Research paper

The origin of $\text{NO}_3^−$ and $\text{N}_2$ in deep subsurface fracture water of South Africa

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**ABSTRACT**

Deep (>0.8 km depth) fracture water with residence time estimates on the order of several Ma from the Witwatersrand Basin, South Africa contains up to 40 μM $\text{NO}_3^−$ up to 50 mM N$_2$ (90% times air saturation at surface) and 1 to ~400 μM NH$_3$/NH$_4^+$+ trapped in fluid inclusions or NH$_4^+$+ concentrations of the pore water and fluid inclusion leachate recovered from the low porosity quartzite, shale and metavolcanic units were ~10$^4$ times that of the fracture water. The δ$^{15}$N–$\text{NO}_3^−$ and δ$^{15}$N–NH$_4^+$ of the pore water and fluid inclusion leachate, however, overlapped that of the fracture water with the δ$^{15}$N–$\text{NO}_3^−$ ranging from 2 to 7% and the δ$^{15}$N–$\text{NO}_3^−$ ranging from 20 to 50%. The δ$^{15}$N–$\text{NO}_3^−$ of the mining water ranged from 0 to 16% and its δ$^{15}$N–$\text{NO}_3^−$ from 0 to 14%, making the mining water $\text{NO}_3^−$ isotopically distinct from that of the fracture, pore and fluid inclusion water. The δ$^{15}$N–$\text{N}_2$ of the fracture water and the δ$^{15}$N–$\text{N}_2$ from the cores ranged from ~5 to 10%, and overlapped the δ$^{15}$N–$\text{N}_2$. The δ$^{15}$N–$\text{NH}_4^+$ of the fracture water and pore water $\text{NH}_4^+$ ranged from ~15 to 4%. Although the $\text{NO}_3^−$ concentrations in the pore water and fluid inclusions were high, mass balance calculations indicate that $\text{NO}_3^−$ accounts for ~10% of the total rock N, whereas $\text{NH}_4^+$ trapped in fluid inclusions or $\text{NH}_4^+$ present in phyllosilicates account for ~90% of the total N. Based on these findings, the fluid inclusion $\text{NO}_3^−$ appears to be the source of the pore water and fracture water $\text{NO}_3^−$ rather than palaeometeoric recharge or mining contamination. Irradiation experiments indicate that radiolytic oxidation of $\text{NH}_4^+$ to $\text{NO}_3^−$ can explain the fluid inclusion $\text{NO}_3^−$ concentrations and, perhaps, its isotopic composition, but only if the $\text{NO}_3^−$ did not attain isotopic equilibrium with the hydrothermal fluid 2 billion years ago. The δ$^{15}$N–$\text{N}_2$, δ$^{15}$N–$\text{N}_2$, and δ$^{15}$N–$\text{NH}_4^+$ suggest that the reduction of $\text{N}_2$ to $\text{NH}_4^+$ also must have occurred in the Witwatersrand Basin in order to explain the abundance of $\text{NH}_4^+$ in the strata. Although the depleted $\text{NO}_3^−$ concentrations in the fracture water relative to the pore water are consistent with microbial $\text{NO}_3^−$ reduction, further analyses will be required to determine the relative importance of biological processes in the subsurface $\text{N}$ cycle and whether a complete subsurface $\text{N}$ cycle exists.

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1. Introduction

With the discovery of widespread microbial life in the deep subsurface, the question of nutrient availability becomes critical to understanding the limits of this ecosystem. In the case of marine sediments, Lipp et al. (2008) reported that the total lipid concentration, a proxy for living biomass, was correlated with the total organic matter concentration (TOC), such that cellular densities diminished to ~10$^6$ cells g$^{-1}$ for sediment with TOC of ~0.2%. A number of recent studies have shown, however, that H$_2$-producing water–rock interactions provide the energy for subsurface autotrophic microbial metabolism deep in the earth’s crust (Stevens and McKinley, 1995; Kelley et al., 2001; Lin et al., 2005b, 2006) and that acetogens could provide the organic carbon substrate necessary to support acetoclastic
heterotrophs (Stevens and McKinley, 1995; Stevens, 1997). The principal N substrate for subsurface ecosystems could be NH₄⁺/NH₃ originating from denitrification of organic photosynthetic or from deep-seated N-containing fluids from silicates (Mysen and Fogel, 2010). Determining the origin and form of N available to the deep biosphere community is critical for understanding how it may influence and limit autotrophic microbial abundance in the deep terrestrial subsurface. Significant N₂ concentrations have been discovered in gas reservoirs < 1 km depth that are typically associated with high ³⁷Ar, indicating a crustal origin and long subsurface residence time for N₂ (Ballentine and Sherwood Lollar, 2002). In deeper crustal environments (T = 400–700 °C) N₂ is the dominant N species unless low N₂O conditions stabilize NH₃ (Haendel et al., 1986; Bebout and Fogel, 1992). The migration of N₂ from deeper to shallower crustal levels could provide an alternative N source for deep subsurface N₂-fixing microbial communities in the absence of NH₃. Autotrophs that are capable of N₂ fixation, such as Candidatus Desulfurinis audaxviator (Chivian et al., 2008), have also been reported from deep subsurface samples despite the fact that N₂ fixation is energetically costly.

N₂O is generally accepted to be an important source of energy and nutrients for microorganisms in shallow aquifers and marine sediments, but not for deeper settings. D’Hondt et al. (2004), however, identified N₂O (≤ 30 μM) in the pore water of seafloor sediments near their contact with the underlying basaltic crust and conjectured that “the transport of O₂ and N₂O through the underlying basaltic aquifer sustains aerobic and nitrate-reducing prokaryotic communities in the deepest (11 to 35 Ma) sediments of these sites, although anaerobic communities are active in the overlying sediment”. Much higher N₂O concentrations have been reported in the pore water of ultra-high-pressure metamorphic rocks in China (~ 20 mM) (Zhang et al., 2005) and in the fluid inclusions in ~ 2.0 Ga hydrothermal veins of the Onal Au deposit in South America (≤ 15 mM) (Voicu and Hallbauer, 2005) and the Witwatersrand Basin of South Africa (~ 70 mM) (Frimmel et al., 1998). Lower N₂O concentrations, ≤ 40 μM, have also been reported for the fracture water emanating from mine boreholes at 0.8 to 3.5 km depth in the Witwatersrand Basin, which is believed to be a mixture of paleo-meteoric water and ~ 2.0 Ga hydrothermal fluid (Onstott et al., 2006). Caution must be exercised, however, when examining N₂O in a mining environment as Onstott et al. (2003), Stros-Gascony and Gascony (1998), Gascony and Thomas (1997) and Stotler et al. (2009) have all stressed that the N₂O may originate from the explosives used in mining. A possible origin for these reported deep N₂O occurrences is suggested by irradiation studies of NH₄⁺-bearing water solutions that have measured the production of NH₂O₂ (Pagsberg, 2001), N₃⁻ (Rigg et al., 1952), NO₂⁻ (Dwebedy et al., 1996) and NO₃⁻ (Shin et al., 2001). If NO₂⁻ and NO₃⁻ are produced within the subsurface from irradiation of NH₃, then they could provide an alternative source of N, an energy-rich electron acceptor and could, in principal, sustain a complete subsurface N cycle.

This study, therefore, sought to determine to what extent the three principal N species, N₂O, NH₃ and N₂, observed in the Witwatersrand Basin fracture water were derived from mining contamination, from the surface biosphere by recent meteoric recharge, from Archean organic-rich shale, or from a ~ 2.0 Ga hydrothermal fluid. This was done by performing N and O isotopic analyses on cores collected from the lithological units in contact with these fractures, the organic-rich Kimberly Shale, the Ventersdorp Supergroup metaultramafics and the Witwatersrand Supergroup quartzite. In addition NH₃ irradiation experiments on anaerobic water were performed to test the possibility of radiolytically produced NO₃⁻.

2. Geologic setting

The Witwatersrand Basin, located in the center of the Kaapvaal Craton, is 300 km long along a NE–SW axis and 100 km wide, and formed 3.1 to 2.7 byr ago. The core samples for this study were collected during exploration coring from the Evander and Kloof Au mines. Evander Au Mine is located on the easternmost edge of the Witwatersrand Basin where the 2.9 Ga Witwatersrand Supergroup is overlain by the 2.7 Ga old Ventersdorp Supergroup. At this location the Upper Witwatersrand Group stratigraphic column includes the 60 m thick Booyens/Kimberley Shale Formation underlain by the 30 m thick Krugersdorp Quartzite Formation and overlain the 6 m thick Kimberley reef of the 14 m thick Kimberley–Elsburg Quartzite Formation (Tweedie, 1986). Kloof Au Mine (S 26°26′; E 27°34′) is located southwest of Johannesburg where the contact between the Ventersdorp Supergroup and the Upper Witwatersrand Group is mined. Metamorphic mineral assemblages are consistent with lower greenschist facies with maximum temperatures of 300 ± 50 °C and pressures of 2.5–3 kbar (Phillips, 1988; Wallm and Meyer, 1990). Fluid inclusion studies indicate a maximum temperature of 250 °C (Hallbauer and Kable, 1979; Hallbauer and Von Gehlen, 1983; Hallbauer, 1986).

3. Materials and methods

3.1. Core collection

On August 13, 2002 at the 24th level, 1.83 km bls., of #2 shaft of Evander Au mine (S 26°30′36″; E 29°08′40″) a very short exploratory borehole was drilled downwards for 20 m through the Kimberley reef and the resulting quartzite and conglomerate rock core, labeled EV224, was collected. No fracture water was associated with the EV224 borehole; only mining water was obtained. On October 25, 2002 during an exploratory coring operation on the 18th level of the #5 shaft of Evander Au mine (S 26°27′13″; E 29°03′56″), 1.83 km bls., a 2 m core of the Kimberley–Elsburg Quartzite Formation, labeled as EV818, was collected from a vertical borehole at a depth of 60 m below the tunnel floor. This sampling was followed by collection of numerous fracture water samples. Samples were collected of the calcareous siltstone and the upper laminated sections of the Kimberley Shale, as well as from the quartzite and conglomerate overlying and underly ing it. These samples were taken from an exploration core through the Upper Witwatersrand Group drilled from #3 shaft of Evander Au mine (S 26°31′5″; E 29°07′40″) at a depth comparable to that of the other cores, and, hence, referred to as core EV3.

On July 31, 2003, several core samples along with fracture water and mine water samples were collected from a horizontal borehole drilled into a water bearing fracture at the 39th level, 3.1 km bls., of the #7 shaft of Kloof Au mine (S 26°26′42″; E 27°3′59″). Rock cores, labeled KL739, were collected from the lower greenschist facies, tholeiitic, Ca-rich, porphyritic flood basalt of the Klipriviersberg Group of the Ventersdorp Supergroup (van der Westhuizen et al., 1991).

All freshly drilled rock cores were immediately stored in sterile Whirlpak bags and frozen on dry ice. The rock cores were subsequently shipped frozen to Princeton University where they were stored in a −80 °C freezer. EV3 was collected from the Evander Au mine core library after storage for several weeks and, thus, was not suitable for pore water analyses, but as it is the only organic carbon bearing shale unit in the Witwatersrand strata, it was collected to measure the isotopic composition of any organic N.

3.2. Fracture water sample collection

Mining water was collected from the EV224, and mining water and fracture water were collected from the EV818 and KL739 coring operations on August 13, 2002, October 25, 2002 and July 31, 2003, respectively. An autoclaved Margot-type expansion plug connected to a sampling manifold constructed of Delran plastic and equipped with quick-connect release valves and sterile tubing with a syringe tip was used to collect water and gas samples without exposing them to the mine air, following the procedures described in Onstott et al. (2006).
3.3. Pore water, fluid inclusion, exchangeable NH₄⁺ extractions

Rock cores were 45 to 50 mm in diameter and were split into 20 g nugget (internal) and 20 g paring (outer rim) portions using a custom-made rock splitter. Within an anaerobic glove bag, the nuggets and parings were aseptically broken into smaller pieces and placed into 50 mL centrifuge tubes with 40 mL of deionized, degassed, sterile water. The samples were placed on a rotisserie shaker in the anaerobic glove bag for 5 days. This pore water leachate was frozen and saved for concentration (NH₄⁺) and isotopic analysis (δ¹⁵N–NO₃⁻, δ¹⁸O–NO₃⁻ and δ¹⁵N–NH₄⁺). The rock fragments were subsequently pulverized, sieved at mesh number 200 (pore size of 75 μm) and placed in 40 mL of deionized, degassed water and set on the shaker for 5 days with all steps taking place without rock to determine the trace amounts of NO₃⁻ present in the procedure. Multiple tests revealed that certain 50 mL centrifuge tube caps contained significant NO₃⁻ contamination, and the caps that yielded no detectable NO₃⁻ contamination were then selected for the extraction process.

To determine the exchangeable NH₄⁺ in the residual powder, 20 mL of degassed, pH 7.2 M KCl was added. The solution was shaken for 1 h in the anaerobic glove bag, centrifuged for 5 min at 3400 rpm, the leachate removed, the powder rinsed with 10 mL of deionized, degassed H₂O and centrifuged for 15 min. The deionized, degassed H₂O step was repeated. The DI H₂O rinses were added to the 20 mL KCl solution for a total volume of 40 mL.

3.4. Dissolved N species analyses

All NO₃⁻, NO₂⁻ and NH₄⁺ concentrations for mining water, fracture water, pore water and fluid inclusion extract samples were analyzed at Princeton University (Tables 3–5). NO₃⁻ and NO₂⁻ were measured by ion chromatography (DX-320, Dionex, Sunnyvale, CA) using EG40 and LC25 columns and an AS40 autosampler with detection limits of 0.2 and 0.1 μM, respectively. Pore water and fluid inclusion extract NO₃⁻ concentrations were also measured in duplicate by reduction to nitric oxide (NO) in a heated solution of acidic V²⁺ followed by chemiluminescent detection of the NO (Broman and Hendrix, 1989) with a detection limit of 0.01 μM for 2–3 mL injections. NO₃⁻ concentrations were determined using the Nesslerization method (Eaton et al., 1995) with detection limits of 1 μM and a relative standard deviation of 10% based on a four point calibration curve. NO₃⁻, NO₂⁻ and NH₄⁺ measurements for the extracts were converted into pore water and fluid inclusion concentrations using the following relationship,

\[ [N_x]_{PW,FI} = \left( \frac{W_i}{W_f} \right) \left( [N_x]_{linear}/MW_x \right) \left( \frac{n_{rock}}{\theta_{rock}} \right) \]

(1)

where \([N_x]_{PW,FI}\) is the concentration of NO₃⁻, NO₂⁻ or NH₄⁺ in the pore water or fluid inclusion (in mM) (Table 3), \(W_i\) is the volume of the DI water extract (40 mL), \(W_f\) is the mass of the rock being extracted (2 to 6 g), \([N_x]_{linear}\) is the NO₃⁻, NO₂⁻ or NH₄⁺ concentration in the extract (in mg L⁻¹), \(n_{rock}\) is the density of the rock (in grams cm⁻³), \(\theta_{rock}\) is the

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Table 1

FRACTURE WATER ANALYSES

<table>
<thead>
<tr>
<th>Sample</th>
<th>δ¹⁵N NO₃⁻ (‰)</th>
<th>Salinity (‰)</th>
<th>NO₃⁻ (μM)</th>
<th>δ¹⁸O NO₂⁻ (‰)</th>
<th>NH₄⁺ (μM)</th>
<th>δ¹⁸O NH₄⁺ (‰)</th>
<th>Na (mM)</th>
<th>δ¹⁸O H₂O (‰)</th>
<th>Age (Ma)</th>
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<td>BE16</td>
<td>0.9 ± 0.1</td>
<td>69.0</td>
<td>1.02</td>
<td>1.3 ± 0.1</td>
<td>1.04 ± 0.01</td>
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<td>94</td>
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<td>1.22 ± 0.04</td>
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<td>MS151</td>
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<td>592</td>
<td>2.4 ± 0.1</td>
<td>37.5 ± 0.33</td>
<td>9.9</td>
<td>24.9</td>
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<td>860</td>
<td>2.9 ± 0.1</td>
<td>4.08 ± 0.01</td>
<td>2.3</td>
<td>20.4</td>
<td>6.52 ± 1.6</td>
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<td>2.9 ± 0.1</td>
<td>27.3</td>
<td>5.8 ± 0.1</td>
<td>0.20 ± 0.01</td>
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and introduced into isotope ratio mass spectrometer (ThermoFinnigan). In this procedure, NO$_3^-$ was typically ±0.5 ‰ and δ$^15$N was ±0.5 ‰. NO$_3^-$ was quantitatively converted to N$_2$O using a strain of denitrifying bacteria (Pseudomonas aureofaciens) a naturally occurring mutant that lacks the ability to reduce N$_2$O. The resulting N$_2$O was extracted, purified and measured for δ$^15$N for samples with more than 1 μg of N and for δ$^13$C-C for samples with less than 1 μg of N and for δ$^13$C-C was ±0.1‰ VPDB. Only the δ$^15$N–N of those samples with > 0.5 μg of N are considered precise enough for constraining the isotopic composition of the total rock. N and total carbon, TC in moles. The concentration was determined by subtracting the OC from the TC; and the porosity (or fluid inclusion fractional volume) of the rock and MW$_x$ is the molecular weight of the N species (mg/MM). Rock density and porosity were determined on seven representative 1 in. diameter subcores by Core Petrophysics Inc. (Houston, TX). Fluid inclusion fractional volume was based on thin section examination.

3.5. NO$_3^-$ isotopic measurements

The δ$^15$N–NO$_3^-$ and δ$^18$O–NO$_3^-$ were analyzed using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002). In this procedure, NO$_3^-$ was quantitatively converted to N$_2$O using a strain of denitrifying bacteria (Pseudomonas aureofaciens) a naturally occurring mutant that lacks the ability to reduce N$_2$O. The resulting N$_2$O was extracted, purified and introduced into isotope ratio mass spectrometer (ThermoFinnigan DeltaPlus). Each analysis was referenced to injections of N$_2$O from a pure N$_2$O gas cylinder and then calibrated using an internationally accepted NO$_3^-$ reference material (IAEA-N3, with a δ$^15$N of 4.7 ‰) and a δ$^{10}$O of 25.6‰, VSMOW) with corrections for water–oxygen atom incorporation using the same reference material dissolved in 18O-enriched water (Casciotti et al., 2002). Analytical precision at the time of analysis was typically ±0.5 ‰ for δ$^{18}$O and ±0.2 ‰ for δ$^15$N (Tables 1–3 and 5).

3.6. Solid N and C isotopes and total U analyses

Rock core chunks were crushed under a He atmosphere in an anaerobic chamber and 15 to 20 mg were transferred to Sn capsules, which were then sealed. Samples were then introduced into the autosampler (A2100) of a Carlo Erba Instruments, NA 2500 series, elemental analyzer (EA) (Wooller et al., 2001), where N$_2$ and CO$_2$ are obtained by combustion at 1020 °C. The elemental composition is reported as total nitrogen, N and total carbon, TC in moles. The δ$^15$N and δ$^{13}$C of the total N and C of the rock, δ$^15$N–N and δ$^{13}$C–C, were measured using continuous-flow, stable isotope ratio mass spectrometry (Finnigan MAT, DeltaAutoXL at the Geophysical laboratory, CIW) relative to internal working gas standards (Wooller et al., 2003; Papineau et al., 2009). Precision for δ$^15$N–N was ±0.5‰. AIR for samples with more than 1 μg of N and ±2 ‰ for AIR for samples with less than 1 μg of N and for δ$^{13}$C–C was ±0.1‰ VPDB. Only the δ$^15$N–N of those samples with > 0.5 μg of N are considered precise enough for constraining the isotopic composition of the total rock. N. Samples were then acidified with ultra-pure 6 N HCl, spun down, rinsed with DI water, dried, weighed and combusted in order to obtain the concentration and the δ$^15$N of the organic carbon, δ$^{13}$C–OC. The concentration was determined by subtracting the OC from the TC, and the δ$^13$C of the inorganic carbon, δ$^{13}$C–IC, was determined from the isotopic mass balance (Table 4).

An 80 mg portion of pulverized paring and nugget was dried overnight in an oven at 120 °C. LIBO$_3$ powder was added to the sample at a 5:1 ratio (LIBO$_3$:sample). After the powders were mixed carefully they were transferred to a pre-ignited graphite crucible, which was then placed in an oven at 1100 °C for 10 min. The molten sample was then quickly poured into a 250 mL Teflon beaker with 40 ml 2:25 HNO$_3$ and a stir bar, placed on a stirrer and covered with a watch.
glass for 5 min. The sample was then transferred to a 50 mL volumetric flask and the volume made up to 50 mL with DI water. The U concentration of the solution was measured by ICP-OES (Perkin Elmer) and determined using both standards (IAEA-N1 and IAEA-N2) was applied to obtain final \(\delta^{15}\text{N} \) composition of the procedural blank derived using both standards (IAEA-N1 and IAEA-N2) was applied to obtain final \(\delta^{15}\text{N} - \text{NH}_4^+ \) values.

### 3.7. Nitrogen isotopic analyses of \(\text{NH}_4^+\)

\(\text{NH}_4^+\) was extracted and analyzed using a protocol described by Houlton et al. (2007) that merges the “passive ammonia diffusion” method for \(\text{NH}_4^+\) collection described in Sigman et al. (1997) with the “persulfate/denitrifier” method for analyzing \(\delta^{15}\text{N}\) of reduced N forms (Knapp et al., 2005). Briefly, \(\text{NH}_4^+\) traps were constructed by sealing pre-combusted GP-T filters (with 20 μL of 2 N HSO₄ added to each filter) inside Teflon tape envelopes. Between 5 and 40 mL of sample was aliquoted to acid-washed 50 mL Falcon centrifuge tubes. To avoid rupture of the traps due to the osmotic pressure gradient between the acidified filters inside the Teflon envelope and the ambient water, a NaCl solution (NaCl was pre-combusted at 650 °C) was added to a final concentration of 0.6 M. The Teflon traps were then added to each tube, followed immediately by addition of 20 to 200 mg, depending on a sample volume, of MgO, pre-combusted at 650 °C, so that the final pH of the fluid sample was ~9.7. The tubes were then immediately capped and incubated at room temperature on a shaker for 8–20 days.

After the incubations, the traps were removed from the tubes, briefly rinsed in deionized water of pH ~3, and stored in tightly capped glass for 5 min. The sample was then transferred to a 50 mL volumetric flask and the volume made up to 50 mL with DI water. The U concentration of the solution was measured by ICP-OES (Perkin Elmer) and converted to mol U/kg rock (Table 4).

### 3.8. \(\text{N}_2\) compositional and isotopic analyses

Compositional and isotopic analyses of \(\text{N}_2\) gas samples dissolved in the fracture water were performed at the Stable Isotope Laboratory at the University of Toronto using the method of Ward et al. (2004). A Varian 3800 GC equipped with a micro-thermal conductivity detector (μTCD) and a Varian Molecular Sieve 5A PLOT fused silica column (25 m x 0.53 mm ID) was used to determine the \(\text{N}_2\) concentrations. All analyses were run in triplicate and mean values are reported. Reproducibility for triplicate analyses was ±5%.

The concentrations of dissolved gases were derived from the gas volume abundance, the ratio of water to gas flow rates and Henry’s Law constants following the procedure of Andrews and Wilson (1987). The \(\text{N}_2\) data was first corrected for air contamination based on the \(\text{O}_2\) concentration, which was assumed to be an artifact of sampling, since \(\text{Eh}\) measurements indicated highly reducing borehole environments. \(\text{N}_2/\text{Ar}\) or \(\text{O}_2/\text{Ar}\) ratios cannot be used as a second crosscheck on contamination due to the radiogenic-rich Ar isotopic signature and the depleted atmospheric Ar concentrations reported by Lippmann et al. (2003). The air correction was made using the following equation:

\[
\%\text{N}_2/\text{CORR} = \%\text{N}_2/(\text{MEAS} - 3.73\cdot(\%\text{O}_2/(\text{MEAS}))
\]

(2)

The dissolved \(\text{N}_2\) concentration was calculated according to the following equation,

\[
\text{N}_2 = \left(\frac{V_g}{V_p}\right) \cdot \%\text{N}_2/(\text{CORR}) \cdot 10^{-2} \cdot P/(0.082054 \cdot T) + \%\text{N}_2/(\text{CORR}) \cdot 10^{-2} \cdot P/18000
\]

(3)

where \(V_g\) is in mol L⁻¹, \(V_p\) and \(V_g\) are the gas and water flow rates, respectively, \(P\) is the ambient pressure underground in atm, \(T\) is the ambient water temperature in K and \(H\) is the Henry’s Law constant for \(\text{N}_2\) in mol of \(\text{N}_2\) (mol of \(\text{H}_2\)O)⁻¹ atm⁻¹ (Table 1).

The N isotope analyses were performed using a Varian 3400 Chromatograph coupled with a Finnigan 252 mass spectrometer (GC-IRMS). The \(\text{N}_2\) was separated from other gases using a Varian Molecular Sieve 5A PLOT fused silica column (25 μm x 0.53 mm ID.) and a He flow measured \(\delta^{15}\text{N}\) of IAEA-N2 (reported \(\delta^{15}\text{N} = 20.4 ± 0.2\%\)) was 17.5 ± 0.1% (\(n = 2\)) and 18.8 ± 0.2% (\(n = 2\)) for standards containing 200 and 400 nmol of \(\text{N}_2\), respectively, while average measured \(\delta^{15}\text{N} \) of IAEA-N1 (reported \(\delta^{15}\text{N} = 0.4 ± 0.1\%\)) was 1.2 ± 0.1% (\(n = 2\)) and 0 ± 0.1% (\(n = 2\)). Average \(\delta^{15}\text{N}\) composition of the procedural blank determined using both standards (IAEA-N1 and IAEA-N2) was applied to obtain final \(\delta^{15}\text{N} - \text{NH}_4^+\) values.

### Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rock type</th>
<th>Bulk density</th>
<th>Por. %</th>
<th>U (ppm)</th>
<th>Total N°</th>
<th>(\delta^{15}\text{N} ) (‰)</th>
<th>Organic carbon°</th>
<th>(\delta^{15}\text{C} ) (‰)</th>
<th>Inorganic carbon°</th>
<th>(\delta^{15}\text{C} ) (‰)</th>
</tr>
</thead>
</table>
| EV 3 KS T2Z-Paleo | Kimberley Shale-silt | n.a. | n.a. | 58 ± 11 | 3.79 ± 0.47 | 0.54 ± 0.35 | 11.9 | −28.37 | < dl | n.a.
| EV 3 KS I 9 | Kimberley Shale-Up. Calc. | n.a. | n.a. | 67 ± 6 | < dl | n.a. | 7.39 | −29.97 | 21.3 | −5.62
| EV 3 KS 24.4 | Kimberley Shale-up calc. silt. | 2.76 | 0.5 | 89 ± 14 | 2.14 ± 0.16 | 5.61 ± 4.60 | 5.06 ± 0.24 | −30.17 ± 0.15 | 1.14 ± 0.54 | −6 ± 11
| EV 3 KS T2I | Kimberley Shale-silt | n.a. | n.a. | 144 ± 3 | 2.42 ± 0.23 | 4.93 ± 0.63 | 2.03 ± 0.21 | −25.57 ± 0.15 | 1.38 ± 0.44 | 4.1 ± 2.7
| EV 3 KS Paleo-0.8 | Kimberley Shale-up. laminated | 2.78 | 1.0 | 108 ± 20 | 3.57 ± 0.18 | 6.65 ± 3.83 | 1.53 | −26.4 | 0.954 | −3.83
| EV 3 Cong | LK1 reef chert pebble cong. | 2.66 | 0.7 | 83 ± 6 | < dl | n.a. | 1.68 | −24.16 | 1.39 | −25.92
| EV 3 Qlite | LK1 quartzite | 2.64 | 0.9 | 19 ± 9 | < dl | n.a. | 1.06 | −25.6 | 0.521 | −19.91
| EV 818 | Quartzite | 2.70 | 0.6 | 32 ± 13 | 1.86 ± 0.49 | 9.3 ± 12.46 | 1.81 | −25.5 | < dl | n.a.
| EV 224 | Quartzite | 2.71 | 0.6 | 32 | 1.14 ± 0.88 | −6.2 ± 8.06 | 2.08 | −24.4 | < dl | n.a.
| KL 739 | Metavolcanic w/ calcite veins | 2.73 | 0.7 | 35 ± 18 | 1.79 ± 0.93 | 5.2 | 2.99 ± 0.96 | −25.38 ± 0.27 | 124 ± 86 | −8.3 ± 1.0

° Total N ranged from 16 to 53 ppm.

° \(\delta^{15}\text{N}\) values for Total N are expressed in ‰ with respect to atmospheric \(\text{N}_2\) and \(\delta^{13}\text{C}\) for organic carbon and inorganic carbon are expressed in ‰ with respect to V-PDB.

° Organic carbon ranged from 127 to 1430 ppm.

° Inorganic carbon ranged from 63 to 2520 ppm.
rate of 1.2 mL min$^{-1}$. The temperature program started at 30 °C for 6 min and then ramped up to 230 °C at 20 °C min$^{-1}$. All N$_2$ gas analyses were run in triplicate, and mean values are reported as $\delta^{15}$N–N$_2$ with respect to atmospheric N$_2$ using laboratory characterized air working standards cross-calibrated against international IAEA nitrogen standard materials. Measured $\delta^{15}$N–N$_2$ values are reported in Table 1. To attempt to correct for air contamination (and resultant mixing of the fracture gas N$_2$ with N$_2$ associated with air contamination during sampling, as discussed above), corrected $\delta^{15}$N–N$_2$ values were calculated using the following formula,

$$\delta^{15}\text{N} = \frac{\%N_2 - d^{15}\text{N} - N_2}{\%N_2(AIR\text{,CORR})}$$

The range of corrected values (−0.9% to 5.8%) is somewhat larger than the range of measured values (−0.9% to 2.9%), but the differences are not large enough to affect the interpretations.

### 3.9. Irradiation experiments

Aqueous solutions of NH$_4$Cl were prepared using spectroscopic grade solid salts and deionized H$_2$O in varying concentrations in an anaerobic glove bag (containing 90% N$_2$; 10% H$_2$). Each solution was mixed in a 160 mL serum vial and then injected into separate 30 mL serum vials. With one exception (Solution #4 in Table 1 of Supplemental Information), they were then bubbled with Ar to expel any H$_2$ or N$_2$ in the vial. Anaerobic NaOH solution was added to some of the serum vials in order to bring their pH to 10. This converted the NH$_4^+$ to NH$_3$. Irradiations were carried out at two separate facilities, the Center for Radiological Research at Columbia Medical School and the Notre Dame Radiation Laboratory. The former had a $^{60}$Co gamma source with a LET of 0.2 and a dose rate of 0.36 Gy s$^{-1}$, whereas the latter had a $^{137}$Cs source with an LET of 6.5 and dose rate of 0.2 Gy s$^{-1}$.

Because the concentrations of radiolytically produced NO$_3^-$ were likely to be minute, extreme care was taken to avoid contamination of the solutions. Measurements of NO$_2^-$ and NO$_3^-$ concentration were performed by ion chromatography as described above (Table 2 in Supplemental Section and Table 5). Each set of samples run through the IC was preceded by ion chromatography as described above (Table 2 in Supplemental Section).

### 3.10. Irradiation models

Rigg et al. (1952) originally proposed the following equations as the first two steps in the radiolytic oxidation of NH$_3$.

$$\text{NH}_3 + \text{OH} \rightarrow \text{NH}_2 + \text{H}_2\text{O}$$  

Table 5

<table>
<thead>
<tr>
<th>Dose (kGy)$^a$</th>
<th>NO$_3^-$ (µM)</th>
<th>$\delta^{15}$N (% air)</th>
<th>$\delta^{18}$O (% VSMOW)</th>
<th>$\Delta^{18}$O–NO$_3^-$–H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.89 ± 0.01</td>
<td>1.7</td>
<td>18.1</td>
<td>24.6</td>
</tr>
<tr>
<td>30</td>
<td>242.72 ± 0.01</td>
<td>−25.4</td>
<td>9.9</td>
<td>16.4</td>
</tr>
<tr>
<td>50</td>
<td>253.85 ± 0.01</td>
<td>−23.8</td>
<td>10.4</td>
<td>16.8</td>
</tr>
<tr>
<td>70</td>
<td>222.37 ± 0.01</td>
<td>−22.1</td>
<td>8.9</td>
<td>15.3</td>
</tr>
</tbody>
</table>

$^a$ $^{137}$Cs y rays at a dose rate of 720 Gy h$^{-1}$ of a pH 7.50 mM NH$_4$Cl solution.

$^b$ Calculated assuming the $\delta^{18}$O of the milliQ DI water used in the solution was −6.5%. $\Delta^{15}$N NO$_3^-$–NH$_4$ is equal to $\delta^{15}$N of the nitrate, since the $\delta^{15}$N of the NH$_4$Cl solution −0‰.

NH$_3$ + O$_2$ → NH$_2$O$_2$  

Pagsberg (1972) used pulse radiolysis experiments to prove that NH$_4$O$_2$ radicals are formed by the reaction of NH$_3$ radicals with O$_2$ and that limited destruction of NH$_3$ does occur during the irradiation of solutions in the absence of O$_2$ by forming hydrazine, N$_{2}$H$_4$, by the following reaction.

$$\text{NH}_2 + \text{NH}_3 \rightarrow \text{N}_2\text{H}_4$$  

Dwivedy et al. (1996) detected small amounts of NO$_3^-$, although only in O$_2$ saturated solutions and only at ~4 kGy. Dwivedy et al. (1996) also observed that NO$_2^-$ yields fluctuated according to concentrations of O$_2$ and NH$_3$. T-butanol, an OH scavenger, prevented the accumulation of NO$_2^-$, signifying that reaction (5) was a necessary first step in the oxidation process. N$_2$H$_4$ was not observed as a radiolytic product in air or O$_2$ saturated NH$_3$ solutions, leading Dwivedy et al. (1996) to conclude that dimerization of NH$_2$ does not occur in the presence of O$_2$ due to the much faster formation of NH$_2$O$_2$.

As O$_2$ is depleted, however, NH$_2$O$_2$ becomes the rate-limiting variable so that the rate of reaction (8) eventually decreases. Shin et al. (2001) has proposed that H$_2$O$_2$ reacts with NO$_2^-$ to create NO$_3^-$ by the following reaction,

$$\text{H}_2\text{O}_2 + \text{NO} + \text{H}_2\text{O}$$  

4NO + H$_2$O $\rightarrow$ 2NO$_2^-$ + N$_2$O + 2H$^+$  

In this study, the final yields for NO$_2^-$ and NO$_3^-$ from irradiated NH$_4$Cl solutions of varying pH and dosage levels were used to estimate the rate of reaction (10) by comparing them to those calculated from the integration of the second-order kinetic reactions (5) through (10) over the time span of the irradiation using the Kineticus program (Table 2 of Supplemental Information). In order to apply the yields of NO$_2^-$ and NO$_3^-$ to the in situ oxidation of NH$_3$ in the subsurface, we utilized the expressions of Lin et al. (2005b) to calculate the dosage rate for the Witwatersrand Supergroup quartzite and Ventersdorp Supergroup metavolcanics from their measured U, Th and K concentration, porosity and density. Mineral–water reactions were modeled with the React module of the Geochemist’s Workbench Standard Version 8.0 software package (University of Illinois). Radiolytic reactions were defined as mineral phases, He(s), H$_2$(s), O$_2$(s), H$_2$O$_2$(s) and H$_2$O$_2$(l). The first three phases were dissolved into solution at rates predicted from the dosage rate calculated above. H$_2$O$_2$(l) was dissociated into radiolytically generated OH$^-$ and H$^+$ at the rate predicted from the dosage rate. Oxidation of NH$_3$ to NO$_3^-$ was simulated by the following first order kinetic redox reactions,

$$2\text{NH}_3 + 3.5\text{O}_2^{-} \rightarrow \text{NO}_3^{-} + \text{NO}_2^{-} + 2\text{H}_2\text{O} + 2\text{H}^+$$  

$$\text{H}_2\text{O}_2 + \text{NO}_3^{-} \rightarrow \text{NO}_3^{-} + \text{H}_2\text{O}$$  

where the reaction rates were determined from the Kineticus model.

### 4. Results and discussion

#### 4.1. Evaluating potential mining contamination

The pore water and fluid inclusion NH$_4$NH$_4$NO$_3^-$ and NO$_3^-$ concentrations of this study (Table 3) overlap those published by Frimmel et al. (1999), which were based upon crush and leach analyses of Ventersdorp Supergroup hydrothermal quartz vein samples (Fig. 1). The difference
between this study and that of Frimmel et al. (1999) is that our samples represent the Witwatersrand and Ventersdorp Supergroups host rock lithologies. Additionally, the extraction procedure utilized in this study scrupulously avoided exposing the rock samples to O₂ during the extraction process. This study also utilized core samples that were collected 10 to 50 m beyond the typical tunnel damage zone radius of 2 to 3 m where NO₃-bearing explosive residues in microfractures might be expected to reside (Rubel et al., 2002). Despite these differences, the results from this study are consistent with those of Frimmel et al. (1999) and suggest that NO₃ does occur within fluid inclusions. The NO₃ concentrations are ~30 times less than the NH₄/NH₄⁺ concentrations in the fluid inclusion and pore water of the Ventersdorp Supergroup metvolcanics, whereas the NO₃ and NH₄/NH₄⁺ concentrations are approximately equal in the fluid inclusion and pore water of the Witwatersrand Supergroup quartzite (Fig. 1).

The NO₃ concentration in the fracture water (Table 1) was ~10⁴ times less than that in the pore water and fluid inclusions, whereas the NH₄/NH₄⁺ concentration in the fracture water was ~10³ times less than that in the pore water and fluid inclusions (Fig. 1). The NH₄/NH₄⁺ and NO₃ concentrations of the fracture water overlap that of the groundwater in the overlying Karoo and Transvaal Dolomite aquifers and some of the mining water, but most of the mining water yields NO₃ concentrations that are higher than and NH₄/NH₄⁺ concentrations that are lower than those of the fracture water (Table 2; Fig. 1). NH₃ from explosive residues in the mining water can be nitrified to NO₃ by nitrifiers, which based upon the 16S rRNA gene sequences (Onstott et al., 2003) have been reported in the mining water and, thereby, can account for its high NO₃⁻ concentrations. Fertilizer NH₄NO₃ has a δ¹⁵N-N of −5 to +8‰ (Kendall and Aravena, 2000), and since the manufacturing processes of explosives are similar to that of fertilizers, it seems likely that their isotopic signatures should be similar (Spalding et al., 1982; DiGnazio et al., 1998). Most of the mining water samples in this study are characterized by high NH₄⁺ and NO₃ concentrations, δ¹⁵N-NO₃ values ranging from 0 to 7‰ and δ¹⁸O-NO₃ values less than 3‰ (Fig. 2), which is consistent with contamination by explosive residues (Stroes-Gascogne and Gascoyne, 1998).
Based upon the concentration data alone, therefore, the NO$_3^-$ present in the fracture water could arise from diffusive exchange with the pore water or mixing with mining water or both. But the distinct difference observed between the $\delta^{18}$O–NO$_3^-$ in the mining water versus the more positive $\delta^{18}$O–NO$_3^-$ in the fracture water, which overlaps that of the fluid inclusion and pore water, clearly indicates that in most cases the fracture water NO$_3^-$ originates from diffusive exchange with the pore water and decrепitation of fluid inclusions and not from contamination due to the explosives used in mining (Fig. 2). Recent results from analysis of noble gases dissolved in the fracture water and fluid inclusions also indicate that much of the fracture water Ne originates from Ne from the 2 Ga fluid inclusions via fluid inclusion leakage and decrепitation (Lippmann-Pipke et al., 2011).

Mining water with high NO$_3^-$ concentration injected into diamond drill holes prior to blasting can penetrate fractures and undergo microbial denitrification in situ as noted by Onstott et al. (2003). Because the O-to-N ratio of isotope fractionation by microbial denitrification is ~1 (Granger et al., 2008), however, elevation of $\delta^{15}$N–NO$_3^-$ and $\delta^{18}$O–NO$_3^-$ of the DR 938 mining water NO$_3^-$ ($\delta^{15}$N–NO$_3^-$ = 0.7% and $\delta^{18}$O–NO$_3^-$ = 2.3%) by denitrification would not yield values that coincide with those of the pore water, the fluid inclusion leachate and most of the fracture water NO$_3^-$ (Fig. 2). Moreover, given $^{15}$N and $^{18}$O isotope effect amplitudes of up to 30% for denitrification (Vogel et al., 1981; Granger et al., 2008), the residual NO$_3^-$ concentrations would also be less than those observed in the pore water (Figs. 1 and 2). No obvious mixing lines between the low salinity mining water and the high salinity pore water and fluid inclusions can explain the fracture water data when the $\delta^{18}$O–NO$_3^-$ is plotted versus the Na$^+$ concentration (Fig. 3) or, for that matter, when $\delta^{15}$N–NO$_3^-$ is plotted against [NO$_3^-$]$_f$, natural log [NO$_3^-$]$_f$, or 1/[NO$_3^-$]$_f$ (Mariotti et al., 1988). The fracture water, pore water and fluid inclusion NO$_3^-$ isotopic compositions also do not overlap the $\delta^{18}$O–NO$_3^-$ and $\delta^{15}$N–NO$_3^-$ reported for desert NO$_3^-$ deposits (Heaton et al., 1983; Kendall and Aravena, 2000) or for modern precipitation (Hastings et al., 2003). These lines of evidence indicate that trace amounts of NO$_3^-$ present in the fracture water originate from the fluid inclusions and the pore water of the rock formations in the Witwatersrand Basin rather than from the water used in mining.

4.2. Radiolytic generation of NO$_2^-$ and NO$_3^-$

The pH 10 solutions produced more NO$_2^-$ upon irradiation than pH 7 solutions (Table 2 in Supplemental Section), consistent with NH$_4^+$ being the parent for the NO$_3^-$ (reactions (5) through (9)), because the higher pH increases the concentration of NH$_3$ relative to NH$_4^+$. The irradiated NO$_2^-$ solution confirms that reaction (10) proposed by Shin et al. (2001) does exist and provides an approximate reaction rate coefficient of ~1 L mol$^{-1}$ s$^{-1}$ for NO$_2^-$ conversion to NO$_3^-$, which is greater than the rate coefficient for the conversion of NH$_3$ to NO$_2^-$ (Fig. 4). If the NH$_3$ concentrations are low, then the NO$_2^-$ is created at a rate slow enough that nearly all produced NO$_2^-$ will be converted to NO$_3^-$. As pH 7 solutions have ~1% of the NH$_3$ of pH 10 solutions, the observed NO$_3^-$ concentrations in the pH 7 solutions match the values expected by the radiolytic model. The delayed increase in NO$_3^-$ concentrations (Fig. 5) is consistent with the radiolytic NO$_3^-$ being generated from NO$_2^-$ for which the initial concentrations were negligible. The NO$_3^-$ yields in this study, however, were less affected by the pH than those of the NO$_2^-$, and this may be because the NO$_3^-$ is also removed from solution by an undetermined radiolytic reaction, but at a rate much slower than that determined for reaction (10) (Table 2 in Supplemental Section; Fig. 4).

The isotopic composition of the NO$_3^-$ found in the irradiated vials was distinct from that of the NO$_3^-$ contamination present in the unirradiated vials, indicating that the NO$_3^-$ was produced by radiolysis (Table 5). The $\delta^{13}$N–NO$_3^-$ of $-22$ to $-25$‰ is substantially more negative than that of NH$_4$Cl ($\delta^{15}$N–NH$_4^+$ = +23‰), but similar to what would be expected for NH$_3$ given the $-19$‰ determined experimentally for the $\Delta^{15}$N (NH$_4^+$–NH$_3$) at room temperature (Hermes et al., 1985). This
verifies that the radiolytically produced NO$_3^-$ was likely to have originated from NH$_3$ via reaction (5).

The observed $\Delta^{18}O$ values of NO$_3^--$H$_2$O in irradiated solutions were lower than the −23‰ equilibrium fractionation predicted for 298 K by Bohlke et al. (2003). The $\delta^{18}O$—NO$_3^-$ of contaminating NO$_3^-$ in the unirradiated NH$_4$Cl solution, however, was consistent with this value and with that of industrial NO$_3^-$ (IAEA-N3 has a $\delta^{18}O$—NO$_3^-$ of 25.6‰). The oxygen isotopic analyses suggest that reactions (11) and (12) may have a significant kinetic oxygen fractionation effects associated with them.

If we assume that the net production of radiolytically produced NO$_3^-$ in our experiments can be approximated by Eqs. (11) and (12) alone, then the theoretically estimated $O_2$ and H$_2$O concentrations during the radiolysis experiments and the measured NH$_3$ and NO$_2^-$ concentrations constrain the NO$_2^-$ and NO$_3^-$ production rates to be 0.6 to $2 \times 10^{-7}$ and 5 to $8 \times 10^{-10}$ mol L$^{-1}$ Gy$^{-1}$, respectively, for the pH 7 radiolysis experiments where the NH$_3$ concentration was $-2.5 \times 10^{-4}$ mol L$^{-1}$ or $-2.0$ to $3.2 \times 10^{-4}$ mol of NO$_2^-$ L$^{-1}$ (mol of NH$_3$ L$^{-1}$)$^{-1}$ Gy$^{-1}$. Using the fluid inclusion salinities (Table 3), polarities, densities, U concentrations (Table 4), and published K and Th concentrations for the Witwatersrand Supergroup quartzite and Ventersdorp Supergroup metatonic strata, respectively (Nicolaysen et al., 1981), the relationships of Lin et al. (2005a) yield dosage rates of $5 \times 10^{-12}$ and $6 \times 10^{-12}$ Gy s$^{-1}$ for the Witwatersrand Supergroup quartzite and Ventersdorp Supergroup metatonic strata, respectively. The NO$_2^-$ production rates corresponding to these dosage rates are 1 to $2 \times 10^{-16}$ mol of NO$_2^-$ L$^{-1}$ (mol of NH$_3$ L$^{-1}$)$^{-1}$ s$^{-1}$. At these production rates, a 1 mM NO$_3^-$ concentration would require ~17 to 32 myr to be produced from a 10 mM NH$_3$ fluid.

In the presence of Fe$^{3+}$, NO$_2^-$ and NO$_3^-$ will form NH$_3$ (Summers and Chang, 1993) at rates of $4.2 \times 10^{-5}$ and $5 \times 10^{-5}$ (mol Fe$^{3+}$ kg$^{-1}$)$^{-1}$ s$^{-1}$ at 40 °C in the absence of denitrifying microorganisms, respectively, although their NO$_3^-$ reduction rate is poorly constrained. For the fluid inclusions, these reaction rates are comparable to the radiolytic NO$_2^-$ and NO$_3^-$ production rates. The rapid abiotic production of NO$_3^-$ also occurs through the reduction of NO$_2^-$ by Fe$^{2+}$ (Samarkin et al., 2010). The available pool of Fe$^{2+}$ in the fluid inclusions, however, is small, and direct oxidation of Fe$^{3+}$ to Fe$^{4+}$ by radiolysis also occurs (Min and Katsumura, 1997). As a result these reactions relatively quickly diminish the Fe$^{2+}$ concentrations to the point that the NO$_2^-$ and NO$_3^-$ reducing reactions become energetically unfavorable and radiolytically produced NO$_3^-$ would steadily accumulate in the fluid inclusions over geological time.

4.3. The origin of the $\delta^{18}O$—NO$_3^-$

To determine whether the highly positive $\delta^{18}O$—NO$_3^-$ observed in the fluid inclusions, pore water and fracture water could be the result of isotopic exchange with fluid inclusion water over geological time, the temporal evolution of the fluid inclusion $\delta^{18}O$—H$_2$O was constrained using the $\delta^{18}O$ data for fracture minerals (Zha et al., 2006), the published fracture water $\delta^{18}O$—H$_2$O, the basin’s thermal history (Omar et al., 2003) and the water/mineral equilibration model of Onstott et al. (2006). This data indicate that the $\delta^{18}O$—H$_2$O was 5 to 6‰ at 2.0 Ga when the temperature of the fluid was 300 °C (Zha et al., 2006) and the pH was ~5.5 and the $\delta^{18}O$—H$_2$O decreased to the present day value of ~12‰. (Ward et al., 2004) for saline fracture water at temperatures of 40 °C and pH values of ~7.5 to 8.5 (Onstott et al., 2006). Using the kinetic and isotope exchange parameters of Bohlke et al. (2003), which are sensitive to temperature, pH and salinity, the corresponding temporal variation of the $\delta^{18}O$—NO$_3^-$ was modeled and found to range from 8 to 12‰ (Fig. 2). At temperatures greater than 160 °C the rate of isotopic exchange was fast enough for complete oxygen isotopic equilibration to occur in 10 million years, but the $\delta^{18}O$ for isotopic equilibrium, the kinetic rates for isotopic exchange were much slower but the $\delta^{18}O$—H$_2$O became increasingly more negative resulting in a $\delta^{18}O$—NO$_3^-$ of only 8‰ (Fig. 2). Over the last 50 million years when the ambient temperatures yielded a larger $\Delta^{18}O$ for isotopic equilibrium, the kinetic rates for isotopic exchange were much slower but the $\delta^{18}O$—H$_2$O became increasingly more negative resulting in a $\delta^{18}O$—NO$_3^-$ of only 8‰ (Fig. 2). The elevated $\delta^{18}O$—NO$_3^-$ of the fracture, pore and fluid inclusion water, therefore, cannot be reproduced by oxygen isotopic equilibration of the NO$_3^-$ with the fracture water over the last 2.0 byr (Fig. 2).

Assuming a $\delta^{18}O$—H$_2$O for the hydrothermal solution of 5 to 6‰ (Zha et al., 2006) and assuming that the measured radiolytic isotopic effect, $\Delta^{18}O$—H$_2$O—NO$_3^-$ = +16‰, applies at this temperature, the $\delta^{18}O$—NO$_3^-$ would be −2.1 to 22‰, close to but still somewhat less than the observed values (Fig. 2). The $\delta^{18}N$—NO$_3^-$ would reflect the NH$_3$/NH$_4^+$ and the $\Delta^{15}N$—NH$_2^+$—NH$_3$, which ranges 13 to 39‰, from temperatures of 300 to 45 °C. Geochemical modeling indicates that NH$_3$/NH$_4^+$ would have been 4 at 300 °C and assuming that its $\delta^{15}N$ equaled that of the Kimberley Shale, 6‰, the $\delta^{15}N$—NO$_3^-$ produced by radiolysis of the NH$_3$ would have been 3‰ close to that observed. As the hydrothermal fluid cools over time and the NH$_2^+$—NH$_3$ boundary increases to a pH of 8.5, greater than that of the fracture water pH, and NH$_3$ becomes the dominant species, the $\Delta^{15}N$—NH$_2^+$—NH$_3$ increases to 39‰ and the radiolytic NO$_3^-$ production rate declines. The $\delta^{18}N$—NO$_3^-$ produced by radiolysis of the NH$_3$ would have decreased rapidly and could be −33‰ at 45 °C with a $\delta^{18}O$—NO$_3^-$ of 8‰, quite far away from the observed values (dashed arrow in Fig. 2). As a result, radiolytically produced NO$_3^-$ could approach the observed isotopic composition, but only if the oxygen isotopic equilibration rate was for some reason considerably slower than predicted by the kinetic parameters published by Bohlke et al. (2003).

Two other conceivable sources for the NO$_3^-$ could be NO$_2^-$ equilibrating with the hydrothermal fluid or NO$_3^-$—bearing paleometeoric water mixing with the hydrothermal fluid. The former would require a $J_{2,0} = 10^{-2}$, which would seem precluded by the Fe—bearing mineral assemblages observed in the fractures (Dremann et al., 1999). The latter would require extremely high NO$_3^-$ concentrations in the surface precipitation during the mid-Proterozoic, which might be possible if, as proposed by Buick (2007), N$_2$O was a major greenhouse gas constituent at that time. At 2.0 Ga any biosphere associated with the paleosurface of the Witwatersrand Basin would have been eradicated by the meteoric impact that led to the formation of the Vredefort Complex, hydrothermal fluid circulation and sterilization of the crust and with it any potential NO$_3^-$—reducing microbial communities. The kinetic parameters of Bohlke et al. (2003) would still predict that the $\delta^{18}O$—NO$_3^-$ of both of these sources would have equilibrated with the $\delta^{18}O$—H$_2$O at that time. One final possibility is that the abiotic
The similarity of the fracture water NO$_3$ isotopic composition with that of the pore water indicates that if dissimilar NO$_3$-reducing microorganisms in the fracture water are producing either NH$_3$ or NH$_4^+$ from the NO$_3^-$ diffusing out from the pore water, then they are doing so without significant isotopic fractionation (Fig. 2). This would be expected for high degrees of NO$_3^-$ consumption (Mariotti et al., 1981) in an environment in which the flux of NO$_3^-$ is very slow and diffusion limited.

4.4. The NH$_3$ and NH$_4^+$ sources

The 25 ± 22 mM NH$_3$/NH$_4^+$ average pore water concentration overlapped those reported for oil reservoirs by Manning and Hutcheon (2004). The shale to rock ratio in the Witwatersrand Supergroup is 0.56% (Tweedie, 1986), so the origin of the NH$_2$-NH$_4^+$, whether it is derived internally from the shale formations or by biological fixation of $N_2$, bears some consideration. The total N, as measured by combustion, was compared to the summation of N from the various components, NH$_4^+$ ex., NH$_4^+$ ri, NH$_3$ r-w, NO$_3^-$ ri, NO$_3^-$ r-w. (Table 6). The calculations reveal that the N mass balance was conserved on all samples within 1 S.D. of reproducibility, with the exception of EV3 TZ2 Paleo, the sample with the highest N and OC concentration (Table 4).

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The mass balance reveals that more than 90% of the total N in the Kimberley Shale, Witwatersrand Supergroup quartzite and Ventersdorp Supergroup Strata the total N is comprised primarily of NH$_4^+$, and 10% of the N is found as NO$_3^-$, bears some consideration. The total N, as measured by combustion, was compared to the summation of N from the various components, NH$_4^+$ ex., NH$_4^+$ ri, NH$_3$ r-w, NO$_3^-$ ri, NO$_3^-$ r-w. (Table 6). The calculations reveal that the N mass balance was conserved on all samples within 1 S.D. of reproducibility, with the exception of EV3 TZ2 Paleo, the sample with the highest N and OC concentration (Table 4).

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<table>
<thead>
<tr>
<th>Table 6</th>
<th>Rock N mass balance.</th>
</tr>
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<tbody>
<tr>
<td>NO$_3^-$ PW $\times 10^{-5}$</td>
<td>NH$_4^+$ PW $\times 10^{-4}$</td>
</tr>
<tr>
<td>EV 3 KS 24.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>EV 3 KS TZ1</td>
<td>0.65 ± 0.20</td>
</tr>
<tr>
<td>EV 3 TZ2 Paleo</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>EV3 Pale 40.8</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>EV818</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>EV224</td>
<td>2.5 ± 1.2</td>
</tr>
<tr>
<td>KL739</td>
<td>1.4 ± 2.7</td>
</tr>
</tbody>
</table>

$^a$ All units are mol N kg$^{-1}$ rock and are based upon the average values reported in Table 3 multiplied by 0.56/1.19.

$^b$ N is total mol N kg$^{-1}$ rock from all the listed components.

$^c$ $N_{max}$ is from Table 4.
If we assume Rayleigh distillation during metamorphism with a kinetic fractionation factor of $-5$, the average of the observed $\Delta^{15}N$ values, then a $+5\%$ enrichment in the $\text{NH}_4^+$ in the Kimberley Shale over a presumed initial value of $0\%$ would correspond to a loss of $63\%$ of the original total N. The corresponding initial N concentration would then be $6.3 \times 10^{-3}$ mol N $\text{kg}^{-1}$, as opposed to the $1.7 \pm 0.6 \times 10^{-3}$ mol N $\text{kg}^{-1}$ assumed above. The initial N concentration corresponding to the kinetic fractionation values of Li et al. (2009) would be even less. The isotopic data indicate that if the nitrogen in Kimberley Shale was transformed to $\text{N}_2$ as suggested by the mineralogical and thermodynamic constraints, then insufficient nitrogen existed within the Kimberley Shale to explain the abundance of nitrogen, primarily $\text{NH}_4^+$, in the Witwatersrand strata and that reduction of $\text{N}_2$ or $\text{NO}_3^-$ to $\text{NH}_3$ during its geological history is required to explain the observed abundances despite their low values. The slightly negative values $\delta^{15}N$ of $-6$ and $-15\%$ in the fracture water at Mponeng and Beatrix, however, are difficult to reconcile with simple kinetic fractionation during thermal oxidation of $\text{NH}_3$ to $\text{N}_2$ and would also seem to require a reductive leg to a subsurface $N$ cycle that kinetically fractionates the $N$ isotopes (Fig. 6).

5. Conclusion

The $\delta^{15}N$ and $\delta^{18}O$ and $\delta^{15}N$ of the fracture water, pore water and fluid inclusion water are required. Experimental determination of the $N$ isotope kinetic fraction factor for abiotic reduction of $\text{N}_2$ and $\text{NO}_3^-$ would provide further constraints on this process. Given that at least one of the genomes of one of the subsurface bacteria does contain $N_2$-fixing genes, some of this reduction may have also occurred through $N_2$ fixation. Experimental data indicates that radiolysis can produce $\text{NO}_3^-$ from $\text{NH}_3$ under anaerobic conditions at rates that can explain the observed concentrations, but not so easily the observed $\delta^{15}N$ and $\delta^{18}O$ values. If true then radiolytically produced $\text{NO}_3^-$ should be found in other continental subsurface sites and could support a complete subsurface $N$ cycle. Further irradiation experiments performed under varying pH conditions are required to fully resolve the complex reactions taking place and to obtain a more reliable estimate of the rate of radiolytic $\text{NO}_3^-$ production. If a similar radiolytically sustained $N$ cycle occurs on Mars, then we would expect to find $\text{N}_2$ or $\text{NO}_3^-$ released into the atmosphere from a deep, anoxic subsurface environment whenever $\text{N}_2$ or $\text{NO}_3^-$ chlorates are destabilized, thereby replacing the $\text{N}_2$ lost to space over time (Capone et al., 2006).

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Appendix A. Supplementary data

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References


