



Deposited via The University of Leeds.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/108920/>

Version: Accepted Version

Article:

Zerkle, AL, Poulton, SW, Newton, RJ et al. (2017) Onset of the aerobic nitrogen cycle during the Great Oxidation Event. *Nature*, 542 (7642). pp. 465-467. ISSN: 0028-0836

<https://doi.org/10.1038/nature20826>

© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved. This is an author produced version of a paper published in *Nature*. Uploaded in accordance with the publisher's self-archiving policy.

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1
2
3
4 **Onset of the aerobic nitrogen cycle during the Great Oxidation Event**
5
6

7 Aubrey L. Zerkle^{1*}, Simon W. Poulton², Robert J. Newton², Colin Mettam¹, Mark W.
8 Claire^{1,3}, Andrey Bekker⁴, and Christopher K. Junium⁵
9

10 ¹*Department of Earth and Environmental Sciences and Centre for Exoplanet Science,*
11 *University of St Andrews, St Andrews, KY16 9AL, Scotland, UK*
12

13 ²*School of Earth & Environment, University of Leeds, Leeds, LS2 9JT, England, UK*
14

15 ³*Blue Marble Space Institute of Science, P.O. Box 88561, 98145, Seattle, WA, USA*
16

17 ⁴*Department of Earth Sciences, University of California-Riverside, Riverside, CA, 92521,*
18 *USA*
19

20 ⁵*Department of Earth Sciences, Syracuse University, Syracuse, NY, 13244, USA*
21
22
23
24
25

26 resubmitted to *Nature* on 17th November, 2016

27 *corresponding author az29@st-andrews.ac.uk

The rise of oxygen on early Earth (~2.4 Ga ago)¹ caused a reorganization of marine nutrient cycles^{2,3}, including that of nitrogen, which is important for controlling global primary productivity. However, current geochemical records lack the temporal resolution to directly address the nature and timing of the biogeochemical response to oxygenation⁴. Here we couple records of ocean redox chemistry with nitrogen isotope ($\delta^{15}\text{N}$) values from ~2.31 billion-year-old shales⁵ of the Rooihogte and Timeball Hill formations in South Africa deposited during the early stages of Earth's first rise in atmospheric oxygen⁶. Our data fill a ~400 million-year gap in the temporal $\delta^{15}\text{N}$ record⁴ and provide evidence for the first pervasive aerobic marine nitrogen cycle. The interpretation of our nitrogen isotope data in the context of Fe speciation and carbon isotope data suggests biogeochemical cycling across a dynamic redox boundary, with primary productivity fuelled by chemoautotrophic production and a nitrogen cycle dominated by nitrogen loss processes utilizing newly available marine oxidants. This chemostratigraphic trend constrains the onset of widespread nitrate availability associated with ocean oxygenation. The rise of marine nitrate could have allowed for the rapid diversification and proliferation of nitrate-utilizing cyanobacteria and, potentially, eukaryotic phytoplankton.

48 Nitrogen (N) is an essential element for all living organisms, required alongside
49 carbon (C) and phosphorus (P) for the formation of nucleic acids and proteins. As a result, N
50 and P are the principal limiting nutrients controlling autotrophic CO₂ fixation, which in turn
51 regulates climate, weathering, and the redox state of Earth's surface on geologic timescales.

52 The marine nitrogen cycle is driven largely by biological processes. The primary
53 source of N to the biosphere is nitrogen fixation, the conversion of atmospheric N₂ to organic
54 nitrogen in its bioavailable form (ammonium, NH₄⁺). In the modern oceans, ammonium is
55 oxidized via the stepwise process of nitrification, producing nitrite (NO₂⁻) and nitrate (NO₃⁻).
56 Nitrate (and nitrite) can be assimilated into organic matter, by both oxygenic
57 photoautotrophic bacteria (cyanobacteria) and eukaryotic phytoplankton. Fixed nitrogen is
58 mostly recycled in the water column, but some sinks to the sediments where it is buried
59 and/or remineralized. Some bioavailable nitrogen in the modern oceans is returned to the
60 atmosphere as N₂ via denitrification (the reduction of NO₃⁻) and anaerobic ammonium
61 oxidation (anammox, the oxidation of NH₄⁺ with NO₂⁻) in oxygen-minimum zones⁷.

62 Each of these transformations can affect the ratio of nitrogen isotopes ($\delta^{15}\text{N} =$
63 $(^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{atmospheric N}_2} - 1$, measured in permil, ‰), producing fractionations
64 between reactant and product N species⁸. Nitrogen fixation produces small negative
65 fractionations from atmospheric N₂, resulting in organic $\delta^{15}\text{N}$ values of -4 to 0‰⁹.
66 Denitrification and anammox preferentially return the lighter isotope to the atmosphere,
67 leaving the residual nitrate and nitrite enriched in ¹⁵N by 10-25‰^{8,10}. Large fractionations can
68 also be produced by nitrification and biological assimilation¹¹ (and possibly by dissimilatory
69 nitrate reduction to ammonium¹²). However, these fractionations are not expressed in most
70 modern environments, since nitrification and the recycling of fixed N compounds occur
71 rapidly and nearly quantitatively. Hence, nitrogen loss via denitrification and anammox
72 dominates the modern nitrogen isotope signal, resulting in sedimentary organic matter with

73 average $\delta^{15}\text{N}$ values of 7‰¹³, due to the uptake of ^{15}N -enriched residual nitrate by primary
74 producers.

75 Beaumont and Robert² were the first to suggest a secular trend in the nitrogen isotope
76 values of organic N for Archean and early Proterozoic sediments. They noted that the $\delta^{15}\text{N}$ of
77 kerogen in Archean cherts centered at ~0‰ (ranging from -6 to +13‰), while the $\delta^{15}\text{N}$ of
78 early Proterozoic kerogens centered at ~5‰, with a total range similar to that in Phanerozoic
79 sediments (~0 to 10‰) (Fig. 1). Our statistical treatment of the temporal $\delta^{15}\text{N}$ record
80 (Extended Data Fig. 1) alongside more recent compilations¹⁴ supports this shift, which occurs
81 broadly coeval with the Great Oxidation Event (GOE) from 2.45-2.32 billion-years-ago
82 (Ga)^{1,15}, although its precise timing remains poorly constrained. As such, the secular rise in
83 $\delta^{15}\text{N}$ is commonly interpreted to reflect the transition from an anaerobic nitrogen cycle
84 dominated by reduced N species (N_2 and NH_4^+), to a modern-style aerobic nitrogen cycle
85 with nitrate as a significant component of dissolved inorganic nitrogen. Small (~2 to 5‰)
86 positive excursions in $\delta^{15}\text{N}$ within older (~2.6-2.5 Ga) sedimentary rocks have been
87 interpreted to represent the temporary onsets of nitrification/denitrification during transient or
88 localized oxygenation events, which were apparently not sufficiently widespread or long-
89 lived for the signal to persist^{16,17}. Alternatively, these small and short-lived positive shifts in
90 $\delta^{15}\text{N}$ recorded exclusively in deep-water facies could reflect the incorporation of ^{15}N -enriched
91 NH_4^+ produced by partial nitrification, assimilation of ^{15}N -depleted NH_4^+ in shallow waters,
92 or nitrogen redox cycling independent of surface oxygenation^{4,18,19}. To date, however, no
93 records of contemporaneous shallow-water sediments linked directly to records of ocean or
94 atmospheric oxygenation have been available to test these alternatives.

95 Here we examine the response of the nitrogen cycle to changing atmosphere and
96 ocean redox conditions during deposition of ~2.31 Ga siliciclastic rocks, filling a ~400
97 million-year gap in the temporal $\delta^{15}\text{N}$ record (Fig. 1), in sediments contemporaneous with the

early stages of the GOE. We focus our analyses on the Rooihoogte and Timeball Hill (R-TH) formations, present in drill core EBA-2 in the Potchefstroom Synclinorium, South Africa (Extended Data Fig. 2 and 3). The R-TH form the basal part of the Pretoria Group in the Transvaal basin, and were deposited on a palaeo-delta slope open to the ocean¹. U-Pb zircon ages for the tuffs in the lower TH give an age of 2.310 ± 0.009 Ga⁵. Atmospheric oxygen content is constrained by the transition from mass-independent to mass-dependent fractionation of sulfur isotopes recently placed within shales of the Rooihoogte Formation⁶ (Fig. 2), indicating a rise in atmospheric O₂ levels to greater than 1 ppm²⁰. $\delta^{34}\text{S}$ data for sedimentary sulfides in the R-TH also indicate a significant rise in seawater sulfate^{1,6}, consistent with an increase in oxidative weathering of sulfide minerals on the continents. Additional sample information and discussion of post-depositional alteration is available in the Methods and Extended Data Figures 4 and 5.

We used a well-established sequential iron extraction technique²¹ (the ratio of highly reactive to total Fe, $\text{Fe}_{\text{HR}}/\text{Fe}_{\text{T}}$, and the ratio of Fe in pyrite to highly reactive Fe, $\text{Fe}_{\text{Py}}/\text{Fe}_{\text{HR}}$) to assess the redox state of the water column during R-TH deposition. Large variations in iron speciation indicate highly dynamic seawater redox conditions during deposition of the Rooihoogte and the lower ~20 m of the TH formations (Fig. 2), with fluctuations between oxic, ferruginous (anoxic and Fe(II)-rich), and euxinic (anoxic and sulfide-rich) states. The rapid changes in water column chemistry suggest that deposition occurred close to a redox interface (chemocline) between oxygenated surface-waters and anoxic deep-waters that were episodically driven euxinic, possibly by variations in organic carbon delivery or seawater sulfate availability. These data also imply the existence of a transiently sulfidic shelf underlying an oxygenated surface ocean, similar to the redox stratification suggested for the Late Archean²². Fluctuations in Fe speciation records are accompanied by a significant increase in total organic carbon (from <1 to ~4%) and a decrease in $\delta^{13}\text{C}_{\text{org}}$ (from -32 to -

36‰) across the R-TH boundary (Fig. 2), consistent with chemoautotrophic carbon fixation at or near a chemocline²³.

The $\delta^{15}\text{N}$ of both bulk nitrogen, $\delta^{15}\text{N}_{\text{bulk}}$, and extracted kerogen, $\delta^{15}\text{N}_{\text{org}}$, show a high degree of variability across this same interval (Fig. 2). When interpreted within the context of the Fe speciation data, these values are consistent with a marine nitrogen cycle developed across a dynamic redox boundary. $\delta^{15}\text{N}$ values of $6.0 \pm 0.5\text{‰}$ in the lower part of the section are similar to those of modern marine organic matter¹³, which reflect a nitrogen cycle dominated by N loss via denitrification and anammox in oxygen minimum zones²⁴. Nitrogen isotope values vary from 1.4 to 12‰ across the R-TH boundary, consistent with a variable input from similar chemotrophic communities across a shifting redox interface. These changes could reflect imbalances in ammonium supply and nitrification-denitrification resulting from periodic upwelling of nutrients and high organic productivity. On a stratified Paleoproterozoic marine shelf, uptake of ammonium from anoxic deep waters would have produced ^{15}N -depleted biomass just below the chemocline. Nitrification with newly available marine oxidants would have further enriched residual ammonium in ^{15}N across the redox interface. Higher $\delta^{15}\text{N}$ in oxygenated shallow waters could result from the uptake of this ^{15}N -enriched ammonium, or by nitrate assimilation once nitrate levels rose high enough to support partial denitrification. The $\delta^{15}\text{N}$ stabilizes at near modern values ($7.2 \pm 1.0\text{‰}$) up-section in the lower TH, in association with Fe speciation data indicative of the onset of pervasively oxygenated shallower water conditions. Oxygenation of surface waters would have supported widespread nitrification and further enhanced nitrate availability.

Notably, within the context of the global $\delta^{15}\text{N}$ record (Fig. 1), the R-TH succession records the first clear evidence for a long-lived aerobic nitrogen cycle in the sedimentary record. The ~2.31 Ga R-TH section, deposited at the heart of the GOE and coincident with the permanent loss of mass independent S isotope fractionation⁶, is bracketed by evidence for

only transient aerobic nitrogen cycling in older sediments (from ~2.7 to 2.5 Ga^{16,17,19}), and the clear isotopic imprint of aerobic nitrogen cycling in records from younger sediments deposited after ~2.0 Ga²⁵⁻²⁸, as confirmed by statistical analysis of the global database (Extended Data Fig. 1). Available data suggests that earlier transient oxygenation events were insufficient to establish the modern nitrogen cycle, as marine nitrate was not pervasive in the oceans before the GOE^{16,17}. In addition, $\delta^{15}\text{N}$ values > 2‰ are typical for the remainder of the Precambrian record (Fig. 1), indicating that aerobic nitrogen cycling became at least locally widespread enough to impart a long-lived isotopic imprint on marine $\delta^{15}\text{N}$ during the GOE.

The build-up of a significant marine nitrate reservoir would have provided an important evolutionary driver, as prokaryotes and eukaryotic phytoplankton that were able to utilize nitrate as a primary nutrient source could have diversified to fill this new ecological niche. The co-occurrence of this event with other geochemical changes indicative of the first significant oxygenation of Earth's atmosphere provides a crucial constraint on the surprisingly rapid response time of the global biosphere to this major transition in Earth surface chemistry.

References

- 1 Bekker, A. *et al.* Dating the rise of atmospheric oxygen. *Nature* **427**, 117-120 (2004).
- 2 Beaumont, V. & Robert, F. Nitrogen isotope ratios of kerogens in Precambrian cherts: a record of the evolution of atmosphere chemistry? *Precambrian Research* **96**, 63-82 (1999).
- 3 Bekker, A. & Holland, H. D. Oxygen overshoot and recovery during the early Paleoproterozoic. *Earth and Planetary Science Letters* **317-318**, 295-304 (2012).
- 4 Stueken, E. E., Kipp, M. A., Koehler, M. C. & Buick, R. The evolution of Earth's biogeochemical nitrogen cycle. *Earth-Science Reviews* **160**, 220-239 (2016).

173 5 Rasmussen, B., Bekker, A. & Fletcher, I. R. Correlations of Paleoproterozoic
174 glaciations based on U-Pb zircon ages for tuff beds in the Transvaal and Huronian
175 Supergroups. *Earth and Planetary Science Letters* **382**, 173-180 (2013).

176 6 Luo, G. *et al.* Rapid oxygenation of Earth's atmosphere 2.33 billion years ago. *Science*
177 *Advances* **2**, 1-9 (2016).

178 7 Dalsgaard, T., Thamdrup, B., Farias, L. & Revsbech, N. P. Anammox and
179 denitrification in the oxygen minimum zone of the eastern South Pacific. *Limnology*
180 *and Oceanography* **57**, 1331-1346 (2012).

181 8 Wada, E. in *Isotope Marine Chemistry* (eds E. D. Goldberg, Y. Horibe, & K.
182 Saruhashi) 375-398 (Uchida Rokakuho, 1980).

183 9 Zerkle, A. L., Junium, C. K., Canfield, D. E. & House, C. H. Production of ¹⁵N-
184 depleted biomass during cyanobacterial N₂-fixation at high Fe concentrations.
185 *Journal of Geophysical Research* **113** (2008).

186 10 Brunner, B. *et al.* Nitrogen isotope effects induced by anammox bacteria. *Proceedings*
187 *of the National Academy of Sciences* **110**, 18994-18999 (2013).

188 11 Hoch, M. P., Fogel, M. L. & Kirchman, D. L. Isotope fractionation associated with
189 ammonium uptake by a marine bacterium. *Limnology and Oceanography* **37**, 1447-
190 1459 (1992).

191 12 McCready, R. G. L., Gould, W. D. & Barendregt, R. W. Nitrogen isotope
192 fractionation during the reduction of NO₃⁻ to NH₄⁺ by *Desulfovibrio* sp. *Canadian*
193 *Journal of Microbiology* **29**, 231-234 (1983).

194 13 Peters, K. E., Sweeney, R. E. & Kaplan, I. R. Correlation of carbon and nitrogen
195 stable isotope ratios in sedimentary organic matter. *Limnology and Oceanography* **23**,
196 598-604 (1978).

197 14 Ader, M. *et al.* Interpretation of the nitrogen isotopic composition of Precambrian
198 sedimentary rocks: Assumptions and perspectives. *Chemical Geology* **429**, 93-110
199 (2016).

200 15 Farquhar, J., Zerkle, A. L. & Bekker, A. Geological constraints on the origin of
201 oxygenic photosynthesis. *Photosynthesis Research* **107**, 11-36 (2011).

202 16 Garvin, J., Buick, R., Anbar, A. D., Arnold, G. L. & Kaufman, A. J. Isotopic evidence
203 for an aerobic nitrogen cycle in the latest Archean. *Science* **323**, 1045-1048 (2009).

204 17 Godfrey, L. V. & Falkowski, P. G. The cycling and redox state of nitrogen in the
205 Archean ocean. *Nature Geoscience*, doi:DOI: 10.1038/NGEO633 (2009).

206 18 Thomazo, C., Ader, M. & Philippot, P. Extreme ¹⁵N-enrichments in 2.72-Gyr-old
207 sediments: evidence for a turning point in the nitrogen cycle. *Geobiology* **9**, 107-120
208 (2011).

209 19 Busigny, V., Lebeau, O., Ader, M., Krapez, B. & Bekker, A. Nitrogen cycle in the
210 Late Archean ferruginous ocean. *Chemical Geology* **362**, 115-130 (2013).

211 20 Pavlov, A. A. & Kasting, J. F. Mass-independent fractionation of sulfur isotopes in
212 Archean sediments: Strong evidence for an anoxic Archean atmosphere. *Astrobiology*
213 **2**, 27-41 (2002).

214 21 Poulton, S. W. & Canfield, D. E. Development of a sequential extraction procedure
215 for iron: implications for iron partitioning in continentally derived particulates.
216 *Chemical Geology* **214**, 209-221 (2005).

217 22 Reinhard, C. T., Raiswell, R., Scott, C., Anbar, A. D. & Lyons, T. W. A late Archean
218 sulfidic sea stimulated by early oxidative weathering of the continents. *Science* **326**,
219 713-716 (2009).

220 23 Coetzee, L. L., Beukes, N. J., Gutzmer, J. & Kakegawa, T. Links of organic carbon
221 cycling and burial to depositional depth gradients and establishment of a snowball

- 222 Earth at 2.3Ga. Evidence from the Timeball Hill Formation, Transvaal Supergroup,
223 South Africa. *South African Journal of Geology* **109**, 109-122 (2006).
- 224 24 De Pol-Holz, R., Robinson, R. S., Hebbeln, D., Sigman, D. M. & Ulloa, O. Controls
225 on sedimentary nitrogen isotopes along the Chile margin. *Deep-Sea Research Part II-
226 Topical Studies in Oceanography* **56**, 1100-1112, doi:10.1016/j.dsr2.2008.09.014
227 (2009).
- 228 25 Godfrey, L. V., Poulton, S. W., Bebout, G. E. & Fralick, P. W. Stability of the
229 nitrogen cycle during development of sulfidic water in the redox-stratified late
230 Paleoproterozoic Ocean. *Geology* **41**, 655-658 (2013).
- 231 26 Stueken, E. E. A test of the nitrogen-limitation hypothesis for retarded eukaryote
232 radiation: Nitrogen isotopes across a Mesoproterozoic basinal profile. *Geochimica et
233 Cosmochimica Acta* **120**, 121-139 (2013).
- 234 27 Papineau, D. *et al.* High primary productivity and nitrogen cycling after the
235 Paleoproterozoic phosphogenic event in the Aravalli Supergroup, India. *Precambrian
236 Research* **171**, 37-56, doi:10.1016/j.precamres.2009.03.005 (2009).
- 237 28 Kump, L. R. *et al.* Isotopic evidence for massive oxidation of organic matter
238 following the Great Oxidation Event. *Science* **334**, 1694-1696 (2011).
- 239 29 Farquhar, J., Zerkle, A. L. & Bekker, A. in *Treatise in Geochemistry: Reference
240 Module in Earth Systems and Environmental Sciences* Vol. 6 (eds H. D. Holland &
241 K. Turekian) 91-138 (Elsevier, 2014).

242

243 **Acknowledgements**

244 This study was supported financially by Natural Environment Research Council
245 Fellowship NE/H016805 to AZ. We thank the Council for Geoscience in South Africa and

the staff at the National Core Library in Donkerhoek for facilitating access to the core materials, and Misuk Yun for assistance with stable isotope analyses at U. Manitoba.

Author contributions

AZ and SP conceived the study; SP and AB collected the samples; AZ, SP, RN, CM, and CJ processed samples and performed geochemical analyses; MC provided statistical analyses of the global database; AZ interpreted the data and wrote the manuscript with input from all coauthors.

Declaration of competing interests

The authors declare no competing financial interests.

Figure Legends

Figure 1. Secular trend in sedimentary $\delta^{15}\text{N}$ over early Earth history, from Farquhar et al.²⁹, with references listed therein. “Mineral N” refers to nitrogen extracted as ammonium from phyllosilicates. Red and purple data points are from this study, denoting kerogen and bulk rock analyses, respectively.

Figure 2. Lithological and geochemical data for core EBA-2, illustrating the R-TH overlying the Great Chert Breccia (GCB), which developed at the top of the Malmani carbonate platform. Data include Fe speciation, TOC, and $\delta^{13}\text{C}_{\text{org}}$ spanning the section. $\text{Fe}_{\text{py}}/\text{Fe}_{\text{HR}}$ data are only shown for samples with $\text{Fe}_{\text{HR}}/\text{Fe}_{\text{T}}$ suggesting anoxic deposition. Inset is a blow-up of $\delta^{15}\text{N}$ values, alongside TOC and $\delta^{13}\text{C}_{\text{org}}$, for the R-TH boundary (red bar). For $\delta^{15}\text{N}$, red data points are extracted kerogen, purple data points are bulk rock N, and empty symbols are

nano-EA analyses. For all data, errors are within the size of the symbols. The orange arrows denote the disappearance of S-MIF in EBA-2⁶.

METHODS

Statistical analysis of $\delta^{15}\text{N}$ database

Previous studies have utilized age-binned means of the $\delta^{15}\text{N}$ database over hand-picked geologic intervals to propose changes in this proxy with time^{2,4,14,30}. These studies have provided a qualitative indication that the $\delta^{15}\text{N}$ record appears to vary systematically over geologic time; however, they are not statistically robust, because two samples drawn from a single population will often express different means due to random noise. A Student's T-test is a more statistically robust method for determining if two (otherwise normally-distributed) sample sets are likely to arise from the same population, which is considered the null hypothesis. We therefore performed 754 independent two-tailed T-tests spanning every possible time-weighted binning of the $\delta^{15}\text{N}$ database, assuming unequal variances in the sample sets. In all but a few cases at the extremes (where the bin sizes of one of the sample sets were small), the null hypothesis was rejected at greater than 99% confidence and so the divided sample sets are shown to arise from populations with different means and variances.

The sample sets are defined as ranging from the first database entry from the Proterozoic, at 0.70 Ga, to the entry with the age shown on the horizontal axis of Extended Data Figure 1, and then from the subsequent entry to our final database entry, with age of 3.80 Ga. Extended Data Figure 1 shows the “false-positive” probability that the two samples sets arise from populations of the same mean and variance. Using this method, datasets at 2.31 Ga (this study), 2.50 Ga, and 2.70-2.80 Ga are demonstrated to be the most statistically meaningful pivot-ages which separate the database into distinct samples sets.

As discussed in the manuscript, the large number of database entries from ~2.50 Ga stem from predominantly deep water environments which show small stratigraphic shifts in $\delta^{15}\text{N}$ interpreted to reflect temporary localized nitrification/denitrification in an otherwise reducing ocean^{16,17,19}. As a result, the global database may be slightly biased toward results showing an “oxic” nitrogen cycle at this time period. The data presented in this study are from unequivocally oxic shallow waters, and the statistical analysis confirms that our new data provide a stronger statistical power in separating the data sets, even given the bias in the database at 2.50 Ga. As we note in the main text, additional $\delta^{15}\text{N}$ data from shallow water depositional environments in this crucial interval are required to test alternative hypotheses.

Although beyond the scope of this current study, we additionally note that the most statistically meaningful separation of the $\delta^{15}\text{N}$ database occurs when the sample sets are split between 0.70 – 2.71 Ga and between 2.75– 3.80 Ga. The statistical power for this split is driven primarily by the predominance of extremely ^{15}N -enriched $\delta^{15}\text{N}$ measurements (upwards of +55‰, dominantly in kerogens) from this time period. The origin of these extreme values is highly debated, with hypotheses including the onset of partial nitrification¹⁸, and effects from ammonia degassing under highly alkaline conditions³¹. Regardless, it is clear the ~2.70 Ga data do not represent a modern-style aerobic N cycle, as no such extreme values are seen anywhere in the modern Earth system. These statistical analyses therefore demonstrate that the nitrogen cycle underwent massive changes in both the early Neoproterozoic³¹ and at the GOE, with the data from this study forming the key pivot point for the latter.

Fe and C analyses

Iron speciation was determined by means of the sequential extraction technique described in Poulton and Canfield²¹, with a RSD of <5% for all extraction steps. TOC was measured on a Leco analyzer after decarbonation by treatment with 20% HCl, with a 1 σ of

0.05%. $\delta^{13}\text{C}_{\text{org}}$ was measured at the SIFIR Laboratory at the University of Manitoba. A calibration line was calculated by least squares linear regression of analyses of two international standards (USGS40, USGS41) performed at the beginning, middle and end of each run. Replicate analyses of international standard USGS Green River shale SGR-1b ($\delta^{13}\text{C}_{\text{org}} = -29.3 \pm 0.1\text{‰}$ VPDB) alongside unknown samples yielded the results of $\delta^{13}\text{C}_{\text{org}} = -29.5 \pm 0.2\text{‰}$ (n=29).

Kerogen-N isotope analyses

Kerogen was extracted following a method modified from McKirdy and Powell³² in the Geobiology laboratory at the University of St Andrews. Approximately 100-200 mg of bulk rock powders were decarbonated twice with 10% (v/v) HCl overnight at 40°C in a clean hood, then transferred to Teflon beakers in a dedicated fume cupboard, where 5mL of 10% HCl + 2mL of concentrated HF was added and volatilized at 40°C. Residues were rinsed 5x with Milli-Q water. Chloroform was added to the residue, shaken, and allowed to settle in separation funnels for ~30 minutes. Heavy minerals that sank to the bottom were first removed, and then floated kerogen was transferred to a Teflon beaker, dried in a clean hood, and stored in an anaerobic chamber until analysis. A subset of samples were also extracted commercially at Global Geolab Ltd, using techniques similar to those above, except that kerogens were separated out by heavy liquid separation with zinc bromide instead of chloroform. Repeat extracts of the same sample (all plotted in Fig. 2) had consistent $\delta^{15}\text{N}_{\text{org}}$ values between labs, generally within 1‰ (Source Data).

Kerogen N isotope ratios ($\delta^{15}\text{N}_{\text{org}}$) were measured using a Eurovector 3028HT elemental analyser fitted with a Costech Zero Blank autosampler coupled to an Isoprime isotope ratio mass spectrometer, at the University of Leeds. Columns with reagents were fitted to the EA along with either a high-resolution CN GC column (Elemental Microanalysis E3037), or a NCH column (Elemental Microanalysis E3001), as below. A magnesium

perchlorate-carbosorb trap was used to trap water and CO₂. The setup was leak checked and then the combustion and reduction furnaces were heated to operating temperatures and left purging with He overnight. The combustion furnace was held at 1020°C and the reduction furnace at 650°C. The GC column was baked at 190°C with He flowing overnight, and then its temperature was reduced to the normal running temperature (80°C for the NCH column, and 110°C for the high resolution CN column).

Samples were prepared by weighing between 10 and 30 mg of kerogen into 8 x 5 mm tin cups. These were loaded into the autosampler and purged for at least an hour before analyses. Upon sealing the autosampler chamber and opening it to the main He flow, mass 28 was monitored until it returned to a stable background (less than $7e^{-11}$ nA). Samples were combusted in a pulse of pure oxygen (N5.0 grade, BOC, UK) and injected into a stream of helium (CP grade, BOC, UK). The resulting gases were passed through chromous oxide and silvered cobaltous oxide, fine copper wires, and a magnesium perchlorate/carbosorb trap before entering the GC column. The mass 29/28 ratio of the sample N₂ gas was measured relative to a pulse of pure N₂ (Research grade, BOC, UK) and corrected to the AIR scale using the USGS-25 and USGS-26 ammonium sulfate standards, with $\delta^{15}\text{N}_{\text{AIR}}$ values of -30.1‰ and +53.7‰, respectively. Repeated runs of standard materials during each analytical session produced standard deviations of the raw $\delta^{15}\text{N}_{\text{refgas}}$ that were generally between 0.15 and 0.41‰, with the majority $\leq 0.30\%$. Data were corrected with bracketing standards using a simple linear regression equation. Repeats of an in-house yeast standard (7.6 wt% N) gave a long-term average value of $-0.8 \pm 0.31\%$ (1 σ , 37 runs with both NCH and high-resolution CN GC columns), with in-run reproducibility always $\leq 0.2\%$ where 3 or more repeats were measured during the same analytical session. A sample size test using the same yeast standard determined that samples producing peak heights of $< 1\text{nA}$ have larger variability, approaching the blank $\delta^{15}\text{N}$ value as their peak height decreased. Repeat analyses of the yeast

standard with peak height > 1 nA produced $\delta^{15}\text{N}_{\text{refgas}}$ values that differed by $\leq 0.1\%$.

Therefore, analyses that produced peak heights of < 1 nA were discarded in this study.

The analysis of organic materials with low concentrations of nitrogen can be complicated by the production of CO gas (at masses 28 and 29) as a result of incomplete combustion, which can alter the apparent $^{15}\text{N}/^{14}\text{N}$ ratio of the sample. We took the following precautions to ensure that data were not affected by CO production during incomplete combustion: 1) combustion tests using a low-N organic material (cornflower, 0.07 wt% N); 2) mass 30 monitoring; and, 3) use of NCH column to produce a better separation between the N_2 and unwanted CO that might produce a secondary mass 28 peak for samples affected by partial combustion.

Bulk-rock analyses

A subset of R-TH samples was analysed for bulk rock geochemistry (wt % K_2O) to screen for post-depositional alteration at the University of St Andrews, using standard X-ray fluorescence (with 1σ of 0.02 wt%). Bulk nitrogen content (% TN) and bulk $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$, without decarbonation) were measured at the SIFIR Laboratory at the University of Manitoba. Analyses were performed using a CostechTM 4010 Elemental Analyzer (EA) fitted with a Costech Zero Blank autosampler and coupled to a Thermo FinniganTM Delta V Plus isotope-ratio mass-spectrometer via an open-split interface (ConFlo III, Thermo FinniganTM). A magnesium perchlorate-carbosorb trap was placed before the ConFlo III to remove remaining water and CO_2 . In order to improve the efficiency of sample combustion, temperature in the oxidation column was raised to 1050°C, and a ‘macro’ O_2 injection loop was utilized. The setup was leak checked and then the oxidation and reduction columns were heated to operating temperatures and left purging with He overnight. The oxidation column was held at 1050°C and the reduction column at 650°C. The ~3 m-long stainless steel GC column was baked at 100-110°C with He flowing overnight, and then its temperature was

reduced to the normal running temperature (55°C). CO₂ level was monitored during analytical sessions. Sample normalization was performed using the two-point calibration described in Coplen *et al.*³³, by analyzing two international standards (USGS40 and USGS41) at the beginning, middle, and end of each analytical session. Two certified standards were additionally analyzed alongside with samples: B2153, soil, % TN = 0.13 ± 0.02%, $\delta^{15}\text{N}_{\text{air}}$ = +6.70 ± 0.15‰ (Elemental Microanalysis); and SDO-1, Devonian Ohio Shale, % TN = 0.36 ± 0.01%, $\delta^{15}\text{N}_{\text{air}}$ = -0.8 ± 0.3‰ (USGS). The data obtained were % TN = 0.14 ± 0.00% and $\delta^{15}\text{N}_{\text{air}}$ values of +6.76 ± 0.02‰ (n=3) for B2153, and % TN = 0.37 ± 0.00% and -0.32 ± 0.02‰ (n=3) for SDO-1.

Nano-EA-IRMS analyses

A subset of extracted kerogens and bulk rock powders were also run for $\delta^{15}\text{N}$ by nano-EA-IRMS at Syracuse University, following methods outlined in Polissar *et al.*³⁴ The benefit of this approach is that it is specifically designed for analysis of as little as 0.5 mg of kerogen and 50 nanomoles of N, thus limiting some of the complications associated with achieving complete combustion on larger samples. Encapsulated sample powders were evacuated to remove atmospheric N₂ present in capsule pore space and purged with Ar. Sample combustion was performed in an Elementar Isotope Cube elemental analyser with reaction conditions set at 1100°C and 650°C for the oxidation and reduction reactors, respectively. Oxygen flow was set a 30 ml*min⁻¹ and introduced to the helium stream for 90 seconds, initiating when the sample is dropped into the oxidation reactor. The EA is coupled to an automated cryotrapping system that was build using a modified Elementar TraceGas analyser. The generated N₂ gas was trapped in a silica gel-filled, stainless steel trap cooled in liquid N₂. Following complete collection of the N₂ peak from the high-flow EA, the He flow through the cryotrap was switched to a lower flow (2 ml*min⁻¹) via actuation of a VICI Valco 6-port valve. The trap was heated and N₂ was released to a room temperature capillary GC-

column (JW CarboBOND, 25 m, 0.53 mm ID, 5 μ m), and ultimately to the IRMS. The Elementar EA traps CO₂ from combustion in a molecular sieve trap that is released to waste or to the IRMS directly for $\delta^{13}\text{C}$ analyses. This ensures that CO₂ is not trapped in the N₂ cryotrap and mitigates the potential for neo-formed CO within the ion source. All samples were run in triplicate and blank-corrected using Keeling-style plots and normalized using the 2pt-correction scheme detailed in Coplen *et al.*³³ Use of Keeling plots allows for simple estimation of the influence of the N₂ procedural blank on samples and for high fidelity measurements of $\delta^{15}\text{N}$ on the small sample sizes employed. Reproducibility of replicates analyses of standards [IAEA N1 (0.4‰) and N2 (+20.35‰) and NIST Peach Leaves (1.98‰)] and samples was $\pm 0.26\text{‰}$.

Additional analyses and data fidelity

Nitrogen is preserved in the sedimentary rock record primarily as organic N or as ammonium substituting for potassium in phyllosilicates³⁵. The sedimentary N isotope values can be modified by a number of post-depositional processes, including diagenesis, burial, and metamorphism. Therefore, before interpreting sedimentary $\delta^{15}\text{N}$ data, it is first necessary to examine the possible impacts of post-depositional alteration on the primary signal. Here we examine trends in supplementary and bulk-rock data in order to validate our $\delta^{15}\text{N}$ dataset as representing a primary signal.

Degradation of organic matter during early diagenesis can offset primary $\delta^{15}\text{N}$ signals by 2 to 3‰³⁶. High-pressure metamorphism does not impart significant $\delta^{15}\text{N}$ changes³⁷, although high-temperature metamorphism can increase $\delta^{15}\text{N}$ in ammoniated phyllosilicates (and possibly N_{org}; but see Ader *et al.*³⁸) due to volatilization of ¹⁵N-depleted nitrogen^{35,37}. Since the R-TH has only experienced lower greenschist facies metamorphism²³, this mechanism would be expected to produce at most a 1-2‰ positive shift in $\delta^{15}\text{N}_{\text{org}}$. Cross-plots demonstrate no correlation between % N in kerogen (N_{org}) and $\delta^{15}\text{N}_{\text{org}}$ values (Extended

Data Fig. 4A), rendering no evidence for metamorphic devolatilization of ^{15}N -depleted nitrogen from organics. $\delta^{15}\text{N}_{\text{bulk}}$ and % total nitrogen (TN) show only a loose positive correlation (with $R^2 = 0.34$; Extended Data Fig. 5A), in the opposite direction of what would be expected from significant loss of ^{15}N -depleted N from whole rocks via devolatilization. Only a weak negative correlation exists between wt % TOC and $\delta^{13}\text{C}_{\text{org}}$ ($R^2 = 0.42$; Extended Data Fig. 4C), also inconsistent with significant devolatilization of ^{13}C -depleted carbon during metamorphism. These data indicate that loss of N during metamorphism and deep burial did not significantly alter the primary $\delta^{15}\text{N}$ (or $\delta^{13}\text{C}$) values.

Nitrogen isotope exchange can occur between rocks and N-containing compounds when fluids migrate during organic matter maturation³⁹. Similar to metamorphism, offset during thermal maturation generally results from preferential volatilization of ^{15}N -depleted nitrogen from organic molecules. The $\delta^{15}\text{N}$ of the natural gas is highly variable, but can have $\delta^{15}\text{N}$ as low as -12‰^{40,41}. Nitrogen isotope exchange during fluid migration would tend to homogenize the isotopic composition of participating N pools, decreasing the isotopic range within the organic N pool and differences between organic and inorganic N pools³⁹. Bulk-rock $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$) covers the measured range of $\delta^{15}\text{N}_{\text{org}}$, but are generally more positive than $\delta^{15}\text{N}_{\text{org}}$, inconsistent with complete isotopic homogenization.

We observe only a very weak negative correlation between $\delta^{15}\text{N}_{\text{bulk}}$ and TOC:TN ($R^2 = 0.29$; Extended Data Fig. 5B), suggesting that some ^{15}N -enriched ammonium could have been sorbed onto and/or incorporated into clay minerals in very low-TOC sediments, presumably during exchange with post-depositional fluids. The % TN (but not $\delta^{15}\text{N}_{\text{bulk}}$) indeed shows a significant positive correlation with % K_2O ($R^2 = 0.81$; Extended Data Fig. 5C), supporting incorporation of N into illites during K-metasomatism; however, there is no correlation between $\delta^{15}\text{N}_{\text{bulk}}$ and % K_2O ($R^2 = 0.10$; Extended Data Fig. 5D), suggesting that this exchange did not significantly affect bulk $\delta^{15}\text{N}$ values.

469

470 **Data Availability Statement**

471 All data generated or analysed during this study are included as source data in this
472 published article.

473

474 **Extended Data Legends**

475 Extended Data Figure 1. Results from statistical analysis of the $\delta^{15}\text{N}$ database, as detailed in
476 the Methods.

477

478 Extended Data Figure 2. Stratigraphic context for the Rooihooigte and Timeball Hill
479 formations within the Eastern Transvaal basin, South Africa, and associated ages. “MIF”
480 denotes the disappearance of the mass independent fractionation of sulfur isotopes in the
481 underlying Duitschland Formation (now known to reappear in the Rooihooigte Formation⁶).
482 Modified from Rasmussen *et al.*⁵.

483

484 Extended Data Figure 3. Simplified geologic map of the Transvaal Supergroup outcrop area
485 (modified from Guo *et al.*⁴²), showing the location of drill-core EBA-2. The core is currently
486 stored at the National Core Library at Donkerhoek, which is managed by the Council for
487 Geoscience in South Africa.

488

489 Extended Data Figure 4. Cross-plots of kerogen N abundance (% N_{org}) and $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{org}}$, in
490 ‰), total organic carbon (% TOC) and organic $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{org}}$, in ‰). For all datapoints, errors
491 are within the size of the symbols.

492

Extended Data Figure 5. Cross-plots of bulk-rock data, including A. bulk-rock $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{bulk}}$, in ‰) versus total nitrogen (% TN), B. $\delta^{15}\text{N}_{\text{bulk}}$ versus TOC:TN atomic ratios, C. % TN versus K_2O content (%), and D. $\delta^{15}\text{N}_{\text{bulk}}$ versus K_2O content. For all datapoints, errors are within the size of the symbols.

Additional References

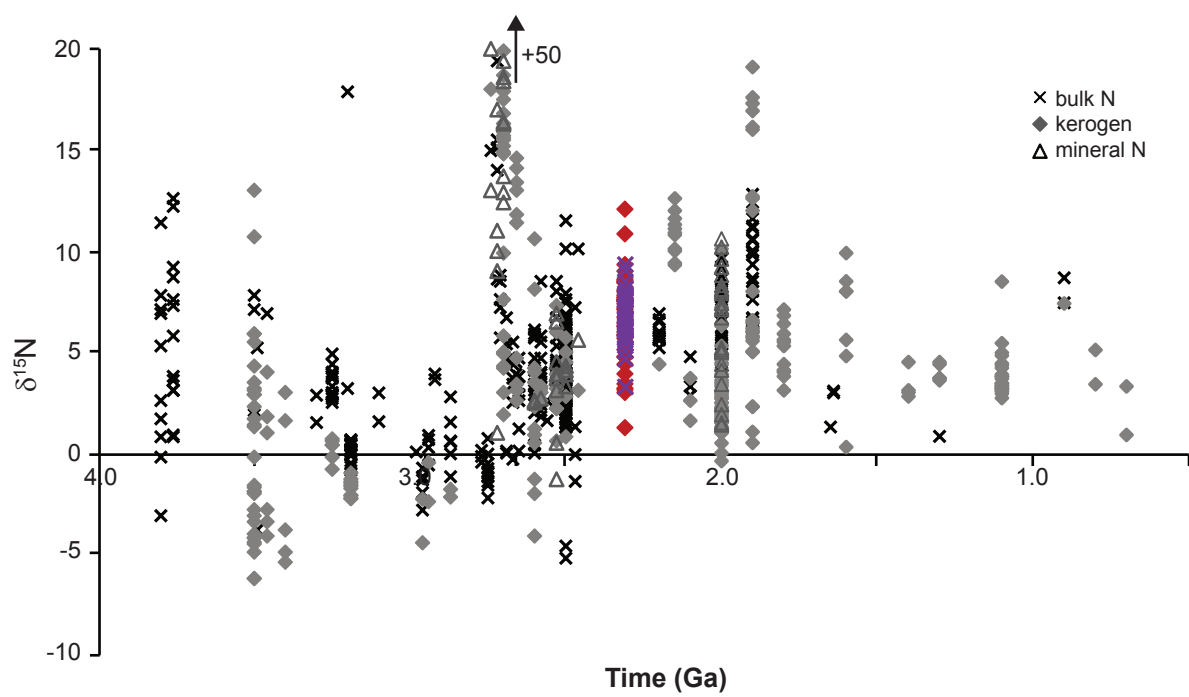
- 30 Thomazo, C. & Papineau, D. Biogeochemical cycling of nitrogen on the early Earth. *Elements* **9**, 345-351 (2013).
- 31 Stueken, E. E., Buick, R. & Schauer, A. J. Nitrogen isotope evidence for alkaline lakes on late Archean continents. *Earth and Planetary Science Letters* **411**, 1-10 (2015).
- 32 McKirdy, D. M. & Powell, T. G. Metamorphic alteration of carbon isotopic composition in ancient sedimentary organic matter: New evidence from Australia. *Geology* **2**, 591-595 (1974).
- 33 Coplen, T. B. *et al.* New guidelines for delta C-13 measurements. *Analytical Chemistry* **78**, 2439-2441, doi:10.1021/ac052027c (2006).
- 34 Polissar, P. J., Fulton, J. M., Junium, C. K., Turich, C. H. & Freeman, K. H. Measurement of ^{13}C and ^{15}N isotopic composition on nanomolar quantities of C and N. *Analytical Chemistry* **81**, 755-763 (2009).
- 35 Boyd, S. R. & Philippot, P. Precambrian ammonium biogeochemistry: a study of the Moine metasediments, Scotland. *Chemical Geology* **144**, 257-268 (1998).
- 36 Robinson, R. S. *et al.* A review of nitrogen isotopic alteration in marine sediments. *Paleoceanography* **27**, doi:10.1029/2012pa002321 (2012).

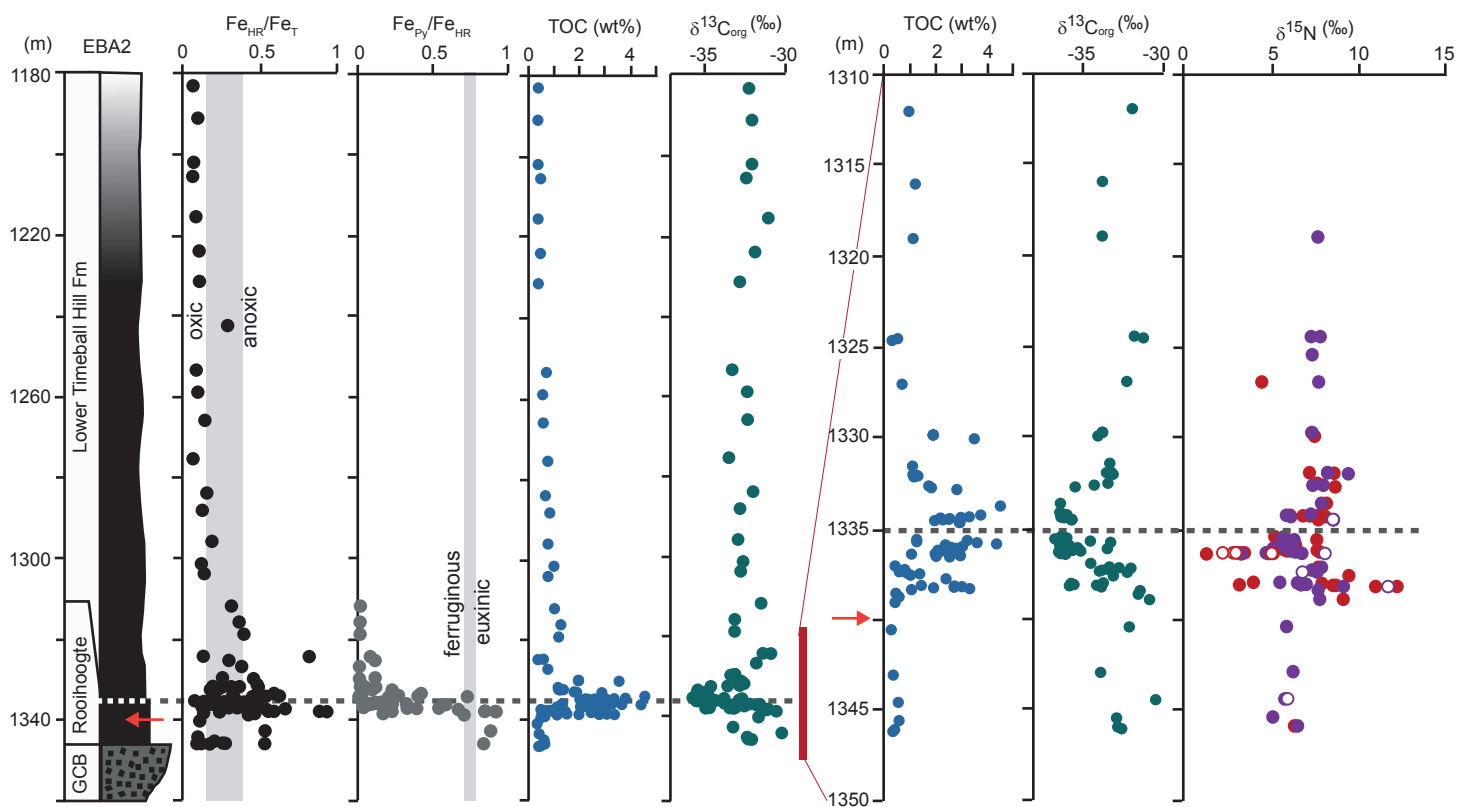
- 516 37 Bebout, G. E. & Fogel, M. L. Nitrogen-isotope compositions of metasedimentary
517 rocks in the Catalina Schist, California: Implications for metamorphic devolatilization
518 history. *Geochimica et Cosmochimica Acta* **56**, 2839-2849 (1992).
- 519 38 Ader, M., Boudou, J.-P., Javoy, M., Goffe, B. & Daniels, E. Isotope study of organic
520 nitrogen of Westphalian anthracites from the Western Middle field of Pennsylvania
521 (U.S.A.) and from the Bramsche Massif (Germany). *Organic Geochemistry* **29**, 315-
522 323 (1998).
- 523 39 Schimmelmann, A. & Lis, G. P. Nitrogen isotopic exchange during maturation of
524 organic matter. *Organic Geochemistry* **41**, 63-70,
525 doi:10.1016/j.orggeochem.2009.01.005 (2010).
- 526 40 Hoering, T. C. & Moore, H. E. The isotopic compositions of the nitrogen in natural
527 gases and associated crude oils. *Geochimica et Cosmochimica Acta* **13**, 225-232,
528 doi:10.1016/0016-7037(58)90024-3 (1958).
- 529 41 Murty, S. V. S. Noble-gases and nitrogen in natural gases from Gujarat, India.
530 *Chemical Geology* **94**, 229-240, doi:10.1016/0168-9622(92)90015-3 (1992).
- 531 42 Guo, Q. J. *et al.* Reconstructing Earth's surface oxidation across the Archean-
532 Proterozoic transition. *Geology* **37**, 399-402, doi:10.1130/g25423a.1 (2009).

533

534

535





KEY:  chert breccia  black shale to siltstone