# Geochemical Tracers in Earth System Science: Nitrogen Isotopes in Deep Time

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Geochemical Tracers in Earth System Science: Nitrogen Isotopes in Deep Time

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Abstract

Nitrogen is an essential nutrient for life, and its sources and cycling have varied over Earth history. Stable isotope ratios of nitrogen compounds (expressed as $\delta^{15}N$, in ‰) are preserved in the sedimentary record and track these changes, providing important insights into associated biogeochemical feedbacks. Here we review the use of nitrogen stable isotope geochemistry in unravelling the evolution of the global N cycle in deep time. We highlight difficulties with preservation, unambiguous interpretations, and local versus global effects. We end with several case studies illustrating how depositional and stratigraphic context is crucial in reliably interpreting $\delta^{15}N$ records in ancient marine sediments, both in ancient anoxic (Archean) and more recent well oxygenated (Phanerozoic) environments.

1. Introduction – Nitrogen in Life

Nitrogen (N) is an essential nutrient for life, as it is critical in the formation of biomolecules, including nucleic acids and proteins. Despite an abundant reservoir of N$_2$ in the atmosphere, only a limited number of micro-organisms (nitrogen-fixing organisms, or “diazotrophs”) have evolved the ability to directly assimilate di-nitrogen. All other organisms rely either on ammonium (NH$_4^+$) or ammonia (NH$_3$) released during the remineralization of biomass, or on the products of oxidized ammonium/ammonia generated by biologically mediated transformations in the nitrogen cycle, such as nitrate (NO$_3^-$) and nitrite (NO$_2^-$).

Deciphering the evolution of the nitrogen cycle through geological time and the relative abundances of different bioavailable nitrogen compounds is therefore critical to understanding the emergence and radiation of early life, and to elucidating key biological and environmental transitions in the Phanerozoic. Such changes can be tracked by measuring the two stable isotopes of nitrogen in Earth materials ($^{14}N$ and $^{15}N$ – expressed as $\delta^{15}N$ in ‰, and described below), since different nitrogen cycling reactions express different isotopic fractionations in $\delta^{15}N$. The resulting N isotope ratios are reflected in biomass, which in turn can be archived in the sedimentary record (e.g., as reviewed in Stüeken et al., 2016) (Figure 1).

A seminal paper analysing changes in the marine nitrogen cycle through the Precambrian from temporal $\delta^{15}N$ trends was published by Beaumont and Robert (Beaumont and Robert, 1999). These authors found a shift from $\delta^{15}N$ values in kerogen centered around 0‰ in the Archaean, to positive $\delta^{15}N$ values centered around +5‰ from the Paleoproterozoic onwards. They suggested that these records were broadly indicative of an anaerobic NH$_4^+$-based nitrogen cycle in the Archean, giving way to an aerobic nitrogen cycle with available nitrite and nitrate after the Great Oxidation Event (GOE). In the ensuing two decades, the $\delta^{15}N$ proxy has become increasingly utilized for paleoenvironmental and paleoredox studies in deep time (here defined as pre-Cenozoic; e.g., Ader et al., 2016; Stüeken et al., 2016a), with more recent targeted studies largely supporting these broad temporal trends (e.g., Kipp et al., 2018; Yang et al., 2019; Zerkle et al., 2017).
Notwithstanding the general step-wise expansion of aerobic nitrogen cycling, nitrogen isotope studies of sediments spanning Earth’s history have revealed spatial and temporal nuances within this narrative. For example, some δ¹⁵N trends in Late Archean sediments have been interpreted to represent periods of temporally and spatially constrained aerobic N cycling prior to the GOE (Garvin et al., 2009; Godfrey and Falkowski, 2009; Yang et al., 2019; Mettam et al., 2019). More recent δ¹⁵N records have identified periods of time when anaerobic nitrogen cycling dominated in the Phanerozoic, including during the Latest Permian Extinction Event (LPEE; e.g., Saitoh et al., 2014) and Cretaceous Ocean Anoxic Events (OAES; e.g., Junium and Arthur, 2007).

Interpretations of these records, and the biogeochemical feedbacks they imply, critically rely on the ability of sedimentary δ¹⁵N to reliably record the isotopic fingerprints of the coeval marine nitrogen cycle. Here we discuss the state of the field in low-temperature N isotope biogeochemistry, including susceptibility to syn- and post-depositional alteration, ambiguities with respect to interpretations, and local versus global effects. Notably, we argue that stratigraphic and depositional context is crucial in reliably interpreting nitrogen stable isotope records in ancient marine sediments.

Figure 1. Temporal trends in δ¹⁵N over Earth history, as preserved in the sedimentary rock record (updated from Yang et al., 2019, and references therein).

2. The δ¹⁵N Proxy in a Nutshell

Multiple biologically mediated redox transitions occur between nitrogen-containing compounds in the marine N cycle. These transitions and their associated δ¹⁵N fractionations are summarised in Figure 2. The δ¹⁵N values of nitrogen compounds are expressed relative to the isotopic composition of atmospheric nitrogen, using the standard delta notation:

\[
\delta^{15}N (\text{‰}) = \left( \frac{^{15}N/^{14}N}_{\text{sample}} / \frac{^{15}N/^{14}N}_{\text{air}} - 1 \right) \times 1000,
\]

with isotopic fractionations for specific reactions shown as \( \varepsilon \approx \delta^{15}N_{\text{product}} - \delta^{15}N_{\text{reactant}} \).

N₂ fixation is the primary source of nitrogen input into the marine system and is regulated by the availability of phosphorous and micro-nutrients, such as Mo and Fe (e.g., Zerkle et al., 2006). Diazotrophs incorporate nitrogen into their biomass directly from atmospheric or dissolved N₂. Remineralization of this biomass in the water column and in sediments releases bioavailable ammonium or ammonia, which can in turn be assimilated by non-diazotrophic organisms. N₂ fixation
and the recycling of NH$_4^+$ generally impart only small fractionations in $\delta^{15}$N ($\varepsilon \leq 2\%$). Therefore, in an environment where N$_2$ fixation and the recycling of NH$_4^+$ are the dominant N sources, the $\delta^{15}$N of biomass broadly reflects the atmospheric input value ($\delta^{15}$N $\approx 0\% \pm 2\%$). However, if NH$_4^+$ or other forms of dissolved inorganic nitrogen (DIN) are readily available, diazotrophy will be suppressed, as N$_2$ fixation is energetically expensive in comparison to assimilation.

In the presence of oxygen, ammonium/ammonia undergoes microbially-mediated sequential oxidation to nitrite (NO$_2^-$) and nitrate (NO$_3^-$). In modern oxygen-rich settings this biological nitrification process is rapid and quantitative, making NO$_3^-$ the largest reservoir of bioavailable DIN in the oceans. Although nitrification can produce large fractionations in $\delta^{15}$N, the quantitative nature of NH$_4^+$ oxidation in the modern oceans means that these isotopic fractionations are suppressed in the resultant nitrate pool.

Nitrate and nitrite both form important sources of nutrient N in modern oceanic settings; however, they can also be utilized as electron acceptors in chemotrophic metabolisms. Nitrate can be reduced to N$_2$ during heterotrophic denitrification, a form of anaerobic respiration of organic carbon with NO$_3^-$, which is second only to aerobic respiration in reduction potential. This canonical form of denitrification proceeds through NO$_2^-$ and a number of intermediate N phases that can also build up in the environment (e.g., N$_2$O). Nitrate reduction can further be coupled to the oxidation of reduced compounds like sulfide, methane, or hydrogen during chemoautotrophy. Nitrate reduction can also proceed via dissimilatory reduction to ammonium (DNRA), which provides a competitive advantage under nitrate-limiting conditions since it requires less nitrate per mole of organic substrate. Finally, some organisms can utilize nitrite to oxidize NH$_4^+$ during anaerobic ammonium oxidation (anammox). The reduction of NO$_3^-$/NO$_2^-$ to N$_2$ during denitrification and anammox can have important implications for the oceanic nitrogen budget, as these processes remove bioavailable nitrogen from the oceanic reservoir. In the case of DIN loss, and if other nutrients remain available, diazotrophs will have a competitive advantage, and can proliferate to restore the balance of fixed N to the marine system. (e.g., Megonigal et al., 2003; Voss et al., 2012).

These N loss processes can all produce large fractionations in $\delta^{15}$N ($\varepsilon = +20$ to +30\%; Brunner et al., 2013; Granger et al., 2008). In marine sediments denitrification is generally quantitative such that no fractionations are expressed. However, in the water column, denitrification is constrained to low-oxygen settings such as oxygen minimum zones (OMZs; Figure 3.). This results in incomplete denitrification in the water column, leaving a residual pool of NO$_3^-$ that carries a positive $\delta^{15}$N value. Anammox produces a similar fractionation effect for $\delta^{15}$N, but its role in the global N cycle and in the N isotope budget is somewhat less well-constrained and the relative contributions of anammox and canonical denitrification remain an area of active research.

The balance between N$_2$ fixation and the assimilation of NO$_3^-$ carrying the positive $\delta^{15}$N signature of denitrification/anammox controls the $\delta^{15}$N values of modern marine biomass. Today, aerobic N loss processes produce organic matter with an average $\delta^{15}$N of $\sim +6\%$ (Peters et al., 1978). However, the $\delta^{15}$N of particulate organic matter and underlying sediments is spatially and temporally heterogeneous, following subtle redox and productivity variations in the modern marine system (e.g., De Pol-Holz et al., 2009). In addition, changes in the redox state and nutrient dynamics of marine settings over Earth’s history have seemingly allowed for unique configurations of the nitrogen cycle that produce both highly positive and highly negative $\delta^{15}$N values not seen on Earth today (Figure 1). We explore some of these records, and their potential (largely non-unique) interpretations in the case studies below.
Figure 2. Major transformations in the marine nitrogen cycle, showing average values for fractionations (adapted from Granger et al., 2008, Ader et al., 2016, and references therein). Fractionations not specifically shown (e.g., for assimilation and mineralization), are generally 0‰.

Figure 3. Nitrogen cycling in a Phanerozoic Oxygen Minimum Zone (OMZ) (adapted from Pinti and Hashizume, 2011). (1) Fixation of gaseous nitrogen (N₂) into biomass (organic matter, OM) by diazotrophs; (2) transport of OM to the sediments, i.e., via the biologic pump; (3) OM can be preserved in the sediments or remineralized to NH₄⁺, which can then be reassimilated into OM by benthic organisms or adsorbed onto clay minerals; (4) Remineralization of OM to NH₄⁺ and reassimilation of NH₄⁺ in low-oxygen settings; (5) in oxic settings NH₄⁺ is oxidised to NO₂⁻ and NO₃⁻; (6) upwelling of NO₃⁻ into low-oxygen setting; (7) partial denitrification of NO₃⁻ (and reduction of NO₂⁻ by anammox) in sub- to anoxic settings releases N₂ to atmosphere. Partial denitrification renders the residual pool of NO₃⁻ isotopically heavy (+ δ¹⁵N); (8) assimilation of this residual pool of NO₃⁻ contributes to the positive δ¹⁵N values of Phanerozoic OM; (9) the size of OMZs can expand and contract enhancing or reducing denitrification and the resulting N isotope effects.

3. Methods and Limitations

Nitrogen isotopes are now routinely measured alongside carbon isotopes in sedimentary rocks. Most recent studies have measured sedimentary δ¹⁵N using an elemental analyser coupled to an isotope ratio mass spectrometer running in continuous flow mode (CF-EA-IRMS). This method is more widely accessible and significantly less time consuming than previously developed offline methods.
(see Ader et al., 2016, for a comprehensive review). The CF-EA-IRMS method generally works well for sediments with high N contents (1σ of ±0.25‰ for N > 700ppm; Bahlmann et al., 2010). However, the low N content and high TOC/TN ratios in most Precambrian rocks can lead to some analytical challenges. In this case, additional steps are required to correct for blanks and ensure complete combustion to prevent isobaric interferences, e.g., with CO (Beaumont et al., 1994).

Several new methods have been developed in the past decade that also allow for accurate δ^{15}N measurements on samples with much lower abundances of N. The nano-EA-IRMS method (Polissar et al., 2009) uses a custom cryo-trap and chromatography system to concentrate the evolved N\textsubscript{2} gas before analysis, decreasing the sample size requirements by several orders of magnitude. This method has been successfully applied to analyze δ^{15}N values in deep time sediments (e.g., Mettam et al., 2019; Yang et al., 2019), but blanks remain an issue and must be closely monitored and corrected for. Alternatively, samples can be run in triplicates of varying mass. These triplicates can then be plotted on ‘Keeling’ plots, with the true value calculated as the intercept with the y-axis (see Mettam et al., 2019). An off-line tube cracker combustion system has also been successfully applied as an introduction system to the CF-EA-IRMS to measure δ^{15}N in low N abundance sediments (e.g., Stüeken et al., 2015a), but requires very high vacuum. Ishida et al. (2018) recently described a method for measuring δ^{15}N values in organic matter in situ using secondary ion mass spectrometry (SIMS), with analytical precision on the same order as bulk methods (2σ of ±0.56‰). Spatially resolved δ^{15}N analyses should be useful in discerning δ^{15}N at the single cell or organismal level (e.g., with Precambrian microfossils); however, the SIMS technique measures δ^{15}N on the CN\textsuperscript{-} ion, so cannot be used to measure mineral NH\textsubscript{4}\textsuperscript{+}, e.g., in clay minerals that don’t contain carbon.

Despite a recent upswing in the use of δ^{15}N values in deep time paleoenvironmental and paleoredox studies, post-depositional alteration remains an issue, and the relative ability of different sedimentary N phases to resist alteration is hotly debated. In the following sections we discuss post-depositional alteration of N stable isotope ratios, and the consensus (or lack thereof) for which N phases most faithfully retain their original δ^{15}N signatures.

### 3.1 The Problem with Preservation

In order to reconstruct ancient nitrogen cycling from the sedimentary rock record, the organic matter preserved within these sediments must faithfully record the δ^{15}N values of the coeval marine biomass. Post-mortem, marine phytoplankton will sink to the sea floor, aided by the biological pump (at least in the Phanerozoic). Some of this organic matter will be remineralized in the water column or directly consumed on descent, which could affect δ^{15}N values. Modern N isotope studies have shown that the oxidation of sinking particulate matter can be an important consideration for preservation, as deamination of organic matter can selectively liberate ^{15}N-depleted ammonium, thereby slightly increasing the residual δ^{13}N values (Möbius et al., 2010).

Primary N isotope values can be further modified by syn- and post-depositional processes in the sediments, including diageneis, metamorphism, and hydrothermal or hydrocarbon fluid migration. Numerous theoretical and empirical studies have been utilized to estimate these effects (e.g., see review in Ader et al., 2016). While the detailed findings of these studies differ, the processes that seem to impart the largest changes in the primary δ^{15}N values include aerobic degradation of organic matter during early diageneis, which can increase δ^{15}N values by 2-4‰ (Freudenthal et al., 2001), and high-temperature metamorphism above greenschist facies, which can increase δ^{15}N values by up to 5‰ (Bebout and Fogel, 1992; Boyd and Philippot, 1998).

Nitrogen isotope values can be modified by syn-depositional degradation of organic matter within the sediments, either by aerobic or anaerobic respiration (e.g., sulphate reduction) (Altabet and Francois, 1994; Macko and Estep, 1984). However, these ^{15}N-enrichments can be muted if the evolved NH\textsubscript{4} is quantitatively reincorporated into new benthic biomass or adsorbed onto clay mineral phases.
samples with high abundances of Ca-bearing silicates. An additional digestion using a combination of HF and boric acid to produce BF$_3$ can be prevented to some extent by digestion of sediments in HCl and thorough rinsing. However, concerns remain about the efficiency and selectivity of specific methods for kerogen extraction. Most kerogen extraction techniques use a series of rock powder dissolutions with increasingly corrosive acids to dissolve the carbonate and silicate fractions (often HCl-HF). Notably, recalcitrant minerals that are also resistant to HF digestion, such as pyrite, can be retained through these extraction procedures.

Proponents of analysing organic matter (or kerogen) argue that this archive is less easily altered or exchangeable than more mobile N pools, such as clay-sorbed NH$_4^+$. However, concerns about the efficiency and selectivity of specific methods for kerogen extraction. Most kerogen extraction techniques use a series of rock powder dissolutions with increasingly corrosive acids to dissolve the carbonate and silicate fractions (often HCl-HF). Notably, recalcitrant minerals that are also resistant to HF digestion, such as pyrite, can be retained through these extraction procedures. While these minerals do not generally contain N, they do influence the nitrogen abundance values of kerogen (total organic nitrogen, TON wt. %), complicating interpretations of TON. These minerals can be removed by the inclusion of a heavy mineral separation step after acid digestion, e.g., with chloroform. A greater concern is the formation of neo-minerals, such as fluoride salts, which can alter measured $\delta^{15}$N values during the combustion of samples in EA-IRMS. The formation of calcium fluoride (CaF) can be prevented to some extent by digestion of sediments in HCl and thorough rinsing to remove carbonate-associated Ca$^{2+}$ prior to HF extraction; however, this remains an issue for samples with high abundances of Ca-bearing silicates. An additional digestion using a combination of HF and boric acid to produce BF$_3$ has recently proven effective in removing secondary fluorides (Stüeken et al., 2017).

Proponents of analysing bulk sediments suggest that the offset between $\delta^{15}$N$_{org}$ and $\delta^{15}$N$_{bulk}$ from the same sample indicates that significant amounts of $^{15}$N must have been liberated from kerogen and ultimately captured on clays. Recent work by Stüeken et al. (2017) suggested that metamorphism drives organic $\delta^{15}$N values lighter and silicate-bound $\delta^{15}$N values heavier, resulting in a 3-4‰ offset at greenschist facies or lower. However, the mechanism(s) behind this offset remain unclear. If this difference occurs when NH$_4^+$ is transferred from kerogen to clays during early diagenesis, it could...
result from the preferential removal of isotopically heavy protein-derived organic matter from kerogen or the preferential preservation of isotopically light porphyrins in the kerogen; if it occurs during thermal maturation, it could be caused by changes in bonding as kerogen matures and clays dewater (Stüeken et al., 2017).

These arguments imply that $\delta^{15}$N$_{bulk}$ could provide the most reliable sedimentary archive, particularly when the majority of nitrogen resides in silicates phases. However, the retention of primary $\delta^{15}$N values in bulk sediments require that post-depositional processes occur within a closed-system that captures most of the $\text{NH}_4^+$ evolved from organic matter (e.g. Robinson et al., 2012). In addition, $\delta^{15}$N$_{bulk}$ data is susceptible to overprinting and isotope exchange between clay-bound $\text{NH}_4^+$ and $\text{NH}_4^+$ from migrating metasomatic fluids. Kump et al. (2011) suggested that potential overwriting by metasomatic $\text{NH}_4^+$ should be identifiable by strong correlations between TN, $\delta^{15}$N$_{bulk}$ and potassium (K, wt%), given the abundance of K in metasomatic fluids. Similar correlations should also identify contamination by allochthonous clay-associated $\text{NH}_4^+$ brought to the depocenter from eroding hinterlands, although such contamination with exogenous N is less easily identified. Given the propensity for these post-depositional processes to alter different N phases, and the lack of a consensus from the community as to which record is more reliable, many studies now report both $\delta^{15}$N$_{bulk}$ and $\delta^{15}$N$_{org}$ data, and comparison of these two datasets can reveal further insights into data fidelity (e.g., Koehler et al., 2018; Yang et al., 2019).

4. Case Studies

Taking into account the precautions discussed above, $\delta^{15}$N values provide important insights into past biogeochemical nitrogen cycling in well-preserved sedimentary rocks. Reliable $\delta^{15}$N data have been generated in sediments as old as 3.8 Ga (Papineau et al., 2005), and provide an interesting narrative of marine nutrient cycling following from biological revolutions that occurred over Earth history. Even the earliest $\delta^{15}$N records show evidence for N$_2$ fixation (Stüeken et al., 2015a) consistent with phylogenetic suggestions that diazotrophy arose very early in life’s history (Weiss et al., 2016). As discussed above, the long-term narrative of nitrogen cycling through geological time documents a change from dominantly anaerobic to aerobic N-cycling, consistent with the progressive oxygenation of the biosphere. However, recently published records hint at earlier spatial and temporal heterogeneities, even in a largely anoxic Late Archean world. In addition, records of $\delta^{15}$N are increasingly being utilized to examine the role of nutrients in more recent transitions in Earth history, including mass extinction events in the Phanerozoic. Here we present three case studies illustrating the utility and complexity of the $\delta^{15}$N proxy in deciphering the biogeochemical nitrogen cycling in the Late Archean and Late Permian marine systems.

4.1 Extreme $\delta^{15}$N values at ~2.7 Ga – marine versus terrestrial signals?

Both extremely high and extremely low $\delta^{15}$N values have been measured in sediments from ~2.7 Ga (Figure 1), and variably linked to global oxygenation and primary production. $\delta^{15}$N values as high as +50‰ in the Tumbiana Formation (Fortescue Group, Western Australia) have been interpreted to represent partial nitrification under conditions where ammonium was abundant and oxygen was limited (Thomazo et al., 2011). This interpretation stems largely from laboratory incubations with methanotrophic bacteria that produced extremely $^{15}$N-depleted N$_2$O (-46‰) during the co-oxidization of ammonium with methane (Mandernack et al., 2009). The co-occurrence of these large $\delta^{15}$N values with very low $\delta^{13}$C$_{org}$ values in the Tumbiana Formation supports the incorporation of methane into these sediments. However, this interpretation remains problematic for several reasons (e.g., Stüeken et al., 2015b). Namely, in order for isotopic fractionations this large to be expressed in the residual $\text{NH}_4^+$, a large fraction of the available ammonium (> 70%) would have had to be oxidized. The residual ammonium would still have been assimilated into biomass, while the resulting NO$_2^-$ and NO$_3^-$
would have been respired. Similarly, in order for the high $\delta^{15}N$ values to have been preserved in the resulting organic matter, the product $\text{NO}_2^-$ would have to be lost from the system (e.g., via denitrification or anammox) rather than re-assimilated. Stüeken et al. (2015b) alternatively suggested that the Tumbiana Formation and underlying Kylena Formation represent a lake system that was driven highly alkaline by aqueous alteration of underlying volcanics. In an anoxic alkaline lake setting, the volatilization of NH$_4^+$ to ammonia (NH$_3$) could produce large N isotope fractionations, similar to high $\delta^{15}N$ values in alkaline lakes today.

Extremely low $\delta^{15}N$ values have recently been reported from ~2.7 Ga sediments of the Manjeri Formation (Belingwe Greenstone Belt, Zimbabwe) (Yang et al., 2019). These values, down to -11‰, were interpreted to form from incomplete assimilation of a non-limiting pool of upwelling NH$_4^+$. This scenario was linked to a rise in global primary productivity following the expansion of oxygenic photosynthesis. The associated surge in primary productivity could have enhanced organic matter export and remineralization, leading to the accumulation of NH$_4^+$ in anoxic deep waters. This pool of NH$_4^+$ could have been upwelled into highly productive surface oceans and partially assimilated by primary producers preferentially utilizing isotopically light NH$_4^+$. In addition, uptake of light NH$_4^+$ would have left the residual pool of DIN isotopically heavy. Assimilation of this residual pool of isotopically heavy NH$_4^+$, either separated in time or space, could also have contributed to production of positive $\delta^{15}N$ values in the Late Archean (Ader et al., 2016; Yang et al., 2019).

### 4.2 Pre-GOE positive $\delta^{15}N$ values - alternatives to oxygen?

Despite the general narrative of an anaerobic Archean N cycle giving way to aerobic N cycling sometime later in Earth history, the exact timing of this transition is debated. Some statistical treatments of the secular $\delta^{15}N$ record seem to imply that nitrate was widely available by ~2.5 Ga (Stüeken et al., 2016), while others suggest that a turning point in the N cycle occurred in association with the GOE at ~2.3 Ga (Zerkle et al., 2017). Positive $\delta^{15}N$ values in Late Archean sediments have also been interpreted to imply the early onset of aerobic N cycling; however, these data are not without controversy, and lend themselves to alternative interpretations.

Small increases in $\delta^{15}N$ values preserved within ~2.5 Ga sediments from the Mt McRae Formation in Western Australia (Garvin et al., 2009) and the Ghaap Group in South Africa (Godfrey and Falkowski, 2009) have been suggested to record the transient or localized appearance of nitrification and denitrification in association with “whiffs” of oxygen in the marine environment. However, these sediments were deposited in distal and relatively deep waters, separated from oxygen oases in shallow, highly productive shelf environments where oxygen production was most likely to have occurred (e.g., Olsen et al., 2013). Therefore, these sediments could instead represent nitrogen redox cycling in open ocean or deeper water environments independent of surface oxygen. For example, positive $\delta^{15}N$ values preserved in ~2.5 Ga BIFs have been interpreted to reflect the assimilation of NH$_4^+$ enriched in $^{15}N$ by partial oxidation to nitrite. However, these researchers argued that ammonium oxidation could have been driven anaerobically by microbes utilizing Fe(III)-oxyhydroxides formed in the water column, rather than by O$_2$ (Busigny et al., 2013).

$\delta^{15}N$ values of ammonium in the Late Archean could also have been driven higher by partial assimilation, as described for ~2.7 Ga sediments above (Ader et al., 2016). Until recently this interpretation was largely dismissed due to a lack of evidence for a correlative pool of $^{15}N$-depleted biomass. However, recent analyses of ~2.5 Ga rocks have identified comparatively low $\delta^{15}N$ values (< -4‰) in shore-proximal sediments (Mettam et al., 2019). Combined with the ~2.7 Ga data (Yang et al., 2019), these studies suggest that partial NH$_4^+$ assimilation could have been widespread once oxygenic photosynthesis kick-started primary productivity in the Late Archean. These data additionally illustrate the potential for spatial and temporal heterogeneity within the global marine N cycle.
The study by Mettam et al. (2019) further demonstrates how depositional setting can play a key role in spatially partitioning the nitrogen cycle, producing local variations in $\delta^{15}$N values. For example, $\delta^{15}$N values of -4‰ were measured in sediments deposited from relatively deep shelf conditions, where partial assimilation from a pool of upwelling NH$_4^+$ from the open ocean could have occurred. In contrast, sediments deposited in shallower, more restricted lagoonal conditions retained $\delta^{15}$N values of ~0‰, likely reflecting diazotrophy and the efficient recycling of remineralized NH$_4^+$. Only a handful of positive $\delta^{15}$N values greater than 2‰ were reported from the same section, hinting at the possible presence of coupled nitrification and incomplete denitrification (Figure 4). However, these positive values were from shallow-water, carbonate-rich facies, probably indicating very localised oxygenated settings. This spatial complexity highlights the importance of sedimentological and depositional context for understanding local variations in $\delta^{15}$N data.

**Figure 4.** Proposed nitrogen cycle in the Late Archean (adapted from Mettam et al., 2019). Nitrogen cycling was likely spatially heterogenous, as follows: (1) N$_2$ fixation in the open ocean and transport of diazotrophic biomass to the seafloor; (2) remineralization of OM to NH$_4^+$ and shoreward transport; (3) incomplete NH$_4^+$ assimilation, producing OM with negative $\delta^{15}$N values; (4) transport of the residual NH$_4^+$ pool (left with positive $\delta^{15}$N values); and, (5) complete assimilation of NH$_4^+$ in the open ocean, producing OM with positive $\delta^{15}$N values. (6) In a relatively restricted lagoonal environment isolated from marine influence these processes could alternatively go to completion, such that the $\delta^{15}$N of OM would reflect the input (0‰). Alternatively, if oxygen oases are present, coupled nitrification/denitrification could produce OM with positive $\delta^{15}$N values.

### 4.2 The Late Permian Extinction Event – multiple controls on $\delta^{15}$N?

Trends in Phanerozoic $\delta^{15}$N values have also been utilized to examine changes in redox and nutrient feedbacks during more recent events in Earth history. For example, nutrient stress during the Late Permian has been implicated in contributing to the greatest extinction event in the Phanerozoic. Notably, $\delta^{15}$N values near 0‰ in palaeoequatorial Tethyan seas during the LPEE have been interpreted to reflect enhanced denitrification and a proliferation of diazotrophy resulting from NO$_3^-$ limitation (as reviewed by Saitoh et al., 2014).

Decreases of ~1‰ in $\delta^{15}$N values in late Permian Boreal and Panthalassic waters have also been reported (Algeo et al., 2012; Grasby et al., 2015; Knies et al., 2013; Schoepfer et al., 2012). However, with the exception of one study (Schoepfer et al., 2012), these changes are small and $\delta^{15}$N values never fall below +4‰. The persistence of positive $\delta^{15}$N values indicate that these waters probably maintained a robust NO$_3^-$ inventory, which would have precluded an expansion in diazotrophy.
Small decreases in δ¹⁵N values in LPEE sediments could be caused by several other factors. As described above, differing rates of organic matter degradation tied to redox changes can influence the δ¹⁵N values of organic matter during deposition, particularly where fluxes of organic matter are low (Freudenthal et al., 2001; Möbius et al., 2010). Such effects are illustrated by LPEE data from Schuchert Dal, East Greenland (Mettam et al., 2017). C/N ratios in these sediments never exceed 6, indicating little variation in organic matter provenance (e.g., due to terrestrial input). However, higher δ¹⁵N values and lower C/N ratios correspond with oxic, bioturbated depositional horizons, while lower δ¹⁵N values and higher C/N ratios are recorded in oxygen deficient, laminated horizons. Given these observations, a wholesale transition from oxic to anoxic conditions during the LPEE coupled with slower syn-depositional degradation could also contribute to decreases in δ¹⁵N values at some sites during and after the LPEE.

The complexity of interpreting sedimentary nitrogen isotope values is further highlighted by δ¹⁵N data from Buchanan Lake, Canada, which was part of the Sverdrup Basin on the northwest margin of Pangea during the LPEE (Knies et al., 2013). These authors concluded that increased diazotrophy was likely responsible for a small decrease in δ¹⁵N (from ~+9‰ to ~+8‰) in these sediments. However, persistently positive δ¹⁵N values throughout this section suggest continued nitrate availability despite evidence for an intensification of anoxia. A fall in the rate of organic matter degradation associated with enhanced anoxia could be responsible for the small fall in δ¹⁵N; however, this scenario is inconsistent with a coeval decrease in C/N ratios. An alternate explanation could come from increased weathering and transport of exposed soils and terrestrial vegetation, consistent with the reorganisation of terrestrial ecosystems during the LPEE (Algeo and Twitchett, 2010; Looy et al., 2001). Increased fluxes of terrestrial organic matter are unlikely to be the cause of the decrease in δ¹⁵N, as this would most likely increase C/N ratios as well. Alternatively, increased fluxes of clay-adsorbed inorganic nitrogen from the depositional hinterland could change δ¹⁵N whilst reducing C/N ratios, as seen at this site. Given these alternative arguments, an increase in marine diazotrophy is a reasonable, although perhaps not completely unique, interpretation.

5. Future Perspectives

These case studies highlight the potential complexity of the sedimentary δ¹⁵N record and some of the uncertainties associated with interpreting δ¹⁵N values in deep time. In particular, the paucity of well-preserved Archean sediments and inherent analytical limitations mean that the majority of δ¹⁵N studies have focused on organic carbon-rich sediments deposited in low energy, deeper water settings. These studies provide important constraints on local nitrogen cycling processes occurring in these basins. However, the spatial heterogeneity of the marine N cycle, along with the ability of disparate N cycling processes to produce overlapping δ¹⁵N values, suggests that depositional and redox context is critical in interpreting the resulting δ¹⁵N records, and in unravelling local versus global signals. The interpretation of Phanerozoic δ¹⁵N records should similarly be approached with caution given the multiple factors that can modify or overprint the isotopic characteristics of primary marine organic matter. For example, redox variations can also influence organic matter degradation rates in the sediments. Furthermore, the evolution of land plants during the Palaeozoic provide a source of organic matter with distinct δ¹⁵N values and C/N ratios, and the transport of these materials to the sediments, along with nitrogen in eroded soils, can lead to mixing of isotope signals that complicate interpretations. Additional targets for future and ongoing development include:

1. **Analytical advancements** – New methods are being developed to characterize the distribution of δ¹⁵N values in low N abundance sediments and to identify post-depositional alteration. In particular, recent advances in *in situ* δ¹⁵N analyses via SIMS will allow for direct analyses of δ¹⁵N in organic nitrogen (Ishida et al., 2018), which should allow for single-cell δ¹⁵N analyses in Precambrian microfossils.
2. Field studies – Modern anoxic aqueous environments, such as redox-stratified lakes and seas, can provide near analogues to Precambrian marine systems. Thus δ¹⁵N values of aqueous and sedimentary N compounds in these systems can be linked directly to N cycling processes, providing important insights into sedimentary δ¹⁵N values preserved in ancient sediments (e.g., Fulton et al., 2018).

3. Laboratory studies – N isotopic fractionations produced during cyanobacterial N₂ fixation and denitrification are generally well calibrated for modern conditions (Baursachs et al., 2009; Granger et al., 2008), but life and environments have evolved significantly over Earth history. Past conditions could have promoted different N cycling processes, by different groups of organisms and/or the same organisms utilizing different enzymes, but the resulting isotopic fractionations are largely unconstrained (e.g., Nishizawa et al., 2014). Incomplete ammonium assimilation in particular has been implicated in contributing to Late Archean δ¹⁵N records, but our understanding of δ¹⁵N fractionations during this process, and their response to changing environmental conditions are extremely limited (Hoch et al., 1992).

Combined, these types of inter-disciplinary studies could make the forward strides necessary for generating and understanding sedimentary δ¹⁵N records throughout Earth history.

6. References (key references in bold)


