Biogeochemical Cycling of Nitrogen on the Early Earth

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INTRODUCTION

Nitrogen is a major component of biomass and plays important roles in metabolic pathways. Nitrogen is a component of numerous macromolecules, including proteins and nucleic acids, and, together with phosphate and iron, can be a biolimiting nutrient in the environment. On the modern Earth, nitrogen is present in the atmospheric reservoir as di-nitrogen (N₂) and is by far the most abundant gas in the atmosphere. Nitrite (NO₂⁻), nitrate (NO₃⁻), ammonium (NH₄⁺), and particulate and dissolved organic nitrogen are present as bioavailable forms of nitrogen in the oceanic reservoir (referred to as “fixed” nitrogen) and are linked by a complex web of biogeochemical processes (Sigman et al. 2009). In sedimentary rocks, nitrogen is mostly preserved as organic nitrogen and as fixed NH₄⁺ substituting for K⁺ in phyllosilicates (i.e. micas; Boyd and Philippot 1998; Busigny and Bebout 2013 this issue); together, these forms represent the available geological archive of past biogeochemical nitrogen cycling on Earth. In the late 1930s, Vernadski was the first to suggest the potential of nitrogen isotopes for recording past metabolic activity in deep time (Vernadski 1944). Indeed, physical, chemical, and biological processes discriminate the two stable isotopes of nitrogen (¹⁴N and ¹⁵N mass units, respectively), leading to measurable differences in the ¹⁵N/¹⁴N ratios of sedimentary nitrogen.

The modern oceanic biogeochemical nitrogen cycle and its isotopic expression are well documented (Sigman et al. 2009; Hastings et al. 2013 this issue) and mostly reflect the fate and recycling of nitrogen compounds in the ocean. Changes in the nutrient supply and/or redox stratification of the water column can affect the relative importance and spatial distribution of dominant nitrogen metabolic pathways. For instance, the ¹⁵N value of +5 to +7‰ of modern sedimentary organic matter in oceanic sediments reflects the ¹⁵N enrichment of fixed nitrogen during anaerobic ammonium oxidation (anammox) and denitrification in oxygen-minimum zones (Lam and Kuypers 2011). Periods of global change, such as the oceanic anoxic events of the Mesozoic and the protracted oxygenation of the Earth's atmosphere at the Archean–Proterozoic transition, are associated with variations in sedimentary ¹⁵N (Canfield et al. 2010). Consequently, nitrogen isotopes can provide a record of specific biosignatures and are sensitive to environmental redox changes during Earth history.

The detailed evolution of the nitrogen cycle over geologic timescales has remained an elusive target, owing partly to difficulties in interpreting the rock record in deep time. The main challenge is the ubiquitous metamorphism of Precambrian rocks (older than 0.542 billion years, Ga). As nitrogen isotopes are sensitive to thermal effects during postdepositional geological processes, primary signals inherited from ancient environments tend to be obscured. Studies of the Archean (4.0–2.5 Ga) and Proterozoic (2.5–0.542 Ga) thus require careful examination of rock samples before nitrogen isotopes can be used as reliable biosignatures of metabolic pathways and diagnostic paleoenvironmental proxies. In addition, nitrogen is found and preserved in measurable quantities in relatively few phases, which primarily include organic matter, phyllosilicates, feldspars, magnetite, and fluid inclusions. The nitrogen isotope ratios measured in such phases from ancient sedimentary rocks have to be evaluated for isotope fractionations imparted during metamorphism before reasonable interpretations of primary biosignatures can be made.

The lack of oxygen in the atmosphere during the Archean and until the Great Oxidation Event, between about 2.50 and 2.06 Ga, has impacted the long-term evolution of the nitrogen biogeochemical cycle (Canfield et al. 2010). The anoxic Archean atmosphere prohibited the oxygenation of the oceans, except perhaps in localized oases.

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Variations in the nitrogen isotope composition of ancient organic matter and associated sediments provide clues for the early evolution of Earth’s atmosphere–ocean–biosphere system. In particular, large isotopic variations have been linked to the protracted oxygenation of Earth’s atmosphere during the Precambrian. Important problems being investigated include the nature of the variations observed at specific times in Earth’s history and the degree of preservation of ancient nitrogen biogeochemical signatures during diagenesis and metamorphism. Interpreting these records in Archean sedimentary environments and their possible implications for the evolution of Earth’s early atmosphere, ocean, and life is challenging.
of oxygen-producing cyanobacteria. The rise of nitrite-oxidizing bacteria in the Archean ocean was impeded by their need for molecular oxygen to convert $\text{NO}_2^-$ to $\text{NO}_3^-$. Consequently, the growth of denitrifying bacteria and archaea, which breathe $\text{NO}_3^-$ (denitrification sensu stricto), was stilled, leaving nitrogen fixation, anoxic ammonium oxidation to nitrite, anammox and ammonium assimilation as the dominant microbiologically mediated processes in the nitrogen cycle on the early Earth.

The fundamentally different oceanic redox chemistry during the Archean must also have affected key enzymatic systems of the nitrogen cycle because most of them require a range of metal cations to perform redox reactions. The abundance of banded iron formations in Archean supra-crustal belts has given support to the hypothesis that oceans on the early Earth were generally anoxic and ferruginous. Such conditions would have favored an early evolution of Fe-bearing enzymes, such as nitrogenase for biological N$_2$ fixation, glutamate synthetase for NH$_4^+$ assimilation, and nitrite reductase for NO$_2^-$ reduction into NH$_4^+$ (Glass et al. 2009). Since transition metals are required in all nitrogen metabolizing microorganisms must have been modulated by the chemical evolution of the oceans (Anbar and Knoll 2002), which might therefore hold key information on the evolutionary history of the nitrogen biogeochemical cycle.

**NITROGEN ISOTOPES DURING THE PRECAMBRIAN**

Craton formation first began in the Precambrian. Shallow-water shelf deposits along craton margins, such as carbonate platforms and sandstones, are few until the end of the Archean around 2.5 Ga, when 2–3% of the Earth’s surface area consisted of emerged continental crust (Flament et al. 2008). Archean and Paleoproterozoic terrains represent only about 10% of the present exposed continental crust. During the last few decades, nitrogen geochemistry studies have mostly focused on the Pilbara (Western Australia), the Kaapvaal and Zimbabwe (southern Africa), the Superior (Ontario and Quebec), the North Atlantic (Nain) (Labrador, southern Greenland, and Scotland), the Dharwar and Aravalli (India), the West African (Ghana), and the Congo (Gabon) cratons (Fig. 1). It has so far not been possible to obtain unmetamorphosed samples of Archean terrains because all have systematically experienced metamorphism above the subgreenschist facies (at temperatures of more than about 220°C). The degree to which secondary alteration (i.e. during diageneis and metamorphism) may affect nitrogen isotope distribution in sediments and blur primary paleoenvironmental reconstructions is reviewed in Box 1.

Reconstructions of the Archean to Paleoproterozoic nitrogen cycle have been attempted by studies of $\delta^{15}$N in a range of rocks and minerals and are detailed in Table 1 and Figure 2A. In such studies, careful consideration of the metamorphic history of the metasedimentary rocks is necessary to evaluate paleoenvironmental conditions. After all, the nitrogen isotope record is the only direct means of reconstructing the nitrogen cycle on the early Earth. The record is established through the analysis of metamorphosed whole-rock samples (Pinti et al. 2001; Jia and Kerrich 2004; Nishizawa et al. 2005; Garvin et al. 2009; Godfrey and Falkowski 2009; Papineau et al. 2009; Thomazo et al. 2011), sedimentary organic matter (Beaumont and Robert 1999; Godfrey and Falkowski 2009), graphite (Van Zuilen et al. 2005), structural NH$_4^+$ contained in metamorphic minerals such as biotite (Jia and Kerrich 2004; Papineau et al. 2005), impurities in Fe-bearing phases (Pinti et al. 2001), and fluid inclusions (Sano and Pillinger 1990; Nishizawa et al. 2007).

**SECULAR VARIATIONS IN THE ARCHEAN TO PALEOPROTEROZOIC SEDIMENTARY NITROGEN ISOTOPE RECORD**

The Evolution of the Nitrogen Cycle from $\delta^{15}$N

We present an extensive $\delta^{15}$N database spanning time from 3.8 Ga in the Archean to 1.4 Ga in the early Mesoproterozoic (1.6 to 1.0 Ga), and we use statistical methods (see online supplementary information at www.elementsmagazine.org/supplements) applied to geochemical time series in our analysis of the database. The data set presented in Figure 2A (table and references in online supplementary material) is based on a compilation of 874 published $\delta^{15}$N values...
measured on various Precambrian sedimentary lithologies and materials, including kerogen, phyllosilicates, shales, carbonates, cherts, banded iron formations (BIFs), and N₂-bearing fluid inclusions. Various levels of secondary alteration have been reported for the sample set and these have been carefully considered. All samples are included in the statistical analysis of δ¹⁵N values from the Precambrian record, allowing us to test the robustness of single, short-term isotopic excursions suggested in previous studies and to identify possible long-term secular variations (again, the reader is referred to Box 1 for considerations of the degree of δ¹⁵N preservation regarding secondary processes). The “SiZer” statistical approach referred to in Figure 2A is used to detect significant trends in δ¹⁵N variations. This method is based on the construction of curves fitting time series using different levels of smoothing (h); the h values represent different binnings of age ranges. The first derivatives of each curve (i.e. the slopes) are simultaneously computed with their 95% confidence intervals, allowing the signs of the derivatives to be statistically tested. The results of multiple tests are then reported under the form of SiZer maps (Fig. 2A) with different colors; these maps are a graphical representation of increasing (positive slope) and decreasing (negative slope) δ¹⁵N values for variable lengths of time periods. Clearly, this first-order statistical analysis is based on currently available data sets and will evolve with future additional data.

The Eo- and Paleoarchean Nitrogen Cycle (4.0–3.2 Ga)

The available nitrogen isotope data set from Paleoarchean (3.6–3.2 Ga) metamorphosed sedimentary rocks and organic matter (Fig. 2A) shows δ¹⁵N values centered around +3‰ and ranging between −6.2 and +27.5‰. Considering that Eo- and Paleoarchean environments were anoxic and that NH₄⁺ must have been the dominant bioavailable form of nitrogen in the ocean, most workers have interpreted this record to indicate that the nitrogen cycle at that time was dominated by nitrogen-fixing and/or ammonium-assimilating microorganisms (see water column I in Fig. 3; Table 1). Notably, N₂-bearing fluid inclusions rich in the seawater component and preserved in the 3.5 Ga North Pole hydrothermal deposits in the Pilbara craton (Western Australia) have δ¹⁵N values between −0.7 and −2‰ (Nishizawa et al. 2007). These N₂-bearing fluid inclusions data suggest that the δ¹⁵N value of the Paleoarchean...
atmosphere was similar to the value of the present-day atmosphere (i.e. 0‰). Kerogens in Paleoproterozoic cherts from the Kaapvaal Craton have low δ15N values, as low as −6.2‰, which have been interpreted as the result of biological nitrogen fixation and/or NH4+ assimilation at the time of formation (Table 1; Beaumont and Robert 1999). These latter values are similar to the range of values reported from Eoarchean metasediments from the Isua Supracrustal Belt and which collectively vary between −3.7 and +27.5‰ (Table 1). However, high δ15N values from Isua that were measured in biotite and whole-rock samples have been differently interpreted as postdepositional metamorphic fluid alterations of primary compositions (Pinti et al. 2001; Papineau et al. 2005; Van Zuilen et al. 2005). Unless other constraints can be used, δ15N values from highly metamorphosed sedimentary rocks can only be interpreted as maxima of their premetamorphic values (as explained in Box 1). The lighter δ15N values from Isua have thus been interpreted by some workers as possible indications of an Eoarchean biological nitrogen cycle (Nishizawa et al. 2005) dominated by chemosynthetic microorganisms (Pinti et al. 2001) and/or biological nitrogen-fixing microorganisms (Papineau et al. 2005). Importantly, however, these light nitrogen isotope compositions do not necessarily reflect biological processes but could conceivably indicate atmospheric or fluid-based contaminants (Van Zuilen et al. 2005). Therefore, the large range of δ15N values from Isua is challenging to interpret as a primary biosignature because these rocks have experienced a protracted metamorphic history.

The Meso- and Neoarchean Nitrogen Cycle (3.2–2.5 Ga)

During the Meso- and Neoarchean, the average δ15N value increases to +15% and the range of δ15N variations also increases drastically in some paleoenvironments, reaching values up to +50.4‰. However, the detailed structure of the δ15N secular variation during the Meso- and Neoarchean is unknown because of a gap in the record (Fig. 2A). The key observation by Beaumont and Robert (1999) that organic matter in Archean sedimentary rocks commonly has negative δ15N values has thus been supplemented by observations of highly 15N-enriched samples in the Neoarchean around 2.7 Ga (Table 1; Fig. 2A) and a long-term increase in δ15N (Fig. 2A). 15N-enriched values in slightly metamorphosed Neoarchean sedimentary rocks around 2.7 Ga have been mostly interpreted as the record of the initiation of the oxidative part of the nitrogen cycle (i.e. the rise of nitrification and denitrification metabolisms; see Fig. 3) (Garvin et al. 2009; Godfrey and Falkowski 2009; Thomazo et al. 2011). Jia and Kerrich (2004), however, have interpreted the large range of highly positive δ15N values in the Neoarchean to reflect a significant increase in atmospheric δ15N composition resulting from the delivery of CI chondrite meteorites and comets with heavy nitrogen at the end of Earth accretion at about 4.5 Ga. While this hypothesis remains to be tested, evidence based on nitrogen isotope analyses of N2-bearing fluid inclusions in Precambrian cherts indicates
After 2.0 Ga, the precursors of all nitrogenous compounds in the cell. These two key enzymes of the nitrogen cycle require Fe, which results in the synthesis of glutamine and glutamate. The ability to assimilate NH₄⁺ directly from the precursors of all nitrogens in the cell. The rise of atmospheric oxygen during the Paleoproterozoic about 2.5–1.6 Ga must have impacted the nitrogen biogeochemical evolution of early Earth environments. In fact, during the Paleoproterozoic at around 2.0 Ga, both oxygenated and redox-stratified water masses existed during deposition of the Aravalli Supergroup (India), where δ¹⁵N values reach +31.6‰ (Papineau et al. 2009). After 2.0 Ga, δ¹⁵N values are between 0 and +10‰, with a statistical average close to +5‰ and a smaller variability. These data are comparable to the modern nitrogen signal (inset, Fig. 2a) and have been interpreted to reflect the expansion of the redox transition zone (see water column III in Fig. 3). In this paleoenvironment, ammonium (NH₄⁺) would be present in the deep anoxic ocean, NO₃⁻ would accumulate at the oxic–anoxic interface during nitrification and denitrification cycles, and NO₂⁻ would accumulate in the oxygenated surface water in response to increasing atmospheric and oceanic oxygen concentrations. Geochemical data are thus consistent with the view that (1) abundant free oxygen had stabilized NO₃⁻ in the oceans and (2) a complete nitrogen biogeochemical cycle was established by about 2.0 Ga.

**INSIGHTS FROM MOLECULAR BIOLOGY**

Because of the incomplete geological record of the earliest Earth, it is unclear when, exactly, life started to evolve. However, the availability of nitrogen compounds for the synthesis of biomolecules on the early Earth was an important factor in the evolution of the first nitrogen metabolisms. The ability to assimilate NH₄⁺ directly from solution and incorporate it into amino acids and nucleotide bases is indeed likely to have been an ancient metabolic pathway (Glass et al. 2009). Two enzymes catalyze this process (glutamine synthetase and glutamate synthase), which results in the synthesis of glutamine and glutamate, the precursors of all nitrogenous compounds in the cell. These two key enzymes of the nitrogen cycle require Fe for catalysis and are thought to be ancient—perhaps they were already present in the last universal common ancestor (Raymond 2005).

Similarly, biological nitrogen fixation also likely evolved early in the history of life. Nitrogen-fixing enzymes need Fe, although it can be substituted by or combined with Mo or V when these are available (the Fe–Fe nitrogenase enzyme is less efficient, but it is likely that this form was dominant in the anoxic and ferruginous Archean oceans [Glass et al. 2009]). While lateral gene transfer might have occurred for some nitrogen-fixing genes in some microorganisms, nitrogen-fixing microorganisms include a large range of both archaea and bacteria, which suggests that nitrogen fixation was operative very early in the history of life, and possibly at the time of prebiotic chemistry. The relationship between metal-cation availability in the water column and the enzymatic requirement for metal cations in some of the most ancient nitrogen metabolisms suggests that there was a nitrogen-based bioinorganic bridge during the earliest Archean.

**PREBIOTIC AND NONBIOLOGICAL EARLY-EARTH NITROGEN CYCLING**

Several nonbiological geochemical processes fix nitrogen into bioavailable forms or change its redox state (Fig. 4), and these processes have been demonstrated experimentally. Mechanisms such as lightning, mineral catalysis during hydrothermal circulation, and photolysis were operative on the early Earth and could have resulted in the abiotic fixation of nitrogen into bioavailable forms (Fig. 4). Subaerial komatiitic volcanism could have provided additional fixed N oxides to the early-Earth environment (Mather et al. 2004). Experiments with lightning have also shown that nitrogen compounds can be synthesized from precursor N₂ gas, and, depending on the partial pressure of H₂, these compounds can be either reduced or oxidized (Navarro-Gonzales et al. 2001). Other experiments have shown that reduced and sulfidic hydrothermal vent fluids can reduce N₂ to NH₄⁺ (Schoonen and Xu 2001). Various Fe- and Ni-bearing minerals can also catalyze the reduction of N₂ or oxidized nitrogen species to NH₃ under hydrothermal conditions in simulated prebiotic environments (Smirnov et al. 2008), and Ti oxide catalysts can reduce nitrogen compounds under photochemical influence (Bickley and Vishwanathan 1979). The types of reactions noted here could have provided a prebiotic source of NH₃ before N₂ fixation had evolved in Hadean environments. Extraterrestrial input could have provided additional fixed nitrogen to the prebiotic Earth. Carbonaceous chondrite meteorites are usually rich in amino acids and sometimes...
in ammonia (Pizzarello et al. 2011), and these sources of extraterrestrial nitrogen could have periodically delivered fixed nitrogen to the early Earth, although the net fluxes are difficult to quantify. Meteorites of all types have a large range of nitrogen isotope compositions, with $\delta^{15}N$ values between $-65.6$ and $+309.0$‰ in acid-insoluble residues (Alexander et al. 2007). The N/C atomic ratio of these residues is typically greater than 1 (it varies between 0.1 and 6.5), and therefore the extraterrestrial contribution of nitrogen is of the same order of magnitude as that of carbon. In brief, during the initiation of the Hadean–Archean biogeochemical nitrogen cycle, several inorganic processes and nonbiological sources of fixed nitrogen led to the presence of yet-to-be-quantified fixed nitrogen in early oceans, which might have favored the emergence of life.

**FUTURE DIRECTIONS**

In the last decade, our knowledge of the evolution of the biogeochemical nitrogen cycle during the Archean–Paleoproterozoic period has greatly improved thanks to analytical developments that have allowed precise isotopic measurements of low nitrogen contents in rock and mineral samples. The general picture that has emerged is that biogeochemically induced nitrogen fractionations were recorded early in some of the oldest metasedimentary rocks during the Paleorarchean and in the increasingly oxidizing global environments of the Neoarchean–Paleoproterozoic ocean–atmosphere system.

Some of the fundamental questions to be investigated are: Which nitrogen metabolisms operated during the Eoarchean? How did the atmospheric $\delta^{15}N$ value evolve during the Archean, if at all? What is the nature of the Neoarchean and Paleoproterozoic global secular variations in sedimentary $\delta^{15}N$ values? What are the main sedimentary controls on nitrogen isotope compositions? Most of these questions can probably be answered by more thorough sampling strategies applied to Archean sedimentary rocks (shown in pink in Fig. 1) for future biogeochemical analysis.

Finally, because our interpretations of the past $\delta^{15}N$ sedimentary record can only be based on a detailed knowledge of isotopic effects from metabolic pathways and similar observations from modern analogs, such as redox-stratified lakes and basins (Fig. 5), the scientific community will need to continue merging the disciplines of limnology, paleoceanography, geobiology, and global biogeochemical cycles to better decipher the evolutionary history of the ancient nitrogen cycle and the early evolution of life on our planet.

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