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Nitrogen in Ancient Mud: A Biosignature?

Eva E. Stüeken

Abstract

Nitrogen is an essential nutrient for all life on Earth and possibly elsewhere. Burial of nitrogen bound to organic matter constitutes the major flux of nitrogen into sediments today, which has led to the inference that nitrogen enrichments in sedimentary rocks may be a biosignature. However, abiotic processes such as lightning or volcanism can fix atmospheric N_2 and contribute to sedimentary nitrogen burial in the absence of life. It is therefore uncertain whether observed nitrogen enrichments of up to 430 ppm in Paleoarchean metasedimentary biotite grains are indeed biogenic. This study seeks to address that problem with a numerical model. The NH_4^+ concentration of an abiotic ocean is modeled as a function of source fluxes, pH-dependent NH_3 volatilization, and equilibrated adsorption of NH_4^+ onto clay particles. The results suggest that the observed nitrogen concentrations in Paleoarchean biotite can only be reconciled with purely abiotic processes if the ocean was more acidic (pH <6) and/or if the source fluxes from lightning and volcanism were at least an order of magnitude higher ($\geq 10^{12}$ mol/yr) than previously thought. The bulk of the nitrogen is thus most likely of biological origin. While this does not necessitate a particular metabolism such as biological N_2 fixation, the data provide evidence of nitrogen utilization back to 3.8 Gyr. Nitrogen abundances could thus provide useful information in extraterrestrial missions. Key Words: Early Earth—Biosignatures—Nitrogen fixation. Astrobiology 16, 730–735.

1. Introduction

The bioavailability of nitrogen partly controls biological productivity today, and it may have shaped biological evolution in the past (Falkowski, 1997; Stüeken, 2013; Stüeken *et al.*, 2015, 2016). Given the central role of nitrogen in many biomolecules such as DNA, RNA, and proteins, it has almost certainly been a major constituent of biomass since the origin of life. The most widely accepted evidence of life dates back to 3.5 Gyr (Buick, 2007), based on a combination of proxies, including stromatolites and sulfur and carbon isotopes. More controversial evidence based on carbon isotopes has been reported from Paleoarchean rocks of the Isua Supracrustal Belt in Greenland that are 3.7–3.8 billion years old (Mojzsis *et al.*, 1996; Rosing, 1999). The biogenicity of the organic matter has been questioned because abiotically produced Fischer–Tropsch-type hydrocarbons could have had similar $^{13}C/^{12}C$ ratios (McCollom and Seewald, 2006). Nitrogen could potentially serve as an independent biosignature, as has been proposed by previous studies that extracted up to 430 ppm of nitrogen from metasedimentary biotite grains of Isua rocks (Fig. 1, Table 1; Honma, 1996; Pinti *et al.*, 2001; Papineau *et al.*, 2005). Biotite forms during metamorphism of pelitic sediments, in particular from clay mineral precursors. Honma (1996) reported the highest nitrogen concentrations in biotite grains from samples that also contained graphite, but noted that some amount of biotite-bound NH_4^+ may not be biogenic. Similar concerns about the biogenicity of graphite-bound nitrogen have been raised by van Zuilen *et al.* (2005), who invoked hydrothermal sources. Abiotic processes such as lightning, volcanism, impact shocks, and hydrothermal N_2 reduction generate fixed nitrogen that can be incorporated into the rock record. However, it is so far unclear whether those abiotic pathways could indeed mimic biogenic nitrogen enrichments in sedimentary rocks.

NH_4^+ uptake into the crystal lattice of clay minerals is preceded by adsorption onto the mineral surface (Schroeder and McLain, 1998). On modern Earth, this occurs primarily in pore waters of anoxic sediments, where dissolved NH_4^+ can be present in millimolar concentrations due to the degradation of organic matter (*e.g.*, Rosenfeld, 1979; Boudreau and Canfield, 1988). It is also relevant in anoxic water columns such as the Saanich Inlet or the Black Sea where NH_4^+ levels are on the order of 100 μM (Brewer and Murray, 1973). Boatman and Murray (1982) derived a relationship between dissolved and adsorbed NH_4^+ as a function of clay mineralogy (Fig. 2), following standard formulations

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Department of Earth and Space Sciences and Astrobiology Program, University of Washington, Seattle, Washington, USA.
Department of Earth Sciences, University of California, Riverside, California, USA.
Department of Earth and Environmental Sciences, University of St Andrews, St Andrews, Scotland, UK.

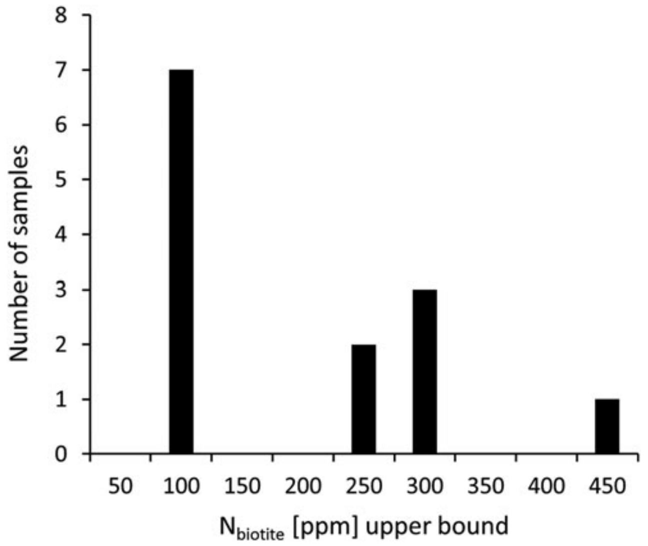

FIG. 1. N concentrations in Paleoarchean biotite from the Isua Supracrustal Belt. Biotite grains were separated from metasedimentary rocks. Data are compiled from the works of Honma (1996), Pinti *et al.* (2001), and Papineau *et al.* (2005). Concentrations tend to be lower in whole-rock samples due to dilution with N-poor minerals such as quartz or volcaniclastic debris.

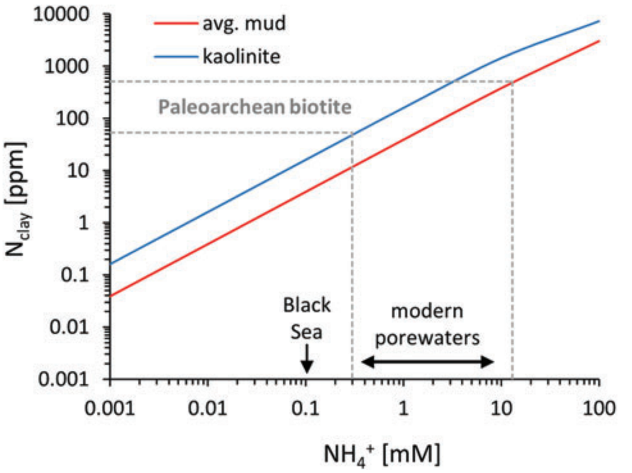

FIG. 2. Adsorbed N content of clay as a function of dissolved NH_4^+. The relationship is modeled after the work of Boatman and Murray (1982). Red = calibrated with average mud from the Saanich Inlet, after removal of organic matter, sulfides, and oxide minerals; blue = calibrated with pure kaolinite, which has the highest adsorption affinity from NH_4^+ among clays. Horizontal gray lines = range of N concentrations in Paleoarchean biotite (Fig. 1). Maximum dissolved NH_4^+ concentrations of modern pore waters are taken from the works of Rosenfeld (1979) and Boudreau and Canfield (1988). The Black Sea (Brewer and Murray, 1973) exemplifies a modern anoxic marine basin with largely biogenic NH_4^+ sources. (Color graphics available at www.liebertonline.com/ast)

of adsorption isotherms (Stumm and Morgan, 1996). This relationship applies over a wide range of NH_4^+ concentrations, and it reproduces measured values in other settings (*e.g.*, Rosenfeld, 1979; van Raaphorst and Malschaert, 1996). [It is preferred here over alternative formulations by Abdulgawad *et al.* (2009) because the experiments by Boatman and Murray (1982) were carried out under more controlled conditions.] Comparison to the Paleoarchean biotite data suggests that the water column or pore fluids from which the preceding clay minerals obtained their NH_4^+ must have had dissolved NH_4^+ concentrations between 0.2 and 10 m*M* (Fig. 2). Importantly, this is a minimum estimate, because some nitrogen may have been lost from the clay minerals during metamorphism. The calculated concentrations are well within the range of modern biologically controlled pore waters (Fig. 2). However, it is unknown whether similar concentrations could have been achieved on an abiotic planet or whether they are indeed a biosignature. This study seeks to address this question with a numerical model of nitrogen sources and sinks.

TABLE 1. AMMONIUM CONCENTRATIONS FROM BIOTITE SEPARATES OF MICA SCHISTS FROM THE ISUA SUPRACRUSTAL BELT

Reference	*Sample*	*Biotite N (ppm)*
Honma, 1996	GGU167682	253
	GGU173019	234
	GGU172999	95
	GGU172997	59
	GGU173067	55
	GGU173140	54
	GGU173164	79
	GGU173153	68
Papineau *et al.*, 2005	GR97im45	298
	GR97im46	233
	GR97im47	288
	GR9817	428
Pinti *et al.*, 2001	SJM/IM/43	73

2. Model Description

A simple one-box model was built with the isee Stella modeling software (Fig. 3). The initial NH_4^+ concentration

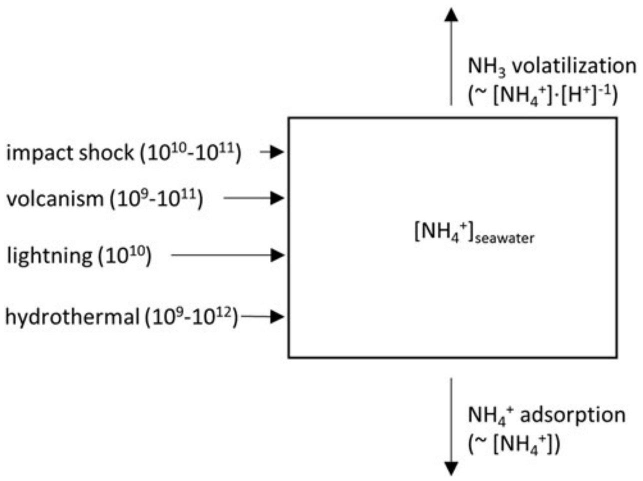

FIG. 3. Schematic of the abiotic box model. Sources (in mol/yr) and sinks are described in the text. Minor source fluxes ($\leq 10^9$ mol/yr) such as extraterrestrial delivery or photochemical reactions are left out.

of the ocean was set to zero; the model was run until the concentration had reached steady state. Source fluxes included hydrothermal input, impact shock heating, lightning, and volcanism. Hydrothermal N_2 reduction to NH_4^+ was varied from 10^9 mol/yr to 10^{12} mol/yr (Smirnov *et al.*, 2008), impact shock heating from 10^{10} mol/yr to 10^{11} mol/yr (Kasting, 1990), volcanism from 10^9 mol/yr to 10^{11} mol/yr (Mather *et al.*, 2004), and lightning was set to 10^{10} mol/yr (Kasting and Walker, 1981; Navarro-González *et al.*, 1998, 2001). Impacts, volcanism, and lightning produce NO_x instead of NH_4^+. It was assumed here that all NO_x is reduced to NH_4^+ during hydrothermal circulation or by reaction with ferrous iron (Summers and Chang, 1993; Brandes *et al.*, 1998). It is possible that some NO_x was instead reduced to N_2, but the relative proportions of NH_4^+ and N_2 production are unknown. The assumption of complete conversion to NH_4^+ is thus conservative and provides an upper bound of the NH_4^+ source flux. The model was run with all fluxes set to either their maximum or their minimum value to explore the full range of total NH_4^+ source fluxes. Further discussion of the source fluxes is given below.

The two abiotic sinks in the model were adsorption of NH_4^+ onto settling clay particles and dissociation of NH_4^+ to NH_3. NH_3 is volatile and can escape into the atmosphere, where it is rapidly photolyzed back to N_2 (Kuhn and Atreya, 1979). For clay adsorption, the settling flux was set to $5 \cdot 10^{15}$ g/yr (Li, 2000, p 326, assuming seafloor area of 3.6 km^2), which is in good agreement with average sedimentation rates of $4 \cdot 10^{15}$ g/yr on continental shelves (Gregor, 1985). The model is fairly insensitive to this parameter above pH 6, where NH_3 volatilization is the dominant N sink from the ocean. Here, an order-of-magnitude change in the clay flux changes the clay-bound N concentration by only a few percent. Below pH 6, a decrease in the clay flux has similarly small effects, whereas an increase leads to a significant drop in clay-bound N. If continental land masses were smaller in the early Archean, then the clay flux is more likely to have been smaller than today, meaning that the N concentration is overall not strongly affected by this parameter. The adsorption process itself was modeled after the work of Boatman and Murray (1982) as $N_{clay} = a_{NH4+}/([1/K_{ads}] + a_{NH4+}) \cdot \Gamma \cdot M_N$ (Fig. 2). Here, a_{NH4+} is the activity of NH_4^+, that is, the molar concentration multiplied by an activity coefficient of 0.57 for seawater. M_N is the molar mass of 14.01 g/mol for nitrogen. K_{ads} is the adsorption constant, which is 5.1 L/mol for average mud from the Saanich Inlet and 21.0 L/mol for kaolinite (Boatman and Murray, 1982). Other clay minerals fall close to, or within, this range (montmorillonite 11.7 L/mol, illite 14.8 L/mol, chlorite 3.8 L/mol). The constant Γ is the cation exchange capacity of clay, which is taken to be 0.96 mmol/g for average surface mud (Boatman and Murray, 1982). Deeper mud samples and some individual clay minerals tend to have lower values for Γ (down to 0.65 mmol/g), making this a conservative estimate. The adsorption isotherm of Boatman and Murray (1982) has not been calibrated for low pH, but other studies have observed a decrease in NH_4^+ adsorption with decreasing pH (Kithome *et al.*, 1999). This is expected, because mineral surfaces become progressively more positively charged with an increase of dissolved H^+ ions (Stumm and Morgan, 1996), leading to increasing repulsion of the positively charged NH_4^+ ion under more acidic conditions. Keeping adsorption constant across pH is thus again a conservative approach. NH_4^+ adsorption has been shown to decrease with increasing salinity (Hou *et al.*, 2003). The salinity of the Paleoarchean ocean is not well constrained but may have been 1.5–2 times higher than it is today (Knauth, 2005). Keeping salinity constant is therefore a conservative choice that may overestimate the abiotic NH_4^+ burial flux.

NH_3 volatilization is a function of pH: $(NH_3) = [(NH_4^+) \cdot K_{NH3}]/(H^+)$, where $K_{NH3} = 10^{-9.25}$ mol/L at standard conditions (Li *et al.*, 2012). It is assumed that NH_3 escape is limited by ocean circulation with an overturn rate of 1000 years as today. This is a conservative estimate, because NH_3 may have escaped more rapidly to the atmosphere if it was produced in shallow water.

3. Results

The major results of the model are depicted in Fig. 4 and summarized in Table 2. The concentration of ammonium in clay minerals shows a strong dependence on pH, because under abiotic conditions, pH-dependent NH_3 volatilization is a major sink of fixed nitrogen from the ocean. (As noted above, this is under the conservative assumption that NO_x is mostly reduced to NH_4^+ instead of N_2.) The higher the pH, the lower the concentration of dissolved NH_4^+ in seawater and the lower the abundance of adsorbed NH_4^+ in clay minerals. The results are also strongly dependent on the total source flux and the mineralogy of the clay mineral. Kaolinite with its higher adsorption constant (Boatman and Murray, 1982) tends to accumulate more nitrogen than other clay minerals. The original mineralogy of the clay minerals that preceded the Paleoarchean metasedimentary biotite grains is unknown. Overall, the dissolved NH_4^+ concentrations that are necessary to explain the nitrogen content of the

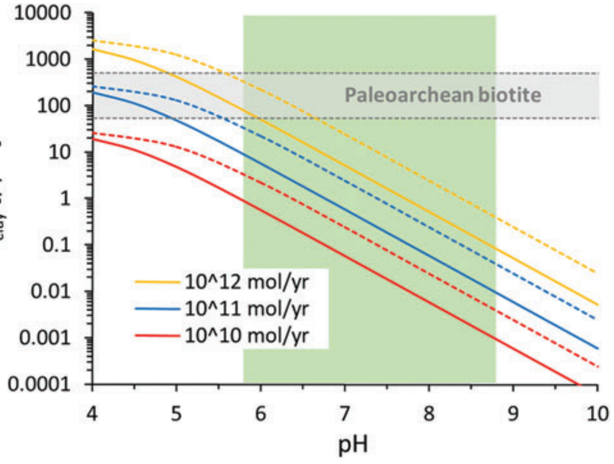

FIG. 4. Modeled N contents of clay as a function of pH and total N source flux. The most realistic source flux is between 10^{10} mol/yr and 10^{11} mol/yr, based on a combination of volcanism, impact shock heating, and lightning (see text). Colored solid lines = average mud; colored dashed lines = kaolinite. Gray horizontal bar = N contents of Paleoarchean biotite (Fig. 1); green vertical bar = modeled pH range for the Archean ocean (Grotzinger and Kasting, 1993). (Color graphics available at www.liebertonline.com/ast)

Table 2. Sink Fluxes, Dissolved NH_4^+ Concentrations, and Total N Concentration in Clay as Derived by the Box Model

pH	*NH_3 volatilization (mol/yr)*	*NH_4^+ adsorption (mol/yr)*	*Seawater NH_4^+ (mM)*	*Clay-bound N (ppm)*
Total source 10^{12} mol/yr:				
4	$3.7 \cdot 10^{11}$	$6.3 \cdot 10^{11}$	$4.9 \cdot 10^{1}$	$1.7 \cdot 10^{3}$
5	$8.4 \cdot 10^{11}$	$1.6 \cdot 10^{11}$	$1.1 \cdot 10^{1}$	$4.2 \cdot 10^{2}$
6	$9.8 \cdot 10^{11}$	$1.9 \cdot 10^{10}$	$1.3 \cdot 10^{0}$	$5.1 \cdot 10^{1}$
7	$1.0 \cdot 10^{12}$	$2.0 \cdot 10^{9}$	$1.3 \cdot 10^{-1}$	$5.2 \cdot 10^{0}$
8	$1.0 \cdot 10^{12}$	$2.0 \cdot 10^{8}$	$1.3 \cdot 10^{-2}$	$5.2 \cdot 10^{-1}$
9	$1.0 \cdot 10^{12}$	$2.0 \cdot 10^{7}$	$1.3 \cdot 10^{-3}$	$5.2 \cdot 10^{-2}$
10	$1.0 \cdot 10^{12}$	$2.0 \cdot 10^{6}$	$1.3 \cdot 10^{-4}$	$5.2 \cdot 10^{-3}$
Total source 10^{11} mol/yr:				
4	$3.8 \cdot 10^{10}$	$7.3 \cdot 10^{10}$	$5.0 \cdot 10^{0}$	$1.9 \cdot 10^{2}$
5	$9.3 \cdot 10^{10}$	$1.8 \cdot 10^{10}$	$1.2 \cdot 10^{0}$	$4.8 \cdot 10^{1}$
6	$1.1 \cdot 10^{11}$	$2.2 \cdot 10^{9}$	$1.4 \cdot 10^{-1}$	$5.7 \cdot 10^{0}$
7	$1.1 \cdot 10^{11}$	$2.2 \cdot 10^{8}$	$1.5 \cdot 10^{-2}$	$5.8 \cdot 10^{-1}$
8	$1.1 \cdot 10^{11}$	$2.2 \cdot 10^{7}$	$1.5 \cdot 10^{-3}$	$5.8 \cdot 10^{-2}$
9	$1.1 \cdot 10^{11}$	$2.2 \cdot 10^{6}$	$1.5 \cdot 10^{-4}$	$5.8 \cdot 10^{-3}$
10	$1.1 \cdot 10^{11}$	$2.2 \cdot 10^{5}$	$1.5 \cdot 10^{-5}$	$5.8 \cdot 10^{-4}$
Total source 10^{10} mol/yr:				
4	$3.7 \cdot 10^{9}$	$7.4 \cdot 10^{9}$	$5.0 \cdot 10^{-1}$	$1.9 \cdot 10^{1}$
5	$9.3 \cdot 10^{9}$	$1.8 \cdot 10^{9}$	$1.2 \cdot 10^{-1}$	$4.8 \cdot 10^{0}$
6	$1.1 \cdot 10^{10}$	$2.2 \cdot 10^{8}$	$1.4 \cdot 10^{-2}$	$5.7 \cdot 10^{-1}$
7	$1.1 \cdot 10^{10}$	$2.2 \cdot 10^{7}$	$1.5 \cdot 10^{-3}$	$5.8 \cdot 10^{-2}$
8	$1.1 \cdot 10^{10}$	$2.2 \cdot 10^{6}$	$1.5 \cdot 10^{-4}$	$5.8 \cdot 10^{-3}$
9	$1.1 \cdot 10^{10}$	$2.2 \cdot 10^{5}$	$1.5 \cdot 10^{-5}$	$5.8 \cdot 10^{-4}$
10	$1.1 \cdot 10^{10}$	$2.2 \cdot 10^{4}$	$1.5 \cdot 10^{-6}$	$5.8 \cdot 10^{-5}$

Data are for average mud from the Saanich Inlet (Boatman and Murray, 1982).

biotite grains by purely abiotic mechanisms can only be achieved if the total source flux of fixed nitrogen was on the order of 10^{12} mol/yr or higher, or if the pH of the ocean was less than 6.

4. Discussion

The results suggest that the observed N concentrations are most probably of biogenic origin for a number of reasons. First, an abiotic source flux of $>10^{11}$ mol/yr seems unlikely. High-temperature volcanism and impact shock heating of N_2 and CO_2 could potentially have maintained a fixed N flux of 10^{11} mol/yr (Kasting, 1990; Mather *et al.*, 2004); however, estimates of up to 10^{12} mol/yr for hydrothermal reduction of N_2 to NH_4^+ (Smirnov *et al.*, 2008) seem implausible. Even modern hydrothermal fluids from unsedimented vents that do not recycle sedimentary nitrogen are noticeably NH_4^+ poor ($<10\,\mu M$, von Damm, 1990; Lilley *et al.*, 1993), despite the fact that the modern ocean is rich in NO_3^-, which can also be reduced to NH_4^+ (Brandes *et al.*, 1998). Given hydrothermal fluid flux rates of $0.5–6 \cdot 10^{13}$ L/yr (Elderfield and Schultz, 1996; Emerson and Hedges, 2008), a hydrothermal NH_4^+ flux of more than 10^9 mol/yr from N_2 reduction is unrealistic. Other studies of the hydrothermal NH_4^+ flux, based on iron-sulfide minerals instead of iron-nickel alloys as a catalyst, derived much lower values (10^8 mol/yr) (Schoonen and Xu, 2001). The low NH_4^+ concentration of $<10\,\mu M$ in hydrothermal fluids further rules out post-depositional alteration of the biotite grains (*cf.* van Zuilen *et al.*, 2005). Nitrogen delivery by extraterrestrial material is thought to have been minor (10^3 to 10^9 mol/yr) (Chyba and Sagan, 1992; Smirnov *et al.*, 2008). The same is true for photochemical HCN production in the absence of biogenic CH_4 (10^9 mol/yr) (Tian *et al.*, 2011). The total source flux, therefore, probably did not exceed 10^{11} mol/yr. Importantly, this limit would be even lower if a significant portion of NO_x was reduced to N_2 instead of NH_4^+.

Second, assuming a total source flux of 10^{11} mol/yr or less, the model indicates that a pH of less than 5.5 would be necessary to explain the observed N enrichments in Paleoarchean biotite (Fig. 4). If the adsorption properties of the initial clay mineral were closer to that of average mud than to kaolinite, then a pH of less than 5 would be required. The pH of the Archean ocean is not well known, but model estimates by Grotzinger and Kasting (1993) suggest a range from roughly 5.8 to 8.8, depending on atmospheric pCO_2. If the nitrogen were completely abiotic, then it would imply a more CO_2-rich atmosphere than previously thought.

Lastly, the required concentrations of dissolved NH_4^+ are well within the range of modern anoxic pore waters (Fig. 2). They are thus plausibly explained by degradation of organic matter.

Isotopic data collected from Paleoarchean metasediments cannot unambiguously resolve this question, but they are consistent with a biological interpretation. Reported values range from −3‰ to +12‰ (Pinti *et al.*, 2001; Nishizawa *et al.*, 2005; Papineau *et al.*, 2005; van Zuilen *et al.*, 2005). Metamorphic overprint up to amphibolite facies has likely increased initial $^{15}N/^{14}N$ ratios through preferential ^{14}N loss (Haendel *et al.*, 1986). It is not possible to correct for the effect, but original $\delta^{15}N$ values were likely lower than measured. The isotopic composition of abiotically fixed N is not well known, but measurements suggest that NO_x produced by lightning may be isotopically light (–5‰ to −15‰, Ingerson, 1953; Moore, 1977), similar to photochemical reaction products (Kuga *et al.*, 2014). The isotopic effects of volcanic and impact-induced nitrogen fixation have not been determined, but the reaction pathway and energy input are perhaps comparable to lightning; hence one may speculate that the product is isotopically light as well. Hence, if nitrogen was fixed abiotically, then dissolved NH_4^+ may have started out with a light $\delta^{15}N$ value of $<-5‰$. Further fractionation may have occurred during NO_x reduction to NH_4^+ (McCready *et al.*, 1983), if the reduction did not go to completion. In the presence of life, a large proportion of this light NH_4^+ would likely have been assimilated into biomass. In a purely abiotic ocean, however, NH_3 volatilization would have been a major sink of fixed nitrogen from the ocean, especially above pH 6, and this process would have imparted a large isotopic fractionation, rendering residual NH_4^+ heavy ($\varepsilon = 45‰$ at standard conditions, Li *et al.*, 2012). It is conceivable that values from the heavier end of the observed spectrum in Paleoarchean metasediments reflect this process. However, none of the reported data points suggest fractionations up to 45‰. Instead, biological NH_4^+ uptake and burial, followed by metamorphic alteration to amphibolite facies, appears to be the more plausible mechanism. Biological N_2 fixation cannot be ruled out from these data.

5. Conclusion

Given our currently best constraints for abiotic source fluxes of fixed nitrogen through volcanism, lightning, impacts, and hydrothermal activity, and current model constraints on the pH of the Archean ocean, the nitrogen contents of the Paleoarchean biotite cannot plausibly be explained by abiotic adsorption of NH_4^+ from seawater. Instead, it is more likely that NH_4^+ was enriched in sedimentary pore waters due to degradation of organic matter, as it is today. The nitrogen in these biotite separates is thus a biosignature and evidence of biological nitrogen utilization at 3.8 Gyr. Although the concentration and isotopic data cannot resolve by which metabolism nitrogen was assimilated into biomass, that is, by N_2 fixation or by uptake of abiotic or biotic NH_4^+ or NO_3^-, the inference of biological involvement supports previous advocates of a relatively early origin of life on Earth dating back to at least the Paleoarchean (Mojzsis *et al.*, 1996; Rosing, 1999). This study further shows that nitrogen abundances can potentially serve as a useful biosignature on extraterrestrial missions, if source fluxes and solution pH can be estimated independently. For example, this proxy could prove useful in upcoming investigations of sediments on Mars.

Acknowledgments

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Address correspondence to:
Eva E. Stüeken
Department of Earth and Space
Sciences and Astrobiology Program
University of Washington
Seattle, WA 98195-1310

E-mail: evast@uw.edu

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