

## 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  $\delta^{15}\text{N}$ , 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  $\delta^{15}\text{N}$  records in ancient marine sediments, both in ancient anoxic (Archean) and more recent well oxygenated (Phanerozoic) environments.

## **1. Introduction – Nitrogen in Life**

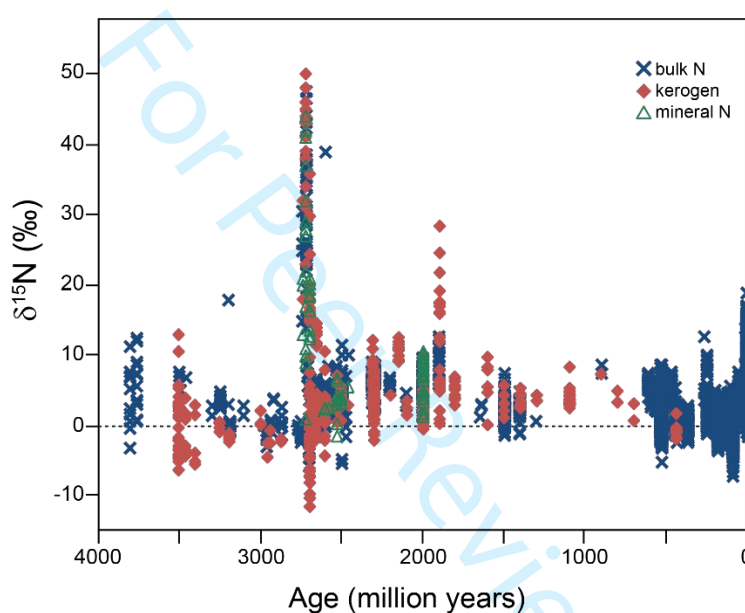
Nitrogen (N) is an essential nutrient for life, as it is critical in the formation of biomolecules, including nucleic acids and proteins. Despite an abundant reservoir of  $\text{N}_2$  in the atmosphere, only a limited number of micro-organisms (nitrogen-fixing organisms, or “diazotrophs”) have evolved the ability to directly assimilate di-nitrogen. All other organisms rely either on ammonium ( $\text{NH}_4^+$ ) or ammonia ( $\text{NH}_3$ ) 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 ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ).

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 ( $^{14}\text{N}$  and  $^{15}\text{N}$  – expressed as  $\delta^{15}\text{N}$  in ‰, and described below), since different nitrogen cycling reactions express different isotopic fractionations in  $\delta^{15}\text{N}$ . 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  $\delta^{15}\text{N}$  trends was published by Beaumont and Robert ([Beaumont and Robert, 1999](#)). These authors found a shift from  $\delta^{15}\text{N}$  values in kerogen centered around 0‰ in the Archean, to positive  $\delta^{15}\text{N}$  values centered around +5‰ from the Paleoproterozoic onwards. They suggested that these records were broadly indicative of an anaerobic  $\text{NH}_4^+$ -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  $\delta^{15}\text{N}$  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](#)).

44 Notwithstanding the general step-wise expansion of aerobic nitrogen cycling, nitrogen isotope studies  
 45 of sediments spanning Earth's history have revealed spatial and temporal nuances within this  
 46 narrative. For example, some  $\delta^{15}\text{N}$  trends in Late Archean sediments have been interpreted to  
 47 represent periods of temporally and spatially constrained aerobic N cycling prior to the GOE (Garvin  
 48 et al., 2009; Godfrey and Falkowski, 2009; Yang et al., 2019; Mettam et al., 2019). More recent  $\delta^{15}\text{N}$   
 49 records have identified periods of time when anaerobic nitrogen cycling dominated in the  
 50 Phanerozoic, including during the Latest Permian Extinction Event (LPEE; e.g., Saitoh et al., 2014)  
 51 and Cretaceous Ocean Anoxic Events (OAEs; e.g., Junium and Arthur, 2007).

52 Interpretations of these records, and the biogeochemical feedbacks they imply, critically rely on the  
 53 ability of sedimentary  $\delta^{15}\text{N}$  to reliably record the isotopic fingerprints of the coeval marine nitrogen  
 54 cycle. Here we discuss the state of the field in low-temperature N isotope biogeochemistry, including  
 55 susceptibility to syn- and post-depositional alteration, ambiguities with respect to interpretations, and  
 56 local versus global effects. Notably, we argue that stratigraphic and depositional context is crucial in  
 57 reliably interpreting nitrogen stable isotope records in ancient marine sediments.



58  
 59 **Figure 1.** Temporal trends in  $\delta^{15}\text{N}$  over Earth history, as preserved in the sedimentary rock record (updated  
 60 from Yang et al., 2019, and references therein).

61

## 62 2. The $\delta^{15}\text{N}$ Proxy in a Nutshell

63 Multiple biologically mediated redox transitions occur between nitrogen-containing compounds in the  
 64 marine N cycle. These transitions and their associated  $\delta^{15}\text{N}$  fractionations are summarised in Figure 2.  
 65 The  $\delta^{15}\text{N}$  values of nitrogen compounds are expressed relative to the isotopic composition of  
 66 atmospheric nitrogen, using the standard delta notation:

$$67 \quad \delta^{15}\text{N} (\text{‰}) = \left( \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{air}}} - 1 \right) \times 1000,$$

68 with isotopic fractionations for specific reactions shown as  $\epsilon \approx \delta^{15}\text{N}_{\text{product}} - \delta^{15}\text{N}_{\text{reactant}}$ .

69  $\text{N}_2$  fixation is the primary source of nitrogen input into the marine system and is regulated by the  
 70 availability of phosphorous and micro-nutrients, such as Mo and Fe (e.g., Zerkle et al., 2006).  
 71 Diazotrophs incorporate nitrogen into their biomass directly from atmospheric or dissolved  $\text{N}_2$ .  
 72 Remineralization of this biomass in the water column and in sediments releases bioavailable  
 73 ammonium or ammonia, which can in turn be assimilated by non-diazotrophic organisms.  $\text{N}_2$  fixation

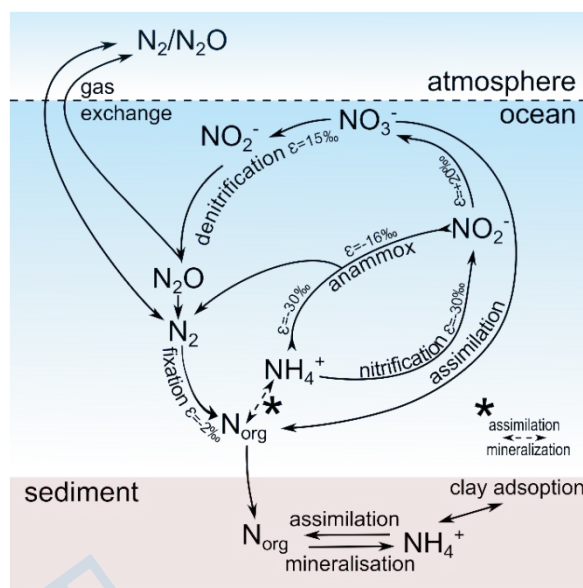
74 and the recycling of  $\text{NH}_4^+$  generally impart only small fractionations in  $\delta^{15}\text{N}$  ( $\epsilon \leq 2\text{‰}$ ). Therefore, in  
75 an environment where  $\text{N}_2$  fixation and the recycling of  $\text{NH}_4^+$  are the dominant N sources, the  $\delta^{15}\text{N}$  of  
76 biomass broadly reflects the atmospheric input value ( $\delta^{15}\text{N} \approx 0\text{‰} \pm 2\text{‰}$ ). However, if  $\text{NH}_4^+$  or other  
77 forms of dissolved inorganic nitrogen (DIN) are readily available, diazotrophy will be suppressed, as  
78  $\text{N}_2$  fixation is energetically expensive in comparison to assimilation.

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

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

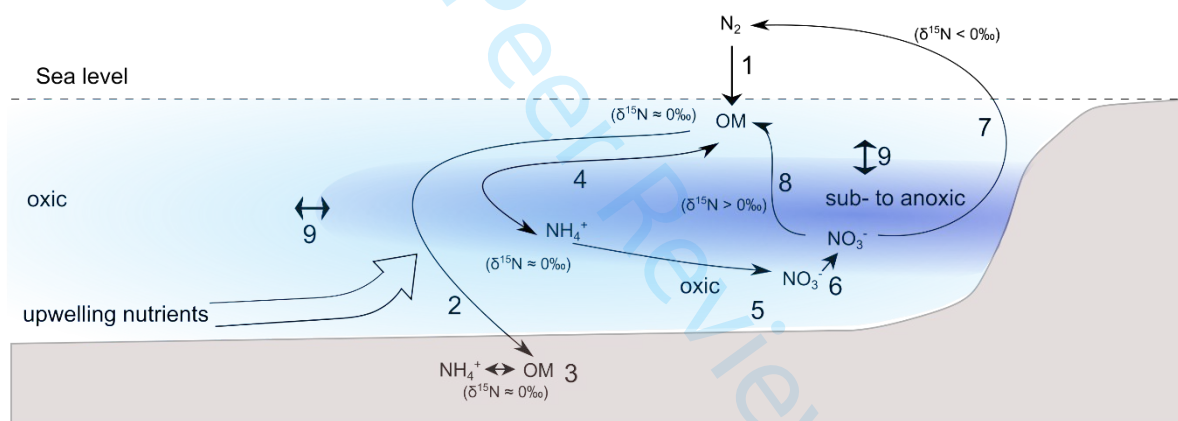
100 These N loss processes can all produce large fractionations in  $\delta^{15}\text{N}$  ( $\epsilon = +20$  to  $+30\text{‰}$ ; [Brunner et al.,](#)  
101 [2013](#); [Granger et al., 2008](#)). In marine sediments denitrification is generally quantitative such that no  
102 fractionations are expressed. However, in the water column, denitrification is constrained to low-  
103 oxygen settings such as oxygen minimum zones (OMZs; Figure 3.). This results in incomplete  
104 denitrification in the water column, leaving a residual pool of  $\text{NO}_3^-$  that carries a positive  $\delta^{15}\text{N}$  value.  
105 Anammox produces a similar fractionation effect for  $\delta^{15}\text{N}$ , but its role in the global N cycle and in the  
106 N isotope budget is somewhat less well-constrained and the relative contributions of anammox and  
107 canonical denitrification remain an area of active research.

108 The balance between  $\text{N}_2$  fixation and the assimilation of  $\text{NO}_3^-$  carrying the positive  $\delta^{15}\text{N}$  signature of  
109 denitrification/anammox controls the  $\delta^{15}\text{N}$  values of modern marine biomass. Today, aerobic N loss  
110 processes produce organic matter with an average  $\delta^{15}\text{N}$  of  $\sim +6\text{‰}$  ([Peters et al., 1978](#)). However, the  
111  $\delta^{15}\text{N}$  of particulate organic matter and underlying sediments is spatially and temporally  
112 heterogeneous, following subtle redox and productivity variations in the modern marine system (e.g.,  
113 [De Pol-Holz et al., 2009](#)). In addition, changes in the redox state and nutrient dynamics of marine  
114 settings over Earth's history have seemingly allowed for unique configurations of the nitrogen cycle  
115 that produce both highly positive and highly negative  $\delta^{15}\text{N}$  values not seen on Earth today (Figure 1).  
116 We explore some of these records, and their potential (largely non-unique) interpretations in the case  
117 studies below.



118

119 **Figure 2.** Major transformations in the marine nitrogen cycle, showing average values for fractionations  
 120 (adapted from Granger et al., 2008, Ader et al., 2016, and references therein). Fractionations not specifically  
 121 shown (e.g., for assimilation and mineralization), are generally 0‰.



122

123 **Figure 3.** Nitrogen cycling in a Phanerozoic Oxygen Minimum Zone (OMZ) (adapted from Pinti and  
 124 Hashizume, 2011). (1) Fixation of gaseous nitrogen ( $N_2$ ) into biomass (organic matter, OM) by diazotrophs; (2)  
 125 transport of OM to the sediments, i.e., via the biologic pump; (3) OM can be preserved in the sediments or  
 126 remineralized to  $NH_4^+$ , which can then be reassimilated into OM by benthic organisms or adsorbed onto clay  
 127 minerals; (4) Remineralization of OM to  $NH_4^+$  and reassimilation of  $NH_4^+$  in low-oxygen settings; (5) in oxic  
 128 settings  $NH_4^+$  is oxidised to  $NO_2^-$  and  $NO_3^-$ ; (6) upwelling of  $NO_3^-$  into low-oxygen setting; (7) partial  
 129 denitrification of  $NO_3^-$  (and reduction of  $NO_2^-$  by anammox) in sub- to anoxic settings releases  $N_2$  to  
 130 atmosphere. Partial denitrification renders the residual pool of  $NO_3^-$  isotopically heavy (+  $\delta^{15}N$ ); (8) assimilation  
 131 of this residual pool of  $NO_3^-$  contributes to the positive  $\delta^{15}N$  values of Phanerozoic OM; (9) the size of OMZs  
 132 can expand and contract enhancing or reducing denitrification and the resulting N isotope effects.

133

134

### 3. Methods and Limitations

135 Nitrogen isotopes are now routinely measured alongside carbon isotopes in sedimentary rocks. Most  
 136 recent studies have measured sedimentary  $\delta^{15}N$  using an elemental analyser coupled to an isotope  
 137 ratio mass spectrometer running in continuous flow mode (CF-EA-IRMS). This method is more  
 138 widely accessible and significantly less time consuming than previously developed offline methods



139 (see [Ader et al., 2016](#), for a comprehensive review). The CF-EA-IRMS method generally works well  
140 for sediments with high N contents ( $1\sigma$  of  $\pm 0.25\%$  for  $N > 700\text{ppm}$ ; [Bahlmann et al., 2010](#)).  
141 However, the low N content and high TOC/TN ratios in most Precambrian rocks can lead to some  
142 analytical challenges. In this case, additional steps are required to correct for blanks and ensure  
143 complete combustion to prevent isobaric interferences, e.g., with CO ([Beaumont et al., 1994](#)).

144 Several new methods have been developed in the past decade that also allow for accurate  $\delta^{15}\text{N}$   
145 measurements on samples with much lower abundances of N. The nano-EA-IRMS method ([Polissar  
146 et al., 2009](#)) uses a custom cryo-trap and chromatography system to concentrate the evolved  $\text{N}_2$  gas  
147 before analysis, decreasing the sample size requirements by several orders of magnitude. This method  
148 has been successfully applied to analyze  $\delta^{15}\text{N}$  values in deep time sediments (e.g., [Mettam et al.,  
149 2019](#); [Yang et al., 2019](#)), but blanks remain an issue and must be closely monitored and corrected for.  
150 Alternatively, samples can be run in triplicates of varying mass. These triplicates can then be plotted  
151 on 'Keeling' plots, with the true value calculated as the intercept with the y-axis (see [Mettam et al.,  
152 2019](#)). An off-line tube cracker combustion system has also been successfully applied as an  
153 introduction system to the CF-EA-IRMS to measure  $\delta^{15}\text{N}$  in low N abundance sediments (e.g.,  
154 [Stüeken et al., 2015a](#)), but requires very high vacuum. Ishida et al. (2018) recently described a method  
155 for measuring  $\delta^{15}\text{N}$  values in organic matter *in situ* using secondary ion mass spectrometry (SIMS),  
156 with analytical precision on the same order as bulk methods ( $2\sigma$  of  $\pm 0.56\%$ ). Spatially resolved  $\delta^{15}\text{N}$   
157 analyses should be useful in discerning  $\delta^{15}\text{N}$  at the single cell or organismal level (e.g., with  
158 Precambrian microfossils); however, the SIMS technique measures  $\delta^{15}\text{N}$  on the  $\text{CN}^-$  ion, so cannot be  
159 used to measure mineral  $\text{NH}_4^+$ , e.g., in clay minerals that don't contain carbon.

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

### 165 3.1 The Problem with Preservation

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

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

182 Nitrogen isotope values can be modified by syn-depositional degradation of organic matter within the  
183 sediments, either by aerobic or anaerobic respiration (e.g., sulphate reduction) ([Altabet and Francois,  
184 1994](#); [Macko and Estep, 1984](#)). However, these  $^{15}\text{N}$ -enrichments can be muted if the evolved  $\text{NH}_4^+$  is  
185 quantitatively reincorporated into new benthic biomass or adsorbed onto clay mineral phases

186 (Robinson et al., 2012). Potential loss of nitrogen during degradation can be qualitatively assessed by  
187 examining the ratios of organic carbon to total nitrogen (C/N ratios) in the sediments. The Redfield  
188 ratio for pristine marine phytoplankton is between ~4 and ~8 (Gao et al., 2012; Meyers, 1997).  
189 Sedimentary values below this lower threshold would indicate that a significant amount of carbon was  
190 lost during degradation, while liberated  $\text{NH}_4^+$  was largely captured and retained on clay surfaces.  
191 These considerations are complicated for many Precambrian sediments, where C/N ratios are  
192 extremely high ( $> 100$ ). In addition, the use of C/N ratios to infer preservation in Phanerozoic  
193 sediments can be complicated by mixing with terrestrial vegetation, which has a higher C/N ratio of  $>$   
194  $\sim 15$  (Gao et al., 2012; Meyers, 1997); such mixed provenance can potentially be untangled by coeval  
195 biomarker analyses, as discussed below.

196 Modification of sedimentary  $\delta^{15}\text{N}$  values by metamorphism is particularly well-documented, and  
197 generally increases with metamorphic grade. Thermal alteration results in the preferential liberation of  
198 the lighter stable isotope, which increases  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. These effects can be identified by a  
199 diagnostic positive correlation between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , and by negative correlations between total  
200 nitrogen (TN) and  $\delta^{15}\text{N}$ , or organic carbon (TOC) and  $\delta^{13}\text{C}$ . Stüeken et al. (2017) further proposed that  
201 C/H ratios in kerogen can be used as a proxy for thermal alteration in metamorphosed sediments.  
202 Regardless, metamorphic effects on  $\delta^{15}\text{N}$  are generally mild (1-2‰) at greenschist facies or below,  
203 thus the majority of sedimentary  $\delta^{15}\text{N}$  studies have focused on low metamorphic grade sediments.

### 204 3.2 The $\delta^{15}\text{N}_{\text{org}}$ versus $\delta^{15}\text{N}_{\text{bulk}}$ Debate

205 Issues surrounding post-burial preservation and alteration are also intrinsic to the debate over whether  
206  $\delta^{15}\text{N}$  values in bulk sediments ( $\delta^{15}\text{N}_{\text{bulk}}$ ) or in extracted organics ( $\delta^{15}\text{N}_{\text{org}}$ ) are a more reliable recorder  
207 of sedimentary organic nitrogen. The former includes a combination of nitrogen contained in organic  
208 matter and  $\text{NH}_4^+$  adsorbed onto clay minerals or substituted into minerals as a replacement for  
209 potassium ( $\text{K}^+$ ), while the latter represents the acid-resistant organic fraction. This debate stems from  
210 discrepancies between  $\delta^{15}\text{N}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{org}}$  values, which often show an offset between isotopically  
211 lighter  $\delta^{15}\text{N}_{\text{org}}$  and heavier  $\delta^{15}\text{N}_{\text{bulk}}$  within the same sample.

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

228 Proponents of analysing bulk sediments suggest that the offset between  $\delta^{15}\text{N}_{\text{org}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  from the  
229 same sample indicates that significant amounts of  $^{15}\text{N}$  must have been liberated from kerogen and  
230 ultimately captured on clays. Recent work by Stüeken et al. (2017) suggested that metamorphism  
231 drives organic  $\delta^{15}\text{N}$  values lighter and silicate-bound  $\delta^{15}\text{N}$  values heavier, resulting in a 3-4‰ offset at  
232 greenschist facies or lower. However, the mechanism(s) behind this offset remain unclear. If this  
233 difference occurs when  $\text{NH}_4^+$  is transferred from kerogen to clays during early diagenesis, it could

234 result from the preferential removal of isotopically heavy protein-derived organic matter from  
 235 kerogen or the preferential preservation of isotopically light porphyrins in the kerogen; if it occurs  
 236 during thermal maturation, it could be caused by changes in bonding as kerogen matures and clays  
 237 dewater (Stüeken et al., 2017).

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

252

253

#### 4. Case Studies

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

##### 268 4.1 Extreme $\delta^{15}\text{N}$ values at ~2.7 Ga – marine versus terrestrial signals?

269 Both extremely high and extremely low  $\delta^{15}\text{N}$  values have been measured in sediments from ~2.7 Ga  
 270 (Figure 1), and variably linked to global oxygenation and primary production.  $\delta^{15}\text{N}$  values as high as  
 271 +50‰ in the Tumbiana Formation (Fortescue Group, Western Australia) have been interpreted to  
 272 represent partial nitrification under conditions where ammonium was abundant and oxygen was  
 273 limited (Thomazo et al., 2011). This interpretation stems largely from laboratory incubations with  
 274 methanotrophic bacteria that produced extremely  $^{15}\text{N}$ -depleted  $\text{N}_2\text{O}$  (-46‰) during the co-oxidation  
 275 of ammonium with methane (Mandernack et al., 2009). The co-occurrence of these large  $\delta^{15}\text{N}$  values  
 276 with very low  $\delta^{13}\text{C}_{\text{org}}$  values in the Tumbiana Formation supports the incorporation of methane into  
 277 these sediments. However, this interpretation remains problematic for several reasons (e.g., Stüeken et  
 278 al., 2015b). Namely, in order for isotopic fractionations this large to be expressed in the residual  
 279  $\text{NH}_4^+$ , a large fraction of the available ammonium (> 70%) would have had to be oxidized. The  
 280 residual ammonium would still have been assimilated into biomass, while the resulting  $\text{NO}_2^-$  and  $\text{NO}_3^-$



281 would have been respired. Similarly, in order for the high  $\delta^{15}\text{N}$  values to have been preserved in the  
282 resulting organic matter, the product  $\text{NO}_2^-$  would have to be lost from the system (e.g., via  
283 denitrification and/or anammox) rather than re-assimilated. Stüeken et al. (2015b) alternatively  
284 suggested that the Tumbiana Formation and underlying Kylene Formation represent a lake system that  
285 was driven highly alkaline by aqueous alteration of underlying volcanics. In an anoxic alkaline lake  
286 setting, the volatilization of  $\text{NH}_4^+$  to ammonia ( $\text{NH}_3$ ) could produce large N isotope fractionations,  
287 similar to high  $\delta^{15}\text{N}$  values in alkaline lakes today.

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

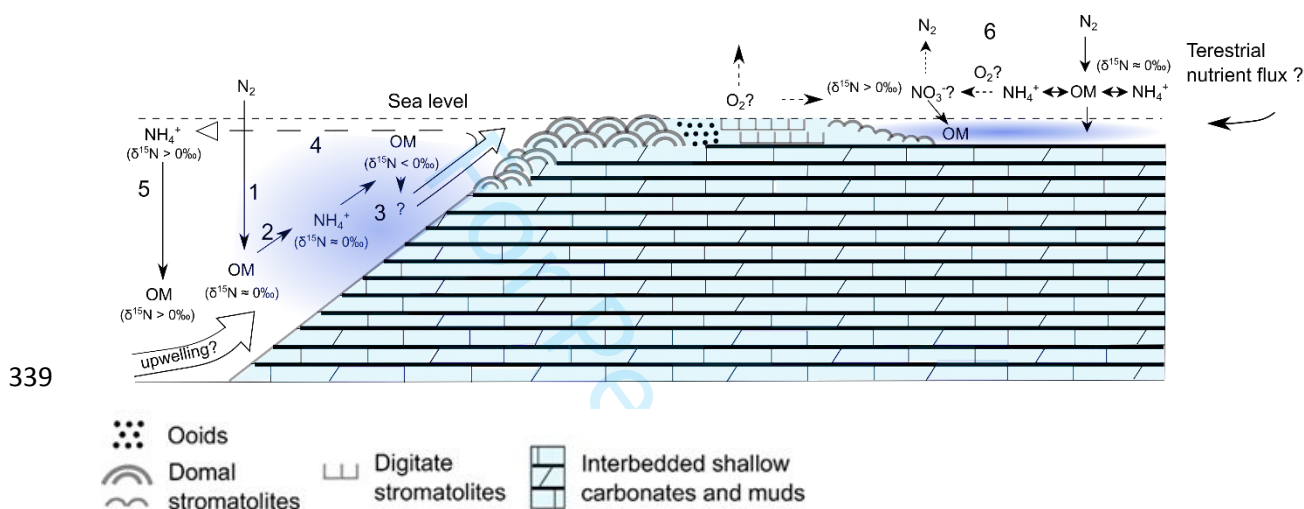
#### 299 4.2 Pre-GOE positive $\delta^{15}\text{N}$ values - alternatives to oxygen?

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

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

319  $\delta^{15}\text{N}$  values of ammonium in the Late Archean could also have been driven higher by partial  
320 assimilation, as described for  $\sim 2.7$  Ga sediments above (Ader et al., 2016). Until recently this  
321 interpretation was largely dismissed due to a lack of evidence for a correlative pool of  $^{15}\text{N}$ -depleted  
322 biomass. However, recent analyses of  $\sim 2.5$  Ga rocks have identified comparatively low  $\delta^{15}\text{N}$  values ( $\leq$   
323  $-4\text{‰}$ ) in shore-proximal sediments (Mettam et al., 2019). Combined with the  $\sim 2.7$  Ga data (Yang et  
324 al., 2019), these studies suggest that partial  $\text{NH}_4^+$  assimilation could have been widespread once  
325 oxygenic photosynthesis kick-started primary productivity in the Late Archean. These data  
326 additionally illustrate the potential for spatial and temporal heterogeneity within the global marine N  
327 cycle.

328 The study by Mettam et al. (2019) further demonstrates how depositional setting can play a key role  
 329 in spatially partitioning the nitrogen cycle, producing local variations in  $\delta^{15}\text{N}$  values. For example,  
 330  $\delta^{15}\text{N}$  values of  $-4\text{‰}$  were measured in sediments deposited from relatively deep shelf conditions,  
 331 where partial assimilation from a pool of upwelling  $\text{NH}_4^+$  from the open ocean could have occurred.  
 332 In contrast, sediments deposited in shallower, more restricted lagoonal conditions retained  $\delta^{15}\text{N}$  values  
 333 of  $\sim 0\text{‰}$ , likely reflecting diazotrophy and the efficient recycling of remineralized  $\text{NH}_4^+$ . Only a  
 334 handful of positive  $\delta^{15}\text{N}$  values greater than  $2\text{‰}$  were reported from the same section, hinting at the  
 335 possible presence of coupled nitrification and incomplete denitrification (Figure 4). However, these  
 336 positive values were from shallow-water, carbonate-rich facies, probably indicating very localised  
 337 oxygenated settings. This spatial complexity highlights the importance of sedimentological and  
 338 depositional context for understanding local variations in  $\delta^{15}\text{N}$  data.



340

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

349

#### 350 4.2 The Late Permian Extinction Event – multiple controls on $\delta^{15}\text{N}$ ?

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

357 Decreases of  $\sim 1\text{‰}$  in  $\delta^{15}\text{N}$  values in late Permian Boreal and Panthalassic waters have also been  
 358 reported (Algeo et al., 2012; Grasby et al., 2015; Knies et al., 2013; Schoepfer et al., 2012). However,  
 359 with the exception of one study (Schoepfer et al., 2012), these changes are small and  $\delta^{15}\text{N}$  values  
 360 never fall below  $+4\text{‰}$ . The persistence of positive  $\delta^{15}\text{N}$  values indicate that these waters probably  
 361 maintained a robust  $\text{NO}_3^-$  inventory, which would have precluded an expansion in diazotrophy.

362 Small decreases in  $\delta^{15}\text{N}$  values in LPEE sediments could be caused by several other factors. As  
363 described above, differing rates of organic matter degradation tied to redox changes can influence the  
364  $\delta^{15}\text{N}$  values of organic matter during deposition, particularly where fluxes of organic matter are low  
365 (Freudenthal et al., 2001; Möbius et al., 2010). Such effects are illustrated by LPEE data from  
366 Schuchert Dal, East Greenland (Mettam et al., 2017). C/N ratios in these sediments never exceed 6,  
367 indicating little variation in organic matter provenance (e.g., due to terrestrial input). However, higher  
368  $\delta^{15}\text{N}$  values and lower C/N ratios correspond with oxic, bioturbated depositional horizons, while  
369 lower  $\delta^{15}\text{N}$  values and higher C/N ratios are recorded in oxygen deficient, laminated horizons. Given  
370 these observations, a wholesale transition from oxic to anoxic conditions during the LPEE coupled  
371 with slower syn-depositional degradation could also contribute to decreases in  $\delta^{15}\text{N}$  values at some  
372 sites during and after the LPEE.

373 The complexity of interpreting sedimentary nitrogen isotope values is further highlighted by  $\delta^{15}\text{N}$  data  
374 from Buchanan Lake, Canada, which was part of the Sverdrup Basin on the northwest margin of  
375 Pangea during the LPEE (Knies et al., 2013). These authors concluded that increased diazotrophy was  
376 likely responsible for a small decrease in  $\delta^{15}\text{N}$  (from  $\sim+9\%$  to  $\sim+8\%$ ) in these sediments. However,  
377 persistently positive  $\delta^{15}\text{N}$  values throughout this section suggest continued nitrate availability despite  
378 evidence for an intensification of anoxia. A fall in the rate of organic matter degradation associated  
379 with enhanced anoxia could be responsible for the small fall in  $\delta^{15}\text{N}$ ; however, this scenario is  
380 inconsistent with a coeval decrease in C/N ratios. An alternate explanation could come from increased  
381 weathering and transport of exposed soils and terrestrial vegetation, consistent with the  
382 reorganisation of terrestrial ecosystems during the LPEE (Algeo and Twitchett, 2010; Looy et al.,  
383 2001). Increased fluxes of terrestrial organic matter are unlikely to be the cause of the decrease in  
384  $\delta^{15}\text{N}$ , as this would most likely increase C/N ratios as well. Alternatively, increased fluxes of clay-  
385 adsorbed inorganic nitrogen from the depositional hinterland could change  $\delta^{15}\text{N}$  whilst reducing C/N  
386 ratios, as seen at this site. Given these alternative arguments, an increase in marine diazotrophy is a  
387 reasonable, although perhaps not completely unique, interpretation.

388

## 5. Future Perspectives

389 These case studies highlight the potential complexity of the sedimentary  $\delta^{15}\text{N}$  record and some of the  
390 uncertainties associated with interpreting  $\delta^{15}\text{N}$  values in deep time. In particular, the paucity of well-  
391 preserved Archean sediments and inherent analytical limitations mean that the majority of  $\delta^{15}\text{N}$   
392 studies have focused on organic carbon-rich sediments deposited in low energy, deeper water settings.  
393 These studies provide important constraints on local nitrogen cycling processes occurring in these  
394 basins. However, the spatial heterogeneity of the marine N cycle, along with the ability of disparate N  
395 cycling processes to produce overlapping  $\delta^{15}\text{N}$  values, suggests that depositional and redox context is  
396 critical in interpreting the resulting  $\delta^{15}\text{N}$  records, and in unravelling local versus global signals. The  
397 interpretation of Phanerozoic  $\delta^{15}\text{N}$  records should similarly be approached with caution given the  
398 multiple factors that can modify or overprint the isotopic characteristics of primary marine organic  
399 matter. For example, redox variations can also influence organic matter degradation rates in the  
400 sediments. Furthermore, the evolution of land plants during the Palaeozoic provide a source of  
401 organic matter with distinct  $\delta^{15}\text{N}$  values and C/N ratios, and the transport of these materials to the  
402 sediments, along with nitrogen in eroded soils, can lead to mixing of isotope signals that complicate  
403 interpretations. Additional targets for future and ongoing development include:

404 1. *Analytical advancements* – New methods are being developed to characterize the distribution of  
405  $\delta^{15}\text{N}$  values in low N abundance sediments and to identify post-depositional alteration. In particular,  
406 recent advances in *in situ*  $\delta^{15}\text{N}$  analyses via SIMS will allow for direct analyses of  $\delta^{15}\text{N}$  in organic  
407 nitrogen (Ishida et al., 2018), which should allow for single-cell  $\delta^{15}\text{N}$  analyses in Precambrian  
408 microfossils.

409 2. *Field studies* – Modern anoxic aqueous environments, such as redox-stratified lakes and seas, can  
 410 provide near analogues to Precambrian marine systems. Thus  $\delta^{15}\text{N}$  values of aqueous and sedimentary  
 411 N compounds in these systems can be linked directly to N cycling processes, providing important  
 412 insights into sedimentary  $\delta^{15}\text{N}$  values preserved in ancient sediments (e.g., [Fulton et al., 2018](#)).

413 3. *Laboratory studies* – N isotopic fractionations produced during cyanobacterial  $\text{N}_2$  fixation and  
 414 denitrification are generally well calibrated for modern conditions ([Boursachs et al., 2009](#); [Granger et](#)  
 415 [al., 2008](#)), but life and environments have evolved significantly over Earth history. Past conditions  
 416 could have promoted different N cycling processes, by different groups of organisms and/or the same  
 417 organisms utilizing different enzymes, but the resulting isotopic fractionations are largely  
 418 unconstrained (e.g., [Nishizawa et al., 2014](#)). Incomplete ammonium assimilation in particular has  
 419 been implicated in contributing to Late Archean  $\delta^{15}\text{N}$  records, but our understanding of  $\delta^{15}\text{N}$   
 420 fractionations during this process, and their response to changing environmental conditions are  
 421 extremely limited ([Hoch et al., 1992](#)).

422 Combined, these types of inter-disciplinary studies could make the forward strides necessary for  
 423 generating and understanding sedimentary  $\delta^{15}\text{N}$  records throughout Earth history.

424

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