

The cycling and redox state of nitrogen in the Archaean ocean

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Organisms that produce oxygen through photosynthesis existed during the late Archaean eon, about 2,500 million years ago, but controversial evidence suggests that they may have evolved several hundred million years earlier. Oxygen is expected to react with oceanic nitrogen, altering its redox state. The reaction leaves a signature in the isotopic composition of the nitrogen bound in organic matter. Here we present a record of the nitrogen isotopic composition of kerogen extracted from minimally altered shales from the Campbellrand-Malmani platform in South Africa. Between the Palaeo-Archaean and about 2,670 million years ago, the $\delta^{15}\text{N}$ values of the kerogen rose by about 2‰. We interpret this increase as an indication of the onset of coupled nitrification and denitrification or anammox reactions in the surface oceans, which require the presence of free oxygen. A second increase in nitrogen isotopic composition around 2,520 million years ago implies instability of the N cycle with loss of fixed N. This evidence for available oxygen in the oceans occurs at least 200 million years before geochemical indications of the presence of significant levels of atmospheric oxygen. We suggest that coupled nitrification and denitrification drove the loss of fixed inorganic nitrogen, leading to nitrogen limitation, and conclude that the low levels of biologically available nitrogen limited the growth of oxygen-producing plankton, delaying the accumulation of oxygen in the atmosphere.

Nitrogen and oxygen are, by far, the two most abundant gases in Earth's atmosphere, and the fluxes of both elements are mediated almost entirely by microbial reactions. N_2 is a relatively inert molecule and has an atmospheric lifetime of the order of ~ 1 billion years (Gyr) (ref. 1). In contrast, O_2 , which has come to comprise between ~ 10 and $\sim 30\%$ of the volume of the atmosphere over the past ~ 500 million years (Myr; ref. 2), is highly reactive and must be produced continuously by oxygenic photosynthesis. It is unlikely that the gas was present above trace levels in the atmosphere of the Earth during the first two billion years of the planet's history, but when oxygenic photosynthesis first arose on the Earth is not known with certainty³⁻⁷. The disappearance of mass-independent fractionation of sulphur isotopes from the geological record (for example, refs 8, 9) and retention of Fe in palaeosol weathering profiles¹⁰ suggests that by 2,300–2,200 Myr ago, the accumulation of O_2 in the atmosphere was $>10^{-5}$ PAL (present atmospheric level). Molecular fossil evidence indicates that oxygenic photosynthetic organisms (that is, cyanobacteria) might have been present and producing O_2 2,800 Myr ago, several hundred million years before the so-called Great Oxidation Event^{3,6} approximately 2,300 Myr ago. Although it is possible that the molecular biomarkers for cyanobacteria have more diverse origins than originally thought⁴, it is also possible that any O_2 produced was consumed within the ocean or troposphere before it escaped to the stratosphere, or initiated a feedback that limited the productivity of photosynthetic organisms. Here, we explore the interaction between the N and O cycles around 2,500 Myr ago at the close of the Archaean eon.

Assuming the early Archaean oceans were devoid of O_2 , the nitrogen cycle would have been relatively simple; N_2 would have been biologically reduced to NH_4^+ , which, in turn, would have become incorporated into organic matter and eventually buried in sediments. Bacterial respiration and grazing would have released NH_4^+ to the water column to be recycled, or within sediments to be fixed in silicates (Fig. 1). During subduction, sedimentary N may

be trapped in melts or returned to the atmosphere predominantly as N_2 because thermal N fixation requires atmospheric O_2 to produce NO_x (ref. 11). This cycle is ultimately driven by tectonics and would have taken millions of years to complete.

The interaction between oxygen and the nitrogen cycle

The evolution of oxygenic photosynthesis allowed the N cycle to completely close within the ocean on much shorter timescales (Fig. 1). Once O_2 became available, chemoautotrophic organisms could oxidize NH_4^+ ; in the contemporary ocean, the oxidation of NH_4^+ is catalysed strictly by microbes that have an absolute requirement for O_2 (ref. 12). Nitrification is divided into two sequential steps, separated at NO_2^- . Members of the Archaea and Bacteria can oxidize NH_4^+ , but the bacteria responsible for NH_4^+ oxidation are not closely related to the NO_2^- oxidizers^{13,14}. This phylogenetic separation suggests that a long period of time could have elapsed between oxidation of NH_4^+ to NO_2^- and oxidation of NO_2^- to NO_3^- . Under suboxic conditions, both oxidized forms of N can be used by facultative anaerobic denitrifying heterotrophs as electron acceptors in the respiration of C_{org} leading to the production of N_2 (with trace fluxes of N_2O). Alternatively, anammox bacteria consume NO_2^- to anaerobically oxidize NH_4^+ to N_2 (without the production of N_2O). If nitrification to NO_3^- was delayed at the first step at formation of NO_2^- , the closure of the N cycle might have first occurred with the formation of NO_2^- through the anammox reaction. Regardless of the pathway, as O_2 was first produced, nitrification followed by rapid denitrification would have led to loss of fixed inorganic N, which would potentially have led to N limitation of primary production¹⁵ if N fixation could not replace the N lost to the atmosphere, a process that short-circuits tectonic processing.

The organisms, the organic molecular remnants of which form the kerogen we analysed, acquired N in the upper ocean. There are two isotopic endmembers for the N sources. If the primary source of fixed N was NH_4^+ , supplied either by local fixation

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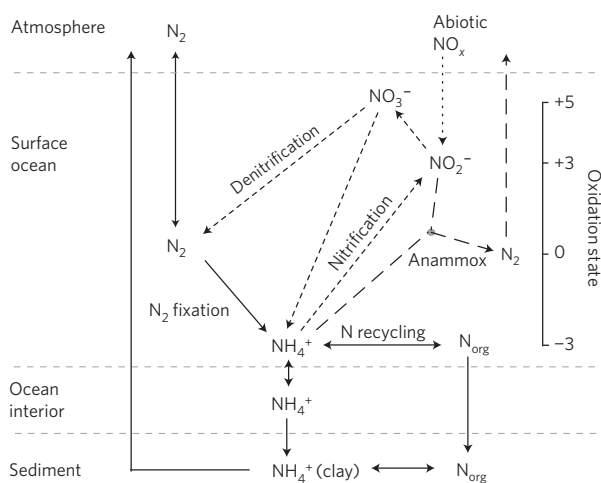


Figure 1 | Proposed late Archaean nitrogen cycle. Upwelling of ocean interior NH_4^+ , N fixation and efficient N recycling support surface productivity. Nitrogen fluxes in a totally anoxic ocean are indicated by solid arrows and the only sink of ocean N is burial in sediments. Once oxygenogenic photosynthesis starts, N can exist in higher oxidation states (fluxes indicated by short-dashed arrows). Coupled nitrification–denitrification allows N to escape from the ocean as N_2 , which causes an increase in the remaining $\delta^{15}\text{N}_{\text{fixed}}$ as well as a decrease in the ocean N inventory. If the rates of oxygen production are sufficiently high, nitrification rates exceed those of denitrification and nitrite and then nitrate can start to accumulate. The fluxes associated with anammox (indicated by long-dashed arrows) are included and the abiotic source of nitrite is shown by the dotted arrow. The isotope signature of surface ocean processes is carried by organic matter sinking to the sediments.

or from isopycnal mixing from the anoxic interior, the isotopic composition would be similar to atmospheric N_2 . If, however, O_2 was present in the upper ocean, subsequent nitrification–denitrification reactions would have led to preferential return of ^{14}N to the atmosphere¹⁶. Under such conditions, the organic matter would become isotopically enriched in ^{15}N . Hence, to first order, the sedimentary record of $\delta^{15}\text{N}$ in organic matter reflects the redox state of N in the upper ocean^{17–19}.

Nitrogen isotope record of late Archaean organic matter

Nitrogen isotopes of bulk rock ($\delta^{15}\text{N}_{\text{bulk}}$), extracted kerogen ($\delta^{15}\text{N}_{\text{kerogen}}$) and non-exchangeable silicate-bound N ($\delta^{15}\text{N}_{\text{silicate}}$) were analysed from a core (GKP01) drilled close to the distal edge of the Campbellrand–Malmani platform (see the Methods section and Supplementary Table S1). The core contains siliclastic material, carbonates and banded iron formations (BIFs) deposited before, during and following the drowning of the Campbellrand–Malmani platform; the youngest samples from the core just pre-date the Great Oxidation Event. The core is described in detail by Schröder *et al.*²⁰ and dated sections are detailed in Knoll and Beukes²¹. The $\delta^{15}\text{N}$ composition of bulk sediments increases from $\sim +2$ to $+10\%$ towards the top of the core, which is consistent with an interaction between the production of O_2 and the N cycle, causing loss of ^{14}N through coupled nitrification–denitrification reactions. However, $\delta^{15}\text{N}$ values in kerogen and fixed sites of siliclastics indicate that there almost certainly was a post-depositional addition of N enriched in ^{15}N , which is only weakly bound to surfaces. Hence, to reduce the potential post-depositional alterations in N isotopes, we specifically analysed the isotopic fractionation of the element in kerogen rather than bulk rock²².

In the absence of terrestrial plants, kerogen formed in the Archaean would have been largely composed of a lipidic material derived from cell outer walls containing N in protected

amide and protein sites and porphyrins. Nitrogen compounds in these sites diagenetically condense to form complex heterocyclic compounds^{23,24}. Although modern kerogen contains carbon enriched in ^{12}C relative to whole cells, no persistent offset has been reported between suspended particulate organic matter and non-hydrolysable $\delta^{15}\text{N}$ in lipid fractions²⁵; we assume this to be true of Archaean systems. The N in tetrapyrroles (for example, porphyrins) derived primarily from pigments is covalently bound to C and is extremely stable, with a $\sim 5\%$ depletion in ^{15}N relative to whole-cell N (ref. 26). Thus, given the available data, proto-kerogen is likely to have similar or lower $\delta^{15}\text{N}$ than whole cells from which it is derived. Early diagenetic changes affecting $\delta^{15}\text{N}$ in organic matter ($\delta^{15}\text{N}_{\text{OM}}$) in the anoxic interior of the Archaean ocean can probably be discounted because there is no difference in $\delta^{15}\text{N}$ of surface sediment organic matter and sinking particulate organic matter in modern anoxic basins²⁷. We infer that the isotopic composition of N in kerogen can be a reliable proxy of $\delta^{15}\text{N}_{\text{OM}}$ sinking from the upper ocean.

Post-depositional processes alter $\delta^{15}\text{N}$ only if circulating fluids add or remove N. As with other Archaean and early Proterozoic sediments, C/N ratios are high, indicating loss of N. The isotopic similarity between $\delta^{15}\text{N}_{\text{kerogen}}$ and $\delta^{15}\text{N}_{\text{silicate}}$ combined with the higher C/N of kerogen versus bulk sediment indicates that N was lost from organic matter during graphitization and incorporated into layered silicates ($\text{N}_{\text{silicate}}$) without isotopic fractionation and without addition of extraneous isotopically distinct N. The remaining kerogen N is probably located at the core of molecular structures of kerogen²⁸. Although N exchange between kerogen and NH_4^+ or condensation of $\text{NH}_4^+ - \text{N}$ to form kerogen-like molecules may also occur, these reactions occur at greater frequency with kerogen derived from terrestrial organic matter²⁹. Furthermore, if the N is derived from organic matter within the studied section, the effect of these N transformations will be to buffer against small-scale fluctuations in $\delta^{15}\text{N}$ (ref. 29) rather than cause changes in average $\delta^{15}\text{N}$.

The values of $\delta^{15}\text{N}_{\text{kerogen}}$ range from $+0.5$ to $+7.3\%$ within the 250 Myr window recorded by the core, with a mean of $3.2 \pm 1.5\%$ (Fig. 2). Nearly all samples more than 1 s.d. from the mean either show evidence of thermal degradation of organic matter, on the basis of elevated $\delta^{13}\text{C}_{\text{org}}$, or are associated with volcanic tuffs or tuff zones. The importance of tuff zones is twofold: (1) they have increased porosity and therefore are susceptible to post-depositional alteration through fluid flow and (2) because these tuffs have high K^+/Al (ref. 20) and the ionic radii of NH_4^+ and K^+ are similar, the two ions can exchange and potentially affect $\delta^{15}\text{N}$ (ref. 30). If these samples are excluded, the $\delta^{15}\text{N}_{\text{kerogen}}$ average and standard deviation remain almost the same, $2.9 \pm 1.0\%$.

Secular trends in $\delta^{15}\text{N}$

A trend of increasing kerogen $\delta^{15}\text{N}$ from the early Archaean to the mid-Proterozoic era is apparent despite a fairly wide range of up to $\pm 5\%$ within a single location and stratigraphic age, which could reflect outcrop heterogeneities arising from post-depositional events (Fig. 3). Incomplete assimilation of fixed N by primary producers can affect $\delta^{15}\text{N}_{\text{OM}}$ but is unlikely to generate the increase in $\delta^{15}\text{N}$ that occurs over hundreds of millions of years. Utilization of N that was advected from regions where N was partially assimilated can enrich organic matter in ^{15}N relative to average ocean. More extreme forms of this condition can occur, but only on timescales of thousands (that is, subglacial) rather than millions of years. If processes operated in the past that allowed N to be a persistently non-limiting nutrient, burial of ^{15}N -depleted organic matter would leave ^{15}N -enriched fixed N in the soluble pool. Assuming fixed N was in the form of NH_4^+ , $\delta^{15}\text{N}_{\text{OM}}$ of 0 to $+2\%$ requires $\delta^{15}\text{N}_{\text{fixed-N}}$ to be considerably higher³¹. This scenario also assumes the N cycle was unbalanced for millions of years with no exchange of N_2

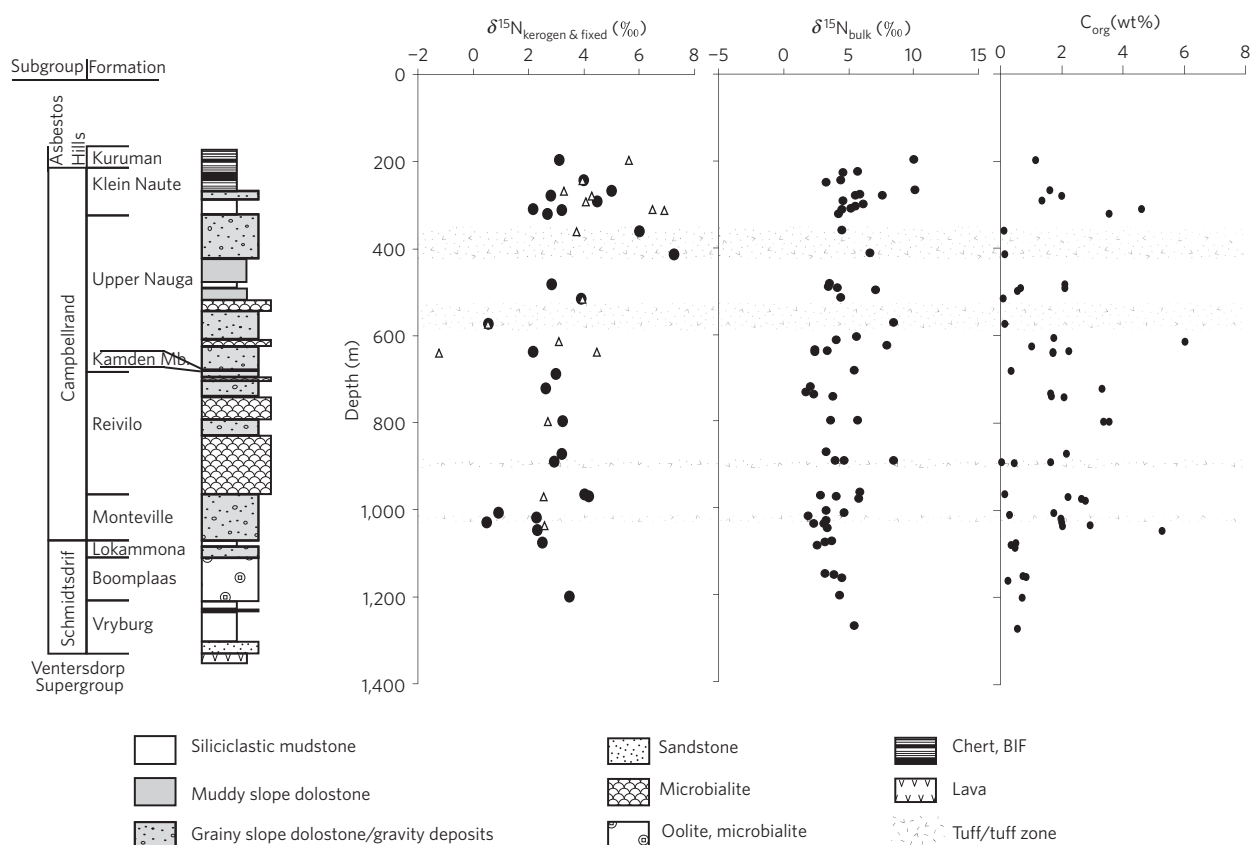


Figure 2 | GKP01 $\delta^{15}\text{N}$ data. Changes in the isotopic composition of nitrogen in kerogen, N fixed in layered silicates and bulk sediments and C_{org} over the last 200 Myr of the Archaean recorded in core GKP01. The difference between bulk and silicate $\delta^{15}\text{N}$ is the inclusion of weakly bound and easily exchanged N in the $\delta^{15}\text{N}_{\text{bulk}}$ measurement. Circulating fluids could have left N on silicate surfaces at any time over the past 2,500 Myr, making the palaeoenvironmental reliability of this measurement questionable. Samples that have not been thermally altered or are associated with volcanic tuffs average $2.9 \pm 1.0\text{‰}$. Samples from the Monteville Formation with low $\delta^{15}\text{N}_{\text{kerogen}}$ may do so due to incomplete N_{fixed} assimilation when flooding occurred over the Kaapvaal craton bringing a new supply of N to this location. There is an increase in $\delta^{15}\text{N}_{\text{kerogen}}$ across the Klein Naute Formation, a time of platform drowning. The increase in $\delta^{15}\text{N}_{\text{kerogen}}$ at this time reflects an enrichment of ^{15}N in the surface ocean due to coupled nitrification–denitrification reaction and loss of ^{14}N to the atmosphere. The decrease in $\delta^{15}\text{N}_{\text{kerogen}}$ into the Kuruman BIF suggests that the availability of O_2 for interaction with the N cycle could reflect rates of O consumption by Fe(II) oxidation, or that N loss during the Klein Naute led to N limitation of oxygenic photosynthetic organisms. Dates for the units are described in Knoll and Beukes²¹ and references therein. These are: Venterdorp Supergroup 2,710–2,700 Myr; Vryburg Formation $2,669 \pm 5$; Lokammona Formation $2,650 \pm 8$; lower Nauga Formation $2,588 \pm 6$ Myr; upper Nauga above Kamden Member $2,552 \pm 11$ Myr and $2,549 \pm 7$ Myr; upper Nauga below Klein Naute Formation $2,521 \pm 3$ and $2,516 \pm 4$; Kuruman Formation $2,460 \pm 5$ Myr.

between the atmosphere and ocean, which is extremely unlikely. Lacking competitive pressure from eukaryotic algae, it is far more likely that autotrophic prokaryotes would have used virtually all available fixed N in the euphotic zone with buried $\delta^{15}\text{N}_{\text{OM}}$ similar to surface ocean $\delta^{15}\text{N}_{\text{fixed-N}}$.

We suggest a more probable explanation for the variations in $\delta^{15}\text{N}_{\text{kerogen}}$ in the late Archaean arises from changes in its redox state in the upper ocean. Between 3,500 and 3,250 Myr ago, in the Palaeo–Archaean, isotopic values are approximately 0‰ (refs 32–34). At 35 the base of GKP01, before the formation of the carbonate platform around 2,600 Myr ago, $\delta^{15}\text{N}_{\text{kerogen}}$ averages $+2.3\text{‰}$ ($\delta^{15}\text{N}_{\text{fixed}}$ is $+2.5\text{‰}$). Nitrogen loss, reflected in high kerogen and bulk C/N values, through devolatilization of N as N_2 is associated with a low isotope fractionation factor, leading to enrichment in ^{15}N by typically less than 2‰ (ref. 35). Although kerogen may have increased up to 2‰, it is also possible that proto-kerogen had lower $\delta^{15}\text{N}$ than whole cells; the two processes may partly counteract each other. The negative to near 0‰ values of early Archaean organic matter are consistent with a N cycle operating in a reducing environment and an isotopic composition of fixed inorganic N reflecting atmospheric N_2 . To explain the modest increase in $\delta^{15}\text{N}_{\text{kerogen}} \sim 2,670$ Myr

ago, there must have been some loss of ^{14}N from the upper ocean. Assuming that the isotopic signal of kerogen-bound N is close to that of the organic matter produced at the time, the most likely mechanism to explain the measured isotopic enrichment is denitrification, a process that can occur only if there is production of free molecular O_2 (ref. 12). This mechanism does not require net accumulation of O_2 , but does require flux of the gas. By far, the largest and most important flux of O_2 would have been oxygenic photosynthesis¹⁸. Although exactly when oxygenic photosynthesis first evolved is not known with certainty^{3–5,18,36}, if our analysis is correct, our results suggest that it arose before 2,670 Myr ago.

Latest Archaean $\delta^{15}\text{N}$ and increased oxygen availability

The uniform $\delta^{15}\text{N}$ values found in kerogen through most of the core suggest that the ocean inventory of fixed N was stable for long periods of time during the last few hundred million years of the Archaean eon. There are a small number of samples in GKP01 that are more than 1 standard deviation from the average $\delta^{15}\text{N}_{\text{kerogen}}$ that are not associated with tuffs and do not show chemical signs of thermal alteration from elevated values of $\delta^{13}\text{C}_{\text{org}}$. The short-term changes in $\delta^{15}\text{N}$ that overlie the long-term trend may reflect changes

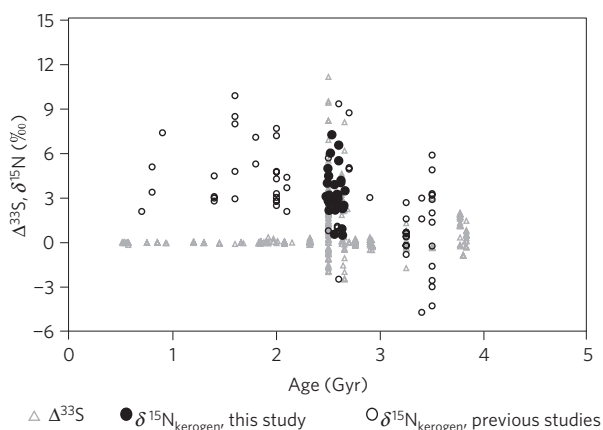


Figure 3 | Secular trends in $\delta^{15}\text{N}_{\text{kerogen}}$ during the Precambrian time. The long-term change in $\delta^{15}\text{N}_{\text{kerogen}}$ through the Precambrian time showing the gradual increase in $\delta^{15}\text{N}_{\text{kerogen}}$ that reflects the increased interaction between the O and N cycles. The data are from this study, Beaumont and Robert³³, Hayes *et al.*³² and Yamaguchi³⁴.

in water depth and position of the redox gradient above the deposition site, exchange with deep-water fixed N or biological responses to nutrient fluxes and volcanism.

Two samples with $\delta^{15}\text{N}_{\text{kerogen}}$ values 2‰ lower than the core-average value of 2.3‰ were in the Monteville Formation, deposited between 2,650 and 2,588 Myr ago^{37,38}. Similarly low $\delta^{15}\text{N}_{\text{kerogen}}$ values of $0.9 \pm 1.0\text{‰}$ in these samples have been obtained from the Oaktree Formation in the Transvaal sub-basin³⁴, which corresponds to the lowermost to middle Reivilo Formation^{20,38}. The Monteville and Oaktree formations represent transgressive events across the Kaapvaal craton^{20,38}, and it is possible that this geological process allowed increased mixing of deep water containing NH_4^+ with low $\delta^{15}\text{N}$ into the upper ocean. We cannot further constrain this process because thermal degradation, indicated by higher $\delta^{13}\text{C}$ values of organic matter in this section of the core, is likely to have compromised $\delta^{15}\text{N}_{\text{kerogen}}$.

Samples with the high, reliable $\delta^{15}\text{N}_{\text{kerogen}}$ values that are greater than +4.5‰ occur between 420 and 267 m in the upper Nauga and Klein Naute formations, which were deposited between 2,560 Myr and $2,465 \pm 4$ Myr ago^{39,40}. These formations are represented by turbidites with increasing chert and mudstone that grade into an iron formation at the top of the Klein Naute, indicating increasing water depths. An increase in water depth may affect $\delta^{15}\text{N}$ by enhancing ^{15}N enrichment at the interface between the anaerobic ocean interior and more oxidized surface ocean owing to greater exchange with open-ocean fixed N, analogous to changes in S isotopes⁴¹, or by an increased biological activity and nitrification–denitrification reactions resulting from increased availability of other nutrients. Regardless, either scenario strongly implies O_2 was present in the upper ocean.

A net flux of O_2 in the upper ocean does not seem to have been translated to an atmospheric signal. It is likely that the concentration of O_2 was extremely low and if a small portion of the gas evaded to the troposphere, it was almost certainly chemically reduced. Whatever the specific mechanisms, our data suggest that oxygenic photosynthesis arose much earlier than the loss of the mass-independent isotopic fractionation of sulphur⁹, which is presumed to be a proxy for the presence of stratospheric ozone⁸.

There is a return to lower values of $\delta^{15}\text{N}$ at the start of deposition of the Kuruman BIF, suggesting that the N_2 fixation may have slightly outpaced the rate of denitrification, reversing the situation recorded in the Klein Naute Formation. The coincidence with the deposition of the Fe formation could be unconnected,

with the decline in denitrification resulting from N limitation of oxygenic photosynthesis and reduction of coupled nitrification–denitrification reactions. Alternatively the increased flux of Fe(II) during a period of enhanced volcanism at the end of the Archaean⁴² sustained high rates of N fixation or competed with the N cycle to consume O_2 and allowed fixed N to accumulate.

In conclusion, the isotopic record of N in kerogen in the late Archaean implies the production of O_2 by oxygenic photosynthetic organisms in the upper ocean. This conclusion is consistent with other, independent, geochemical proxies from South Africa and Australia^{36,43,44}. The production of oxygen precedes the disappearance of the mass-independent isotopic fractionation of sulphur by over 200 Myr, suggesting that the nitrogen cycle may have exerted a negative feedback on the oxidation of the atmosphere and ocean^{15,18}. Regardless, before 2.3 Gyr ago the net flux of O_2 from the surface ocean to the atmosphere must have been trivial. Indeed, the atmospheric concentration of the O_2 seems to have been insufficient to produce ozone⁸. The record of $\delta^{15}\text{N}$ we obtained from the Campbellrand–Malmani platform suggests that the N cycle approached steady-state conditions throughout the 250 Myr interval recorded in the core. However, as the platform drowned, $\delta^{15}\text{N}$ values suggest an increase in denitrification. The data from GKP01 fall on a trend of increasing $\delta^{15}\text{N}$ values that continued to the middle of the Proterozoic era. Whether there are sharp changes marking abrupt changes in oxygen production and denitrification, or whether N isotopes record oxygenation with multiple chemical systems buffering the increase in $p\text{O}_2$ is yet to be revealed.

Methods

Samples for nitrogen and organic carbon isotope analyses were obtained from drill core GKP01, which recovered more than 1,300 m of Transvaal Supergroup sediment from the Griqualand West sub-basin in South Africa. Nitrogen isotope ratios were measured on total ('bulk' N) sediments, refractory organic molecules extracted as kerogen and layered silicates which include clays and micas ('silicate' N) to address post-depositional alterations in the N isotopic signatures, which always present a problem in interpretation of the N cycle from isotopic analyses. Low-temperature regional heating in Griqualand West of 110 to 170 °C (ref. 45) is unlikely to have caused devolatilization of rock-bound N, but a compression-driven fluid flow event around 2,140 Myr ago^{9,46,47} or later circulation of deep groundwater may have altered $\delta^{15}\text{N}$; measurement of $\delta^{15}\text{N}$ in different phases addresses these concerns.

Samples were ground in a ceramic ball mill and used for the bulk measurements or as starting material for extractions of fixed N and kerogen. Fixed N, that is, NH_4^+ or small organic molecules trapped in layered silicates, was measured for concentration and isotope composition after surface-adsorbed N and organic matter were removed by oxidation with KOBBr in a 2N KCl solution following the method described by Silva and Bremner⁴⁸. Nitrogen bound in organic material (kerogen) was isolated from exchangeable or fixed NH_4^+ using a technique modified from McKirdy and Powell⁴⁹. Powdered sample (200 mg) was decalcified in a 50 ml polypropylene centrifuge tube using 25% HCl. The HCl was decanted following centrifugation and 20 ml of 3N HCl–15N HF was added to dissolve layered silicate material. The samples were centrifuged, the acid poured off and the samples rinsed with 0.5N KCl three times to remove any remaining acid and to avoid re-adsorption of NH_4^+ . Kerogen was floated off in chloroform onto precombusted GF/F filters which were dried and segments taken with a clean razor blade for N analysis. For analysis of $\delta^{13}\text{C}_{\text{org}}$, samples were decarbonated in silver capsules with 2 N HCl. Most of the isotopic analyses were carried out using a GVI Isoprime CF-IRMS system; a subset was also analysed using the offline Dumas combustion method and a Finnigan–MAT 252 dual-inlet mass spectrometer to ensure that potential kinetic effects of combustion using a continuous-flow method did not affect our measurement of N isotopes.

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Author contributions

The research focus was identified by P.G.F. and L.V.G. L.V.G. designed and carried out the analytical protocols. The article was written by L.V.G. and P.G.F.

Additional information

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