

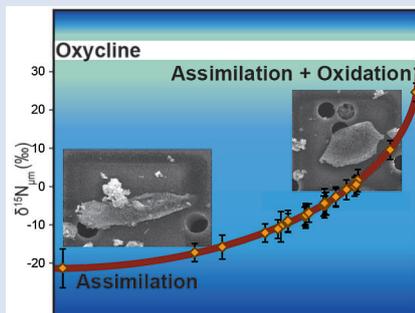
Nitrogen isotope signatures of microfossils suggest aerobic metabolism 3.0 Gyr ago

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Abstract



There is compelling evidence for early oxygenation of mid-Archean oceans. However, the biological use of molecular oxygen is still not ascertained. Here we report the nitrogen isotope composition measured in isolated microfossils ($\delta^{15}\text{N}_{\mu\text{m}}$) from the 3.0 billion years old Farrel Quartzite metasediments. We show that the quasi-null bulk $\delta^{15}\text{N}$ values of Farrel Quartzite organic matter encompass a large ^{15}N isotopic heterogeneity at the scale of isolated microfossils ($-21.6\text{‰} < \delta^{15}\text{N}_{\mu\text{m}} < +30.7\text{‰}$). Rayleigh fractionation is required to yield such large $\delta^{15}\text{N}$ variations. Based on these data, we propose a model in which negative $\delta^{15}\text{N}_{\mu\text{m}}$ values determined on film-like and on spheroidal microfossils are explained by ammonia assimilation in the anoxic deeper levels of the water column, whereas positive $\delta^{15}\text{N}_{\mu\text{m}}$ values determined on lenticular microfossils were driven by both ammonia assimilation and aerobic oxidation close to the sea

surface. Since ammonium aerobic oxidation requires the presence of free molecular O_2 within the water column, we further suggest that positive $\delta^{15}\text{N}_{\mu\text{m}}$ values reflect an ocean redox stratification tightly related to O_2 production by oxygenic photosynthesisers in a mid-Archean ocean 3.0 Gyr ago.

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Introduction

The isotopic composition of nitrogen (N) has been investigated in order to tackle questions related to Archean life (Beaumont and Robert, 1999; Pinti *et al.*, 2001; Thomazo *et al.*, 2011; Stueken *et al.*, 2015a). In the present day natural environment, N is mostly present as di-nitrogen (N_2), ammonium (NH_4^+), organic N, nitrite (NO_2^-) and nitrate (NO_3^-). Within the marine N cycle, N compounds are closely coupled in a redox reaction network including specific isotope fractionation processes (Sigman *et al.*, 2009). Accordingly, $\delta^{15}\text{N}$ of sedimentary organic matter (OM) has been used to infer the evolution of the redox conditions of Archean oceans. For instance, negative $\delta^{15}\text{N}$ values ranging between -7 and 0‰ in the OM isolated from early Archean rocks (3.6 to 3.2 Ga) have been interpreted as reflecting (i) anoxic Archean oceans in which microorganisms consumed N_2 and NH_4^+ (Beaumont and Robert, 1999) or (ii) a consumption of N_2 and NH_4^+ by chemosynthetic bacteria flourishing close to ^{15}N -depleted hydrothermal vents (Pinti *et al.*, 2001; Shen *et al.*, 2006). The late Archean era (2.8 to 2.5 Ga) is characterised by the occurrence of positive OM $\delta^{15}\text{N}$ values (up to *ca.* $+5\text{‰}$) presumably reflecting a rise in the abundance of oxidised N species (Beaumont and Robert,

1999; Garvin *et al.*, 2009; Godfrey and Falkowski, 2009). This suggests that significant free O_2 was present in the oceans at that time, which is also supported by several inorganic palaeoredox proxies (Anbar *et al.*, 2007; Reinhard *et al.*, 2009; Stueken *et al.*, 2015b).

Although early and late Archean sedimentary $\delta^{15}\text{N}$ values suggest a progressive rise in the oxygenation level of Archean oceans, mid-Archean times (3.2 to 2.8 Ga) are characterised by a narrow range of $\delta^{15}\text{N}$ values close to the N isotope composition of the present day atmosphere (0‰), suggesting the dominance of N_2 fixing microorganisms (Stueken *et al.*, 2015a). However, complementary geochemical evidence points toward oxygenic photosynthesis occurring as early as *ca.* 3.0 Ga (Crowe *et al.*, 2013; Lyons *et al.*, 2014; Planavsky *et al.*, 2014) and ocean redox stratification *ca.* 3.2 Gyr ago (Satkoski *et al.*, 2015). If correct, such early oxygenation and ocean redox stratification should be recorded in redox-dependent sedimentary $\delta^{15}\text{N}$ values, implying in turn that the $\delta^{15}\text{N}$ values around $0 \pm 2\text{‰}$ measured on bulk samples might mask isotopic heterogeneities present at smaller scales. To investigate this issue, we have characterised the N isotope composition of organic microfossils (noted $\delta^{15}\text{N}_{\mu\text{m}}$) isolated from the mid-Archean Farrel Quartzite formation (3.0 Ga; Sugitani *et al.*, 2007) using nanoscale secondary ion mass spectrometry (NanoSIMS).

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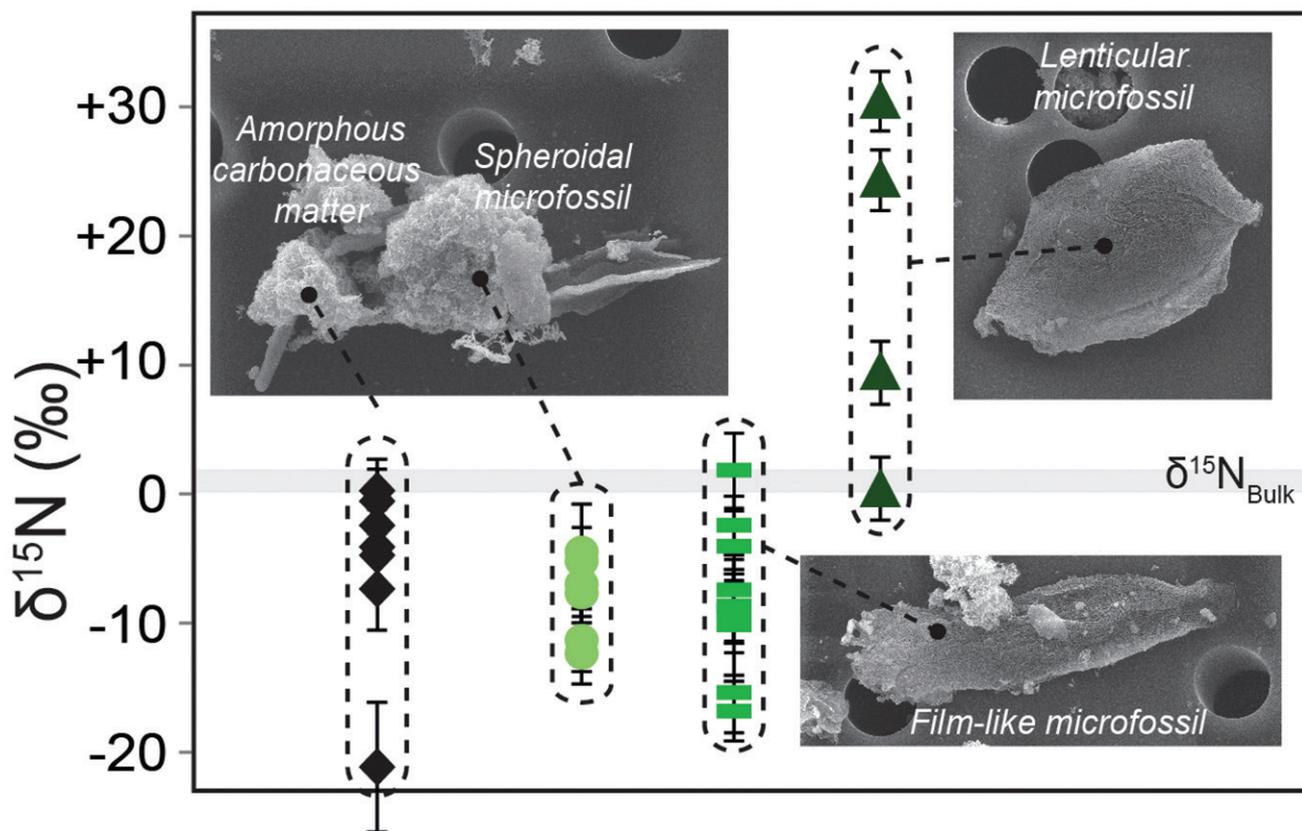


Figure 1 Nitrogen isotopic composition of amorphous carbonaceous matter, and of spheroidal, film-like and lenticular-like microfossils from the 3.0 Ga Farrel Quartzite formation. $\delta^{15}\text{N}_{\text{Bulk}}$ values are provided for comparison with $\delta^{15}\text{N}_{\mu\text{m}}$ values.

Material and Methods

Organic-walled microfossils were isolated by acid maceration using a mixture of hydrofluoric and hydrochloric acids. In the acid maceration residue, we identified lenticular, spheroidal and film-like microfossils following the previously reported taxonomy (Sugitani *et al.*, 2007; see also Supplementary Information). In the absence of unequivocal morphological features, the other organic particles were assigned to the “amorphous carbonaceous matter” group.

From a morphological point of view, previous investigations indicate that acid maceration does not affect the morphological integrity of organic-walled microfossils from the Farrel Quartzite (Grey and Sugitani, 2009; Delarue *et al.*, 2017). From a geochemical point of view, preservation of N content is highly variable across individual microfossils isolated from the Farrel Quartzite (Delarue *et al.*, 2017). This suggests that acid maceration does not cause any significant hydrolysis of N-bearing compounds, which would likely have homogenised the N/C atomic ratios of microfossils through the preferential degradation of chemically labile N organic compounds. Similarly, large variations in $\delta^{15}\text{N}_{\mu\text{m}}$ are observed among the microfossils, which can only be accounted for by Rayleigh distillation. The latter cannot have occurred during the acid treatment, as N isotope exchange through Rayleigh distillation cannot take place between solids (N in microfossils) and a fluid (N dissolved organic compounds in acid solution). Therefore, it is unlikely that acid maceration induced significant modification of both N/C atomic ratios and N isotopic compositions determined at the scale of individual microfossil by NanoSIMS.

Results

The bulk nitrogen isotope composition (noted $\delta^{15}\text{N}_{\text{Bulk}}$) measured on Farrel Quartzite OM ranges between +0.3 ‰ and +2.2 ‰ (see Supplementary Information; $n = 3$; mean $\delta^{15}\text{N}_{\text{Bulk}} = 1.0 \pm 1.1$ ‰; Fig. 1 and Table S-2). These $\delta^{15}\text{N}_{\text{Bulk}}$ values are consistent with the average $\delta^{15}\text{N}$ value calculated from all values determined by NanoSIMS on amorphous carbonaceous matter and microfossils isolated from the mineral matrix ($\delta^{15}\text{N}_{\mu\text{m}} = -3.9 \pm 11.5$ ‰; $n = 27$; Fig. 1 and Table S-2). However, at the microscale, the $\delta^{15}\text{N}_{\mu\text{m}}$ values measured on amorphous carbonaceous matter and microfossils vary from -21.6 ± 5.1 ‰ to $+30.7 \pm 2.3$ ‰. Amorphous carbonaceous matter alone shows $\delta^{15}\text{N}_{\mu\text{m}}$ values ranging between -21.6 ± 5.1 ‰ and 0.0 ± 2.5 ‰ ($n = 8$; Fig. 1). Film-like ($n = 9$) and spheroid-like microfossils ($n = 6$) exhibit mostly negative $\delta^{15}\text{N}_{\mu\text{m}}$ values ranging from -17.2 ± 2.3 ‰ to $+1.6 \pm 2.9$ ‰, while lenticular microfossils are characterised by positive $\delta^{15}\text{N}_{\mu\text{m}}$ values ranging from $+0.5 \pm 2.4$ ‰ to $+30.7 \pm 2.3$ ‰ ($n = 4$; Fig. 1). Based on their nitrogen isotope composition, there is thus a clear discrimination between lenticular microfossils on the one hand and spheroid- and film-like microfossils on the other hand.

Discussion

The quasi-null $\delta^{15}\text{N}_{\text{Bulk}}$ values measured on Farrel Quartzite OM hide unexpectedly large $\delta^{15}\text{N}_{\mu\text{m}}$ variations in isolated microfossils (Fig. 1 and Table S-2). The average of $\delta^{15}\text{N}_{\mu\text{m}}$ values is close to $\delta^{15}\text{N}_{\text{Bulk}}$. This observation can be explained if the bulk value integrates a diversity of organic particles with variable $\delta^{15}\text{N}$.

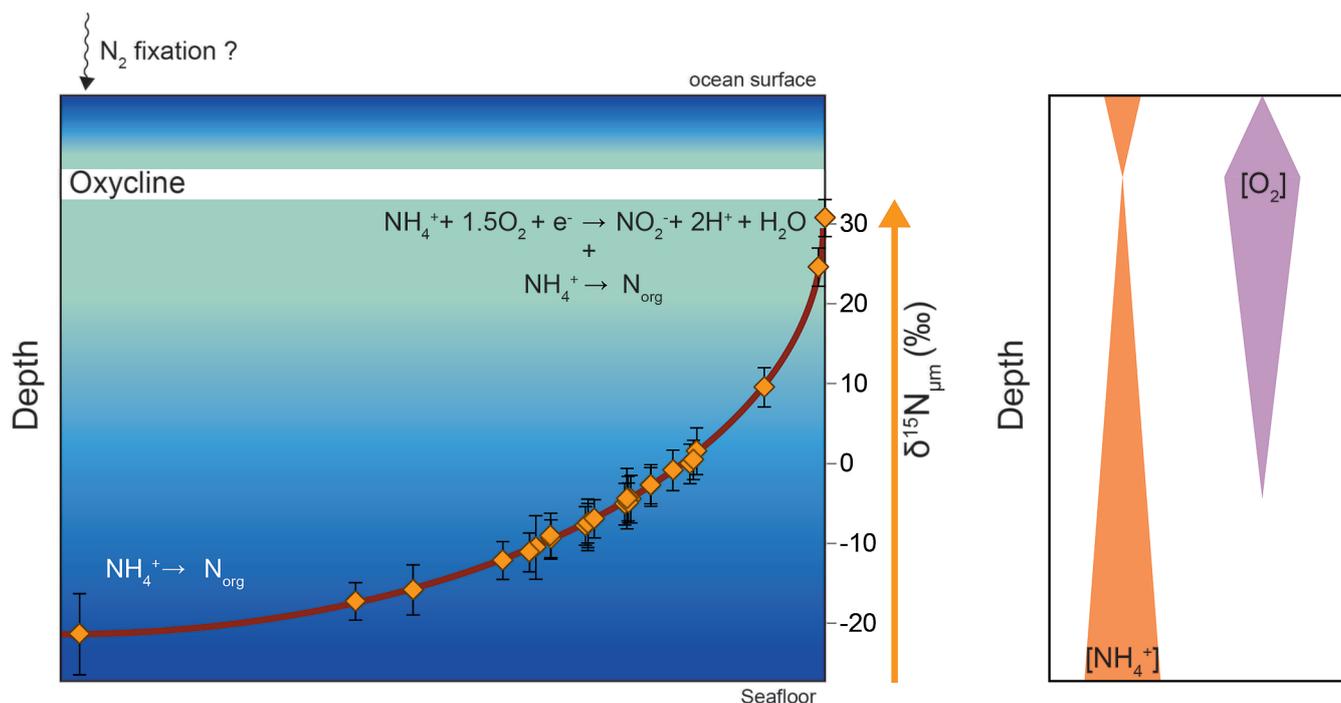


Figure 2 Cartoon illustrating the sedimentary conditions of the Farrel Quartzite formation. The blue gradient in the water column indicates change in NH_4^+ concentration from high (dark) to low (light) concentrations in anoxic zones. $\delta^{15}\text{N}_{\mu\text{m}}$ values determined at the scale of individual microfossil and organic particle are represented by orange diamonds. In the proposed model, negative $\delta^{15}\text{N}_{\mu\text{m}}$ values reflect assimilation of NH_4^+ from benthic efflux whereas positive $\delta^{15}\text{N}_{\mu\text{m}}$ values reflect ^{15}N Rayleigh distillation of NH_4^+ upward through ammonia oxidation when NH_4^+ concentrations were too low to allow the isotopic fractionation of ammonia assimilation to be expressed (see main text).

Before relating measured $\delta^{15}\text{N}_{\mu\text{m}}$ values to potential metabolic effects, it is a prerequisite to ensure that biotransformation and fossilisation processes did not significantly alter the original N isotopic composition. Biomass degradation can cause $\delta^{15}\text{N}$ variations of ca. 3–4 ‰ (Lehmann *et al.*, 2002), while metamorphism up to greenschist facies, as underwent by the Farrel Quartzite samples, can only induce restricted $\delta^{15}\text{N}$ modification of up to ~1 to 2 ‰ (Ader *et al.*, 2006; see Supplementary Information for further details). It thus seems unlikely that secondary processes have largely modified $\delta^{15}\text{N}_{\mu\text{m}}$ values, which, in turn, may be linked with N metabolism(s) in the water column.

In the N cycle, only Rayleigh fractionation has been reported to yield large variations of $\delta^{15}\text{N}$ values through the partial consumption of NO_3^- or of NH_4^+ , leaving the residual dissolved inorganic nitrogen (DIN) enriched in ^{15}N (Sigman *et al.*, 2009). As the NO_3^- concentration was negligible in Archean oceans (Falkowski and Godfrey, 2008), it is unlikely that partial conversion of NO_3^- into N_2 occurred and gave rise to the observed $\delta^{15}\text{N}_{\mu\text{m}}$ variations. Thus, only the partial consumption of NH_4^+ seems to be an acceptable mechanism to explain the large variation in $\delta^{15}\text{N}_{\mu\text{m}}$ values, consistent with an Archean DIN marine reservoir dominated by NH_4^+ (Thomazo *et al.*, 2011). Three different marine metabolisms (referred to as *chemolithoautotrophy*) could then be involved in the nitrogen isotope cycle: (i) NH_4^+ assimilation ($\text{NH}_4^+ \rightarrow \text{N}_{\text{org}}$), (ii) anaerobic NH_4^+ oxidation (anammox; $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}$) and (iii) aerobic NH_4^+ oxidation ($\text{NH}_4^+ + 1.5 \text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$). The N isotope fractionations (noted ϵ , with $\epsilon \approx \delta^{15}\text{N}_{\text{NH}_4^+} - \delta^{15}\text{N}_{\text{Product}}$) associated with these processes have been determined experimentally, and range from +4 ‰ $\leq \epsilon \leq +27$ ‰ for (i) and +14 ‰ $\leq \epsilon \leq +19$ ‰ for (iii), respectively (Hoch *et al.*, 1992; Casciotti *et al.*, 2003). Anammox is not further considered here since its antiquity is still a controversial issue and the resulting $\delta^{15}\text{N}_{\text{Bulk}}$ remains unknown (Stueken *et al.*, 2016; see also Supplementary Information).

In modern environmental settings, $\delta^{15}\text{N}$ variations rarely show large variations. However, in the modern Black Sea environment, large $\delta^{15}\text{N}$ variations ranging from negative up to positive values have been observed to be associated with chemolithoautotrophy along a spatial redox gradient that promotes the progressive oxidation of NH_4^+ in the deep and suboxic level of the Black Sea (Coban-Yildiz *et al.*, 2006). According to this observation, we propose that mineralisation of OM yielded large amounts of NH_4^+ in the deeper level of the ocean (Fig. 2). Preferential assimilation of $^{14}\text{NH}_4^+$ from this benthic efflux may explain the negative $\delta^{15}\text{N}_{\mu\text{m}}$ values measured on spheroids and film/filament-like microfossils. The latter are thus likely remnants of benthic microbial mats, in agreement with their morphology (Westall *et al.*, 2006).

We propose that assimilation of NH_4^+ from benthic efflux in the deep water column led to both a decrease in NH_4^+ concentration and a progressive enrichment in $^{15}\text{NH}_4^+$ in the upper levels of the water column (Fig. 2). Partial NH_4^+ assimilation can yield ϵ values down to -27 ‰ when NH_4^+ concentration is higher than 20 μM , while lower NH_4^+ concentrations are associated with lower ϵ values close to -4 ‰ (see Stueken *et al.*, 2016 and references therein). In this context, such lower concentrations are unlikely to yield $\delta^{15}\text{N}_{\mu\text{m}}$ values up to +31 ‰ as recorded in the lenticular microfossils. These microfossils are interpreted as remnants of pelagic microorganisms based on both their morphology (Sugitani *et al.*, 2007; Oehler *et al.*, 2010) and their carbon isotope composition (House *et al.*, 2013). We then propose that aerobic NH_4^+ oxidation drove ^{15}N enrichment of NH_4^+ upward in the water column (Fig. 2). In this scenario, the positive $\delta^{15}\text{N}_{\mu\text{m}}$ values might be regarded as a redox signal as previously reported (Coban-Yildiz *et al.*, 2006; Granger *et al.*, 2011; Morales *et al.*, 2014). However, to be recorded in OM, aerobic NH_4^+ oxidation should have been coupled with NH_4^+ assimilation, leaving lenticular organic-walled microfossils enriched in ^{15}N (Fig. 2).

In view of the $\delta^{13}\text{C}$ (House *et al.*, 2013) and of the present $\delta^{15}\text{N}$ values along with morphological evidence, this proposed scenario, which combines morphological evidence with C and N isotopic data, suggests that life thrived across the entire water column down to the seafloor, and points to the early existence of aerobic metabolism 3 billion years ago. Since aerobic NH_4^+ oxidation implies the occurrence of free dissolved molecular O_2 in the water column (Wang and Yang, 2004), this finding echoes previous geochemical arguments pointing toward mid-Archean oxygen oases and, therefore, biological O_2 production by oxygenic photosynthesis in stratified mid-Archean oceans (Crowe *et al.*, 2013; Lyons *et al.*, 2014; Planavsky *et al.*, 2014; Satkoski *et al.*, 2015).

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Additional Information

Supplementary Information accompanies this letter at <http://www.geochemicalperspectivesletters.org/article1816>.



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