions then would be sufficient to achieve a denitrifying flux of N2 that is net isotopically positive. To yield surface waters in which NH4+ and N2 are the most important bioavailable sources of N, we assume that nitrification of the upwelling flux to NO3− followed by phytoplanktonic assimilation is much less significant than direct assimilation of concomitant upwelling NH4+ (19). Where NH4+ is available, NO3− is a less favorable nutrient for phytoplankton growth due to the higher energetic costs associated with its reduction (52). Nitrite generally is not believed to be an important source of nutrient N (53), and thus we assume it also is removed by denitrification or, more likely, by anammox. The dominant fractionation and fluxes in the N cycle are then ϵ_{fix} and ϵ_{fix} (N2 fixation), ε_{euk} and ϵ_{euk} (NH4+ assimilation), and ε2 and ε3 assimilation), and ϵ2 and ϵ3 (ammonium oxidation, NH4+ → NO2−), whereas the burial flux is small relative to these internal cycles (Fig. 24). We also specify the flux associated with remineralization of sinking phytoplankton (δ_{phyto}), and assume no fractionation for this process. As stated above, all oxidations and reductions downstream of ϵ2 are quantitative and do not impact further fractionation.

In N-limited surface waters, new production reflects the isotopic signature of the integrated nitrogen budget. The resulting value of δ15N_{TN} will reflect a weighted average of the δ15N values of diazotrophic cyanobacteria (δ_diazo) and of NH4+-consuming phytoplankton (δ_{phyto}). The former will be equal to δ15N_{N2fix}.
of paired values of balance dictates that the source surface waters becomes more organic nitrogen (DIN) accumulates as an ocean system in which the major reservoir of dissolved inorganic nitrogen (DIN) accumulates as a function of percent export from diazotrophs and fractional fluxes \( \varphi_1 \) and \( \varphi_2 \) for three sets of fractionation factors: \( \varphi_1 \) and \( \varphi_2 \) for three sets of fractionation factors: (B) \( \varphi_1 = -5\% \), (C) \( \varphi_1 = 5\% \), (D) \( \varphi_1 = 10\% \). Data for \( \delta^{15}N_{\text{DIN}} \) for OAE 2 from the literature (11 red); (blue, Italy; green, England); (17 yellow); (23 orange), and this study (gray), plotted relative to the range of paired values of \( \varphi_1 \) and \( \varphi_2 \) solved with the model, assuming 20% export of diazotrophic biomass (solid lines), as well as an \( \varepsilon_1/\varepsilon_2 \) offset of 10% assuming 10% export of diazotrophic biomass (dashed line).

Fig. 2. Conceptual model for sedimentary values of \( \delta^{15}N_{\text{DIN}} \) in an ocean in which NH\(_4^+\) is the dominant fixed N species. (A) System in which the \( \delta^{15}N \) values of exported eukaryotic biomass depend on the fractional fluxes to ammonium assimilation (\( \varphi_1 \)), oxidation (\( \varphi_2 \)), and recycling (\( \varphi_3 \)), as well as the difference between the associated fractionation factors \( \varepsilon_1 \) and \( \varepsilon_2 \). (B–D) Calculated \( \delta^{15}N \) values of sedimentary organic matter as a function of percent export from diazotrophs and fractional fluxes \( \varphi_1 \) and \( \varphi_2 \) for three sets of fractionation factors: \( \varepsilon_1 = -5\% \), \( \varepsilon_1 = 5\% \), \( \varepsilon_1 = 10\% \). (E) Data for \( \delta^{15}N_{\text{DIN}} \) in pelagic locations with lesser apparent bacterial biomass burial (16) and more positive values of \( \delta^{15}N_{\text{DIN}} \) in epicontinental environments with higher apparent bacterial flux (16).

As this NH\(_4^+\) upwells into the photic zone, it again becomes \( ^{15}N \)-enriched and the system maintains steady-state.

The resulting value for total buried organic matter (\( \delta^{15}N_{\text{NTN}} \)) is tempered by the percent contribution of diazotrophic biomass (Fig. 2 B–D) such that values of \( \delta^{15}N_{\text{NTN}} \) approach \(-1\%\) when there is greater burial of diazotrophs, but decrease as the ratio \( \varphi_2/\varphi_1 \) increases and diazotrophic burial decreases. This is consistent with records showing the most negative values of \( \delta^{15}N_{\text{TN}} \) in pelagic locations with lesser apparent bacterial biomass burial (16) and more positive values of \( \delta^{15}N_{\text{TN}} \) in epicontinental environments with higher apparent bacterial flux (16).

The model thus depends on the relative magnitudes of \( \varepsilon_1 \) and \( \varepsilon_2 \) compared to the N deficit and resulting diazotrophic contribution. It is possible that the fractionation associated with NH\(_4^+\)-assimilation (\( \varepsilon_1 \)) by the enzyme glutamine synthetase (GS) may exceed that of NH\(_4^+\)-oxidation (\( \varepsilon_2 \)) by the enzyme ammonium monooxygenase (AMO) under some circumstances. The observed value of \( \varepsilon_1 \) (\( +27\% \)) will depend on NH\(_4^+\) concentration, with larger fractionations expressed under NH\(_4^+\)-rich conditions (54). In the modern ocean, NH\(_4^+\)-concentrations are low and \( \varepsilon_1 \) is confined to the lower end of this range. Under the NH\(_4^+\)-replete conditions that we propose for OAE 2, assimilation using different enzymatic controls may lead to expression of \( \varepsilon_1 \) with a larger magnitude, although to date very little information is available about fractionation during NH\(_4^+\)-assimilation by natural planktonic assemblages (55).

The value of \( \varepsilon_2 \) also remains poorly constrained. The relative fraction of aerobic ammonia oxidation by archaea vs. bacteria during OAE 2 is not known, but \( \delta^{13}C \) and archaeal biomarker data measured in black shales deposited during the Albian OAE1b (approximately 112 Ma) suggest that Crenarchaeota
(now called Thaumarchaeota; ref. 56) that are believed to be responsible for most ammonium oxidation in the modern ocean (57), were abundant in the Cretaceous (58). Values of $e_2$ for bacterial AMO are approximately 14–38‰, for a variety of species grown on 1–2 mM NH$_4^+$ (59). Recent measurements of isotope effects associated with archaeal ammonia oxidation show a similar range of values, from 10–37‰ (60). In all cases, the relative contributions of fractionations associated with transport of NH$_4^+$ or diffusion of NH$_4^+$ through membranes and equilibrium of NH$_4^+/NH_3$ are uncertain. It is thus difficult to extrapolate these cultures to natural systems, except to suggest that bacterial and archaeal AMO results are similar.

If $e_1$ was large due to elevated NH$_4^+$ concentrations (54) upwelling to the base of the photic zone from a large, deep NH$_4^+$ pool, the condition of $e_1 > e_2$ could be met. For example, if $e_1 = 22\%$ (average archaeal value) and $e_2 = 27\%$ (maximum enzymatic effect on NH$_4^+$-assimilation), then $e_1 - e_2 = 5\%$. This results in values of $\delta^{15}N_{TN}$ for export production that will be $-2\%$ (Fig. 2C) if NH$_4^+$ oxidation consumes at least one-tenth of the upwelling NH$_4^+$ flux ($\phi > 0.1$) and the burial contribution of diazotrophs is 20%, the upper limit based on our data for $\epsilon_{pore}$. Other versions of the model that impose larger differences between $e_1$ and $e_2$ (e.g., $e_1 - e_2 = 10\%$, Fig. 2D) are also compatible with some of the data from OAE 2, in particular a few of the very negative values of $\delta^{15}N_{TN}$ for the sections from England and Italy (Fig. 2D and E) (16). Analogous models with $e_1 < e_2$ can produce only positive values of $\delta^{15}N_{TN}$, as would be seen in the modern ocean (Fig. 2F). Using our conceptual model, most data for $\delta^{15}N_{TN}$ compiled from OAE 2 (11, 16, 17, 23, this paper) fall within isotope space corresponding to ranges of $e_1 - e_2 = 5\%$ (Fig. 2E).

We further tested the plausibility of our conceptual framework using a simplified steady-state model that calculates $\delta^{15}N$ values of biomass N, NH$_4^+$, NO$_3^-$, and NO$_2^-$ in a two-box (surface and deep) ocean. The model was optimized to reproduce known modern values using estimates of fluxes and fractionation factors from the literature. To run the model subsequently for the OAE, we modified nitrogen-redox partitioning (more NH$_4^+$, less NO$_3^-$) and changed the magnitude of associated fluxes proportionally. Rates of upwelling and the total N inventory remained the same in both cases. By changing these parameters, the model generated sedimentary $\delta^{15}N_{TN}$ Values of -4.4‰ for the OAE and +4.9‰ for modern sediments. For a complete model description, results, and sensitivity analysis, see Supplementary Information.

Implications

Our model implies a widespread and well-mixed “ammonia ocean” for the proto-Atlantic and Western Tethys because it requires a sustained source of upwelling NH$_4^+$ that can be used for biological assimilation. This can be achieved if nitrate production is limited by severe demands on NO$_3^-$, possibly through enhanced anammox. In such an ocean, ammonia assimilators and N fixers both could out-compete assimilatory NO$_3^-$ reducers due to the dominance of NH$_4^+$ and a limited rate of NO$_3^-$ generation. Postulated high rates of upwelling, combined with nutrient trapping under estuarine circulation in the North Atlantic (8), may explain why these negative $\delta^{15}N$ signals are widespread during OAEs, yet are regionally variable (16). The trapping of quantitatively significant levels of NH$_4^+$ in deep waters during OAEs also helps preserve the total pool of marine N, alleviating the need for excessive rates of nitrogen fixation. Extreme anoxia may therefore exert a natural, negative feedback on the nitrogen cycle by preventing the ocean from denitrifying completely. Our proposed model for the N cycle during OAE 2 also helps to explain why extreme N isotopic depletion is not seen in modern anoxic basins like the Black Sea and the Cariaco Trench, where $\delta^{15}N$ values of particulate organic nitrogen are >0‰ throughout the water column (41). The nutrient sources and circulation patterns in these two systems are not analogous to anoxic oceans. The Cariaco Trench is a silled basin that receives NO$_3^-$ from the Atlantic, and sedimentary organic nitrogen in the Cariaco basin carries an isotopic signature that reflects a mass balance between Atlantic NO$_3^-$ that has been influenced by N$_2$ fixation (approximately 3‰) and N$_2$ (local nitrogen fixation) (61). In the Black Sea, a commonly used analog for anoxic oceans, the supply of N to surface waters is largely sourced from continental rivers, whereas the intense salinity stratification limits the upwelling of deep NH$_4^+$ and promotes formation of NO$_3^-$ followed by nearly quantitative loss via the anammox process (62). The nutrient N cycle of the modern Black Sea, therefore, primarily is analogous to a large lacustrine system with severe stratification. In contrast, we envision OAE 2 as a time of sustained upwelling.

The ammonia ocean scenario also may help to explain the temporal evolution of N isotope patterns seen in our data. Values of $\delta^{15}N_{pore}$ and $\delta^{15}N_{TN}$ are out of phase with carbon isotopes. They do not begin to decrease until the middle of the OAE interval, and their minimum persists past the traditionally defined termination of the event. This phase lag may reflect the balance of oxidants in the marine system. Enhanced burial of N in OAEs due to overoxygenation during OAEs should be associated with accumulation of oxygen in the ocean and atmosphere. This in turn would increase the ratios of ammonium oxidation and nitrification, eventually suppressing anammox and allowing NO$_3^-$ to accumulate. Indeed, our predicted values of $\delta^{15}N_{TN}$ only would “flip” to positive values when the nitrification flux ($\phi_2$) was sufficiently high to accumulate excess NO$_3^-$, allowing subsequent denitrification to enrich $^{15}N$ in the accumulating NO$_3^-$ reservoir. These results highlight the importance and promise of using temporal records of $\epsilon_{pore}$ in conjunction with $\delta^{15}N_{TN}$ values to examine both the succession of marine ecosystems and the redox state of the ocean.

In sum, a mid-Cretaceous deep ocean dominated by reduced rather than oxidized nitrogen species, normal rates of ocean circulation (63), and enhanced input of nutrients (5, 6, 8) together could yield negative values of biomass $\delta^{15}N$ and sustain a primary producer community that remained rich in eukaryotes. Although the oxidation state and temperature of OAE oceans was very different from the modern ocean, the persistent dominance of eukaryotes and dependence of primary producers on upwelled nutrients suggests that the balance between gross and net production was not greatly dissimilar from the present-day. Our results imply that additional feedbacks act under oxygen-limited conditions to maintain nitrogen balance, thereby limiting the extent of denitrification and the compensatory expansion of diazotrophy during OAEs.

Materials and Methods

Sediments were obtained from Ocean Drilling Program Leg 207, Site 1258A, from the Demerara Rise, offshore from modern Surinam. Samples spanned 415–428 m composite depth (mcd). Forty samples were analyzed for bulk $\delta^{15}N_{TN}$, $\delta^{15}N_{N_{pore}}$, and $\delta^{15}N_{N}$ at approximately 0.5-m spacing. Sampling resolution was higher leading into and coming out of the OAE, which spanned approximately 422–426 mcd (Table S1). Sample preparation and isotopic analysis followed established methods (43); details are given in SI Text.

ACKNOWLEDGMENTS. We thank Roger Summons, Carolyn Colonero, Amy Kelly, Noreen Tuross, and Kyle McElhenny for assistance with sample preparation and analysis and machine use. We thank Chris Junium and Julian Sachs for helpful discussions and comments, and Don Canfield, the PNAS editorial staff, and two anonymous reviewers for their valuable input. This work was supported by the National Science Foundation Grant OCE-0825269 (to A.P. and R.S.R.) and by the National Aeronautics and Space Administration Astrobiology Institute and the David and Lucille Packard Foundation (A.P.)