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## Nitrogen isotopic composition of ammoniated phyllosilicates: case studies from Precambrian metamorphosed sedimentary rocks

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This paper is dedicated to the late Stuart R. Boyd.

### Abstract

Ammonium concentrations in phyllosilicates from Archean and Proterozoic schists and carbonates were evaluated by Fourier Transform Infrared microspectrometry ( $\mu$ FTIR). The  $\delta^{15}\text{N}_{\text{air}}$  values of structural nitrogen in these phases were subsequently measured by laser mass spectrometry. A rapid estimate of the concentration of  $\text{NH}_4^+$  in biotite by  $\mu$ FTIR allows the preselection of the samples to be analyzed for their isotopic composition. Micaceous from 1.90–2.09 Ga metasediments from Finland and from 1.025–1.500 Ga biotite schists from the Moine Succession in Scotland have high concentrations of structural ammonium (between 176 and 1549 ppm), suggestive of a biological origin for the nitrogen. The heavy nitrogen isotopic signatures ( $\delta^{15}\text{N}=+4.9\text{‰}$  to  $+19.7\text{‰}$ ) of structural  $\text{NH}_4^+$  in these micaceous are considered consistent with biological denitrification in operation at the time of sedimentation. Evidence for denitrification in the Mesoproterozoic is in agreement with geological and geochemical data for abundant free oxygen in the atmosphere, facilitating the stability of marine  $\text{NO}_3^-$ . Sequences of early Archean (ca. 3.8 Ga) garnet–mica schists from the Isua Supracrustal Belt (ISB) in southern West Greenland contain biotite rich in  $\text{NH}_4^+$  (between 233 and 512 ppm) and nitrogen isotopic signatures ( $\delta^{15}\text{N}=-1.9\text{‰}$  to  $+5.9\text{‰}$ ), which possibly point to an early evolution of ammonium assimilation and/or nitrogen fixation. This result appears to be consistent with molecular phylogenetic studies in the bacterial and archaeal domains, which suggest that nitrogen fixation and ammonium assimilation were metabolic features of the last common ancestor (LCA) of all organisms.

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**Keywords:** Ammonium; Nitrogen isotopes; Biosignature; Biotite; Infrared spectroscopy; Precambrian

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## 1. Introduction

Nitrogen and nitrogenous compounds are crucial to biochemistry, as they are key components of amino acids and nucleotide bases in the cell. Because there exists no other significant abiogenic source of reduced nitrogen on Earth (Miller and Orgel, 1974), the biological assimilation of nitrogen via the fixation of atmospheric  $N_2$  to  $NH_4^+$  (ammonium), followed by the production of organic nitrogen compounds, maintains the long-term existence of the biosphere. Exploring the chemical and biological evolution of the nitrogen cycle in the geological record is challenging since few opportunities exist to retain nitrogen in stable mineral phases, especially through long crustal residence times. Most of the Precambrian geological record has endured a protracted metamorphic history that tends to obscure or erase information not carried by geologically robust minerals. Structural  $NH_4^+$  contained in metasedimentary phyllosilicates, such as biotite (e.g., Boyd and Philippot, 1998; Boyd, 2001a,b), as opposed to nitrogen in sedimentary organic matter (Beaumont and Robert, 1999), potentially transcends issues of metamorphism and holds promise for studying a record of ancient nitrogen cycling. This work explores how the concentration of ammonium in micas ( $[NH_4^+]_{mica}$ ), evaluated by Fourier Transform Infrared microspectrometry ( $\mu$ FTIR) and coupled with nitrogen isotopic measurements by laser mass spectrometry (as expressed in the conventional delta notation:  $\delta^{15}N = ((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{air} - 1) \times 1000\text{‰}$ ), can be used as a tool to evaluate the early record of nitrogen biogeochemistry.

The decomposition of marine sedimentary organic matter, including maturation processes mediated by biochemical transamination and deamination reactions on amino acids, results in the release of  $NH_4^+$  into sediment pore water solutions (Macko and Estep, 1984; Williams et al., 1989; Lilley et al., 1993; Boyd, 2001b). The degradation of organic matter by heterotrophic organisms leads to only small ( $<1\text{‰}$ ) nitrogen isotopic fractionation effects on total nitrogen (Freudenthal et al., 2001), while other early diagenetic reactions in oxic or anoxic settings can lead to small nitrogen isotopic variations (Lehmann et al., 2002). Later diagenetic processes appear to have a negligible effect on  $^{15}N/^{14}N$  fractionation between sedimentary

organic matter (kerogens) and  $NH_4^+$  adsorbed and incorporated into marine clays, since fluid exchanges are sluggish in such systems (Williams et al., 1995). Ammonium cation in sixfold coordination has an ionic radius of 1.61 Å (Khan and Baur, 1972), similar to that of  $K^+$  with 1.46 Å (Whittaker and Muntus, 1970). Therefore,  $NH_4^+$  can become structurally incorporated into clay minerals by occupying the  $K^+$  interlayer octahedral sites of, e.g., smectite and illite (Williams et al., 1989; Williams and Ferrell, 1991; Hall, 1999). The intrinsic thermal stability of substituent  $NH_4^+$  in K-containing phyllosilicates (e.g., biotite) promotes the retention of nitrogen in these phases, even during metamorphic recrystallization. Structural  $NH_4^+$  in common phyllosilicates, such as biotite, can therefore provide a robust biosignature in metamorphosed sediments (Boyd, 2001b) when other traces of past life, such as morphological fossils, organic biomarkers, or light stable isotope fractionations (e.g.,  $^{13}C/^{12}C$ ;  $^{34}S/^{32}S$ ) have been obscured or erased. Although different views on possible nitrogen isotope fractionation during metamorphism have recently been proposed (Busigny et al., 2003b), high-temperature metamorphism has been shown to increase the  $^{15}N/^{14}N$  ratio (Haendel et al., 1986; Bebout and Fogel, 1992; Bebout, 1997; Mingram and Bräuer, 2001). This effect must therefore be taken into consideration when interpreting data derived from metamorphic rocks, regardless of age.

The nitrogen data reported here are from a suite of samples dating from the beginning of the sedimentary record at  $\sim 3.8$  Ga and from Paleo- and Mesoproterozoic sequences. The molecular phylogenetic record of extant organisms reveals that some nitrogen metabolisms evolved very early in the history of life. Ammoniated minerals as geochemical biosignatures provide an additional tool in the search for life on other planetary surfaces, such as the ancient crust of Mars, where a seemingly well-preserved record of the first billion years of solar system history awaits retrieval.

## 2. Analytical techniques

### 2.1. Description and composition of reference biotites

To estimate  $[NH_4^+]_{biotite}$  and to select sample aliquots for mass spectrometric analysis used in this

study, it is advantageous to precharacterize samples and compare them with an existing baseline of well-characterized minerals. We used biotite separates from the Moine schists (Scotland) for which the concentration and isotopic composition of  $\text{NH}_4^+$  has previously been reported (Boyd and Philippot, 1998). These reference samples were used in the development of a calibration curve for the estimation of  $[\text{NH}_4^+]_{\text{biotite}}$  and to compare our isotopic data obtained with a two-step laser-heating technique. The Moine Succession is part of a suite of Mesoproterozoic sediments located in Moine Thrust and Great Glen Fault, northern Highlands, Scotland (Johnstone and Mykura, 1989). The rocks experienced two Neoproterozoic thermal events that brought them to the amphibolite grade (Johnstone, 1975). We obtained hand samples for extraction of the calibrant biotites for our analyses from the well-characterized pelitic and banded schists of the Glenfinnian Division, Moine Succession, originally collected by S.R. Boyd (samples MS-2, MS-4 and MS-15). Biotite in these rocks is rich in ammonium and contains between 1220 and 1739 ppm  $\text{NH}_4^+$  (Boyd and Philippot, 1998). The rocks are predominantly garnet bearing, banded and little-deformed muscovite+biotite schists; their protolith is interpreted to be shales and sands deposited in a shallow marine shelf environment (Johnstone, 1975).

### 2.1.1. MS-2

MS-2 is a relatively undeformed garnet-bearing muscovite+biotite+quartz+plagioclase schist. There is no evidence for the chloritization of garnet. From the data reported in Boyd and Philippot (1998), the weighted mean  $[\text{NH}_4^+]_{\text{biotite}}$  in MS-2 is  $1464 \pm 52$  ppm ( $2\sigma$ ), and the weighted mean  $\delta^{15}\text{N}$  value for biotite is  $+15.4 \pm 0.4\%$  ( $2\sigma$ ).

### 2.1.2. MS-4

MS-4 is a retrogressed, undeformed garnet-bearing muscovite+biotite+chlorite+epidote (after garnet and plagioclase) banded schist. The weighted mean  $[\text{NH}_4^+]_{\text{biotite}}$  in MS-4 is  $1672 \pm 84$  ppm ( $2\sigma$ ), and the weighted mean  $\delta^{15}\text{N}$  value for biotite is  $+12.8 \pm 0.5\%$  ( $2\sigma$ ; Boyd and Philippot, 1998).

### 2.1.3. MS-15

MS-15 is a banded, garnet-bearing quartz+plagioclase+biotite+muscovite schist. The measurements

reported for MS-15 in Boyd and Philippot (1998) were  $[\text{NH}_4^+]_{\text{biotite}} = 1220 \pm 61$  ppm and  $\delta^{15}\text{N} = +7.7 \pm 0.5\%$ .

## 2.2. Sample preparation

All rocks were manually separated, coarsely crushed and split into grain-size fractions using 250- and 425- $\mu\text{m}$  sieves, and for some samples, a Franz magnetic separator was used to concentrate biotite from rock powders. Separate sample aliquots were obtained by hand picking individual biotite grains under a stereo microscope for examination by light microscopy prior to  $\mu\text{FTIR}$  analysis. In metapelitic rocks rich in ammonium, biotite is known to have the highest  $\text{NH}_4^+$  concentration, followed by muscovite (Boyd and Philippot, 1998), K-feldspar and plagioclase (Honma and Itihara, 1981). Before mass spectrometric analysis, the grains were washed in a  $\text{H}_2\text{O}_2$  solution, instead of the commonly used acetone, in an effort to decrease isobaric interferences of  $^{14}\text{N}_2$  from, e.g.,  $^{12}\text{C}^{16}\text{O}$  caused by incomplete oxidation of contaminant organic matter. The presence of 1% carbon monoxide in the analysis can change the  $\delta^{15}\text{N}$  value by up to  $+6.5\%$  (Beaumont et al., 1994). Biotite grains were sonicated in a 30% dilute solution of  $\text{H}_2\text{O}_2$  (Fisher Scientific©,  $<1$  ppm  $\text{NH}_4^+$ ,  $<0.4$  ppm  $\text{NO}_3^-$ ) for 2 min at room temperature and oven dried at  $80^\circ\text{C}$ . The hydrogen peroxide washing step resulted in a color change in the samples that may be due to leaching of Fe-oxide coatings and other contaminants. Pre and post peroxide wash measurements by  $\mu\text{FTIR}$  confirm that this cleaning step has no detectable influence on  $[\text{NH}_4^+]_{\text{biotite}}$ .

## 2.3. Fourier Transform Infrared microspectrometry ( $\mu\text{FTIR}$ )

The concentration and bond characteristics of structural  $\text{NH}_4^+$  in micas can be directly studied by infrared spectroscopy (Vedder, 1965; Karyakin et al., 1973; Shigorova, 1982; Duit et al., 1986; Boyd, 1997). Separated biotites were placed on a 2-mm-thick  $\times$  13-mm-diameter NaCl plate and analyzed using a Thermo Nicolet© Continuum® microscope linked to a Nexus® 670 FTIR spectrometer at the University of Colorado. Standard operating conditions were as follows: The infrared beam was collimated by

a 100×100 μm window and focused on the flat surface regions of the biotites (i.e., normal to the *c*-axis). Infrared absorbance spectra were collected on a liquid nitrogen-cooled MCTA detector to minimize electronic noise and water absorption in the detector. Spectra were acquired in transmitted mode between 4000 and 800 cm<sup>-1</sup>, and each composite spectrum obtained represents an integration of 120 spectral scans, with a wavenumber resolution of 4 cm<sup>-1</sup>. Background corrections were applied to the data following each measurement to compensate for instrumental noise and contributions from atmospheric CO<sub>2</sub> and H<sub>2</sub>O by dividing the absorbance of the sample spectrum by the background spectrum at each data point.

The goal of our μFTIR analyses was to perform rapid and nondestructive estimates of ammonium concentrations in biotite grains prior to mass spectrometry. The Beer–Lambert law can be used to evaluate the [NH<sub>4</sub><sup>+</sup>]<sub>mica</sub> and is expressed as:

$$\text{Absorbance} = \log\left(\frac{I_0}{I}\right) = \epsilon cl$$

where  $I_0$  and  $I$  are the incident and transmitted light intensities, respectively,  $\epsilon$  is the molar extinction coefficient,  $c$  is the concentration, and  $l$  is the thickness of the material. Agrinier and Jendrzewski (2000) have shown that the thickness of a grain can be inferred from its infrared spectrum and that direct measurement of grain thickness is not necessary to estimate [NH<sub>4</sub><sup>+</sup>]<sub>mica</sub> (Busigny et al., 2003a). The thickness of a grain of mica is related to the amount of network-forming bonds, such as Si–O (Agrinier and Jendrzewski, 2000; Busigny et al., 2003a). Since the absorbance of the N–H bending vibration at 1430 cm<sup>-1</sup> can be used to quantify the concentration of ammonium (Shigorova, 1982), and the absorption of the Si–O combinations between ~1650 and 1850 cm<sup>-1</sup> (Bastoul et al., 1993) can be used as an indicator of thickness, the Beer–Lambert law can be rewritten to estimate the [NH<sub>4</sub><sup>+</sup>]<sub>biotite</sub> from the ratio of the absorbances of N–H and Si–O:

$$\frac{A(\text{N-H})}{A(\text{Si-O})} = \left(\frac{\epsilon_{\text{N-H}}}{\epsilon_{\text{Si-O}}c_{\text{Si-O}}}\right) [\text{NH}_4^+]_{\text{biotite}}$$

Here,  $\epsilon_{\text{N-H}}$  and  $\epsilon_{\text{Si-O}}$  are the molar extinction coefficient of N–H and Si–O, respectively, and  $c_{\text{Si-O}}$

is the concentration of Si–O<sub>2</sub> and is taken to be a constant for simplicity (Al substitutions may vary by a few percent). Note that the term in parenthesis is assumed constant but that variations in  $c_{\text{Si-O}}$  can potentially affect the linearity of the equation. The absorbances of the N–H bending at 1430 cm<sup>-1</sup> and of the Si–O combinations between 1650 and 1850 cm<sup>-1</sup> are directly obtained from the spectra by integrating the area under the curve at these peaks, as shown in the FTIR spectra for selected Moine biotites in Fig. 1. Interference with the H<sub>2</sub>O bending mode at 1630 cm<sup>-1</sup> (Bastoul et al., 1993) was considered minimal in these samples, although it could also potentially affect the linearity of the above equation. Based on these results, a preliminary calibration curve was developed based on more than 30 separate μFTIR measurements, each of MS-2, MS-4 and MS-15, that relate the ratio of the IR absorbance of the N–H bending mode and of the Si–O combinations (A(N–H)/A(Si–O)) to the reported [NH<sub>4</sub><sup>+</sup>]<sub>biotite</sub> values for these samples (Boyd and Philippot, 1998; Fig. 2).

#### 2.4. CO<sub>2</sub> laser extraction and static mass spectrometric analysis

All micas in this study were measured for their nitrogen isotopic composition using a laser mass spectrometric technique at the C.R.P.G. (Nancy, France), originally developed for high-precision analyses on samples with low nitrogen concentrations (Humbert et al., 2000). In the analysis, a CO<sub>2</sub> infrared laser (λ=10.6 μm) guided by a visible He–Ne laser shines through a ZnSe window and is directed over the sample. To avoid sample loss by rapid thermal expansion and “grain jump” out of the sample holder under intense illumination, the CO<sub>2</sub> laser was slightly defocused and power output slowly increased to allow the grains to heat gradually. Evolved gases from the laser heating were purified by oxidation through a CuO oven at 720 °C. Most oxidized compounds were removed by a –168 °C cold trap with only NO<sub>x</sub> compounds, noble gases and minor amounts of CO and organic volatiles remaining in the purification line. The CuO oven was then cooled to 450 °C to reduce NO<sub>x</sub> to N<sub>2</sub> in the extraction line prior to expansion into the mass spectrometer. After each new installation of the sample chamber, the samples were kept in vacuum overnight at 100 °C.

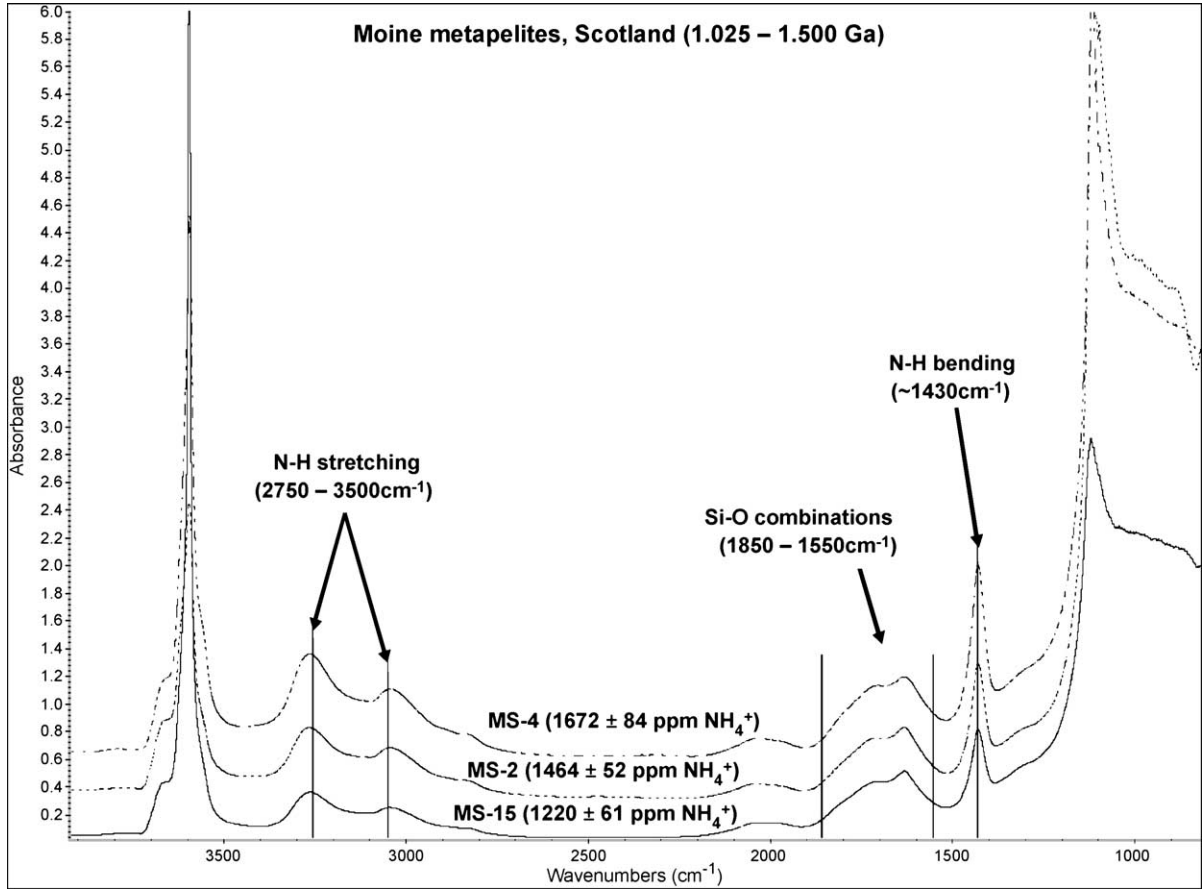


Fig. 1. Spectra of biotite grains from Moine metapelites showing N–H bending and stretching modes (Boyd, 1997) and Si–O combinations (Bastoul et al., 1993). The  $[\text{NH}_4^+]$  data shown are from Boyd and Philippot (1998), and the absorbance scale is normalized for the spectrum of MS-15.

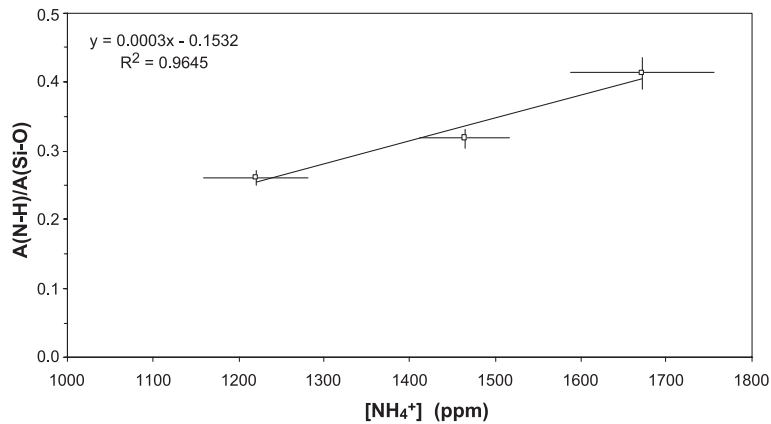


Fig. 2. Preliminary curve for FTIR estimates of  $[\text{NH}_4^+]_{\text{biotite}}$  with the concentration data from Boyd and Philippot (1998).



Isotopic data were collected on a Micromass® VG5400 mass spectrometer following usual procedures (Marty and Zimmermann, 1999; Humbert et al., 2000), using a Faraday cup for masses 28 ( $^{14}\text{N}^{14}\text{N}$ ) and 29 ( $^{14}\text{N}^{15}\text{N}$ ), and an electron multiplier for masses 29 ( $^{14}\text{N}^{15}\text{N}$ ), 30 ( $^{15}\text{N}^{15}\text{N}$ ), noble gases (Ar) and minor organic contaminant isobars at masses 29 (e.g.,  $^{12}\text{C}_2\text{H}_5$ ) and 30 (e.g.,  $^{12}\text{C}_2\text{H}_6$ ). Minor amounts of organic compounds sometimes escape oxidation in the purification step and, therefore, can contribute to measurement error. To compensate for this, an algorithm using the  $^{12}\text{C}_2\text{H}_5$  and  $^{12}\text{C}_2\text{H}_6$  signals was used to correct for isobaric interferences from  $^{12}\text{C}^{16}\text{O}$ ,  $^{13}\text{C}^{16}\text{O}$  and  $^{12}\text{C}^{1}\text{H}^{16}\text{O}$  (Hashizume and Marty, in press). The calculated mean sensitivity for  $\text{N}_2$  in these measurements was approximately  $2.0 \times 10^{-5}$  A/Torr, resulting in a precision of  $<0.3\%$  on the determination of  $^{15}\text{N}/^{14}\text{N}$  ratios (Marty and Zimmermann, 1999). Combined with the error on sample mass measurements, we arrived at error estimates in the determination of the  $[\text{NH}_4^+]_{\text{biotite}}$  in individual sample aliquots of 3–10%. Air standards were measured approximately every second analysis, and the errors on the  $\delta^{15}\text{N}$  values of the samples were derived from the standard deviation of the weighted mean.

Two types of experiments were performed on the samples: either a single heating step consisting in the fusion of the sample, or a double heating step that comprises preheating plus a separate fusion step. During the preheating of the double heating step experiment, the laser illumination was slowly increased until the mineral grains began to emit an orange glow. Under these conditions, the temperature of the sample was held at approximately 700–800 °C for ~5 min. Micas begin to decompose by losing their  $\text{OH}^-$  at temperatures between 850 and 940 °C (Smykatz-Kloss, 1974) and then rapidly release structural  $\text{NH}_4^+$  around 900 °C (Boyd et al., 1993; Sadofsky and Bebout, 2000). The fusion step results in complete melting and in the formation of a molten sample glass bead where bubbles of gas are observed to leave the surface.

Finally, a crushing experiment was performed on selected samples to determine the amount of unbound nitrogen in the analyses. The crushing of biotite grains took place in a high-vacuum cylinder, in which 10 strokes were applied at room temperature. The gas purification protocol was the same as described above.

### 3. Samples and results

#### 3.1. Estimation of $[\text{NH}_4^+]_{\text{biotite}}$ by $\mu\text{FTIR}$

Prior to nitrogen isotopic measurements, our preliminary calibration curve in Fig. 2 was used to estimate  $[\text{NH}_4^+]_{\text{biotite}}$  based on the  $\mu\text{FTIR}$  spectra collected on a sample suite of micas from Paleoproterozoic schists from Finland (Fig. 3) and early Archean (~3.7–3.8 Ga) metapelites from southern West Greenland (Fig. 4). A preselection criterion was established such that biotites with the highest  $[\text{NH}_4^+]$  were chosen for subsequent nitrogen isotopic analysis. Results from the mass spectrometric measurements thereby permitted the further refinement of our  $\mu\text{FTIR}$  calibration.

#### 3.2. Nitrogen in precambrian phyllosilicates

Nitrogen concentrations and isotopic data for the single heating step experiment of this study are reported in Table 1, and the results for the double heating step experiment are shown in Table 2. The results of the double heating step experiment provide some information on potentially different components of nitrogen in the biotite. For instance, nitrogen evolved at low temperature could represent weakly bound structural ammonium of potential metamorphic origin, while nitrogen evolved at high temperature is likely structural ammonium representing the metamorphic alteration of primary nitrogen (e.g., Pinti et al., 2001).

Our data for Moine Formation biotites, used as a baseline, exhibit values for  $[\text{NH}_4^+]_{\text{biotite}}$  and  $\delta^{15}\text{N}$  similar to those previously reported. The two heating steps performed on MS-2 and MS-4 reveal that the first nitrogen component is isotopically lighter than the second, probably reflecting preferential devolatilization of isotopically light structural  $\text{NH}_4^+$  in the first heating step, whereas the second (fusion) step liberates the residual (heavier) structural  $\text{NH}_4^+$  (Boyd et al., 1993). This interpretation implies that  $[\text{NH}_4^+]_{\text{biotite}}$  can be determined by combining the results from the two heating steps, as shown in the last column of Table 2. Modern contaminants not removed during the overnight incubation at 100 °C potentially contribute small quantities of foreign N in the preheating step (e.g.,  $<2$  ppm, Pinti et al., 2001).

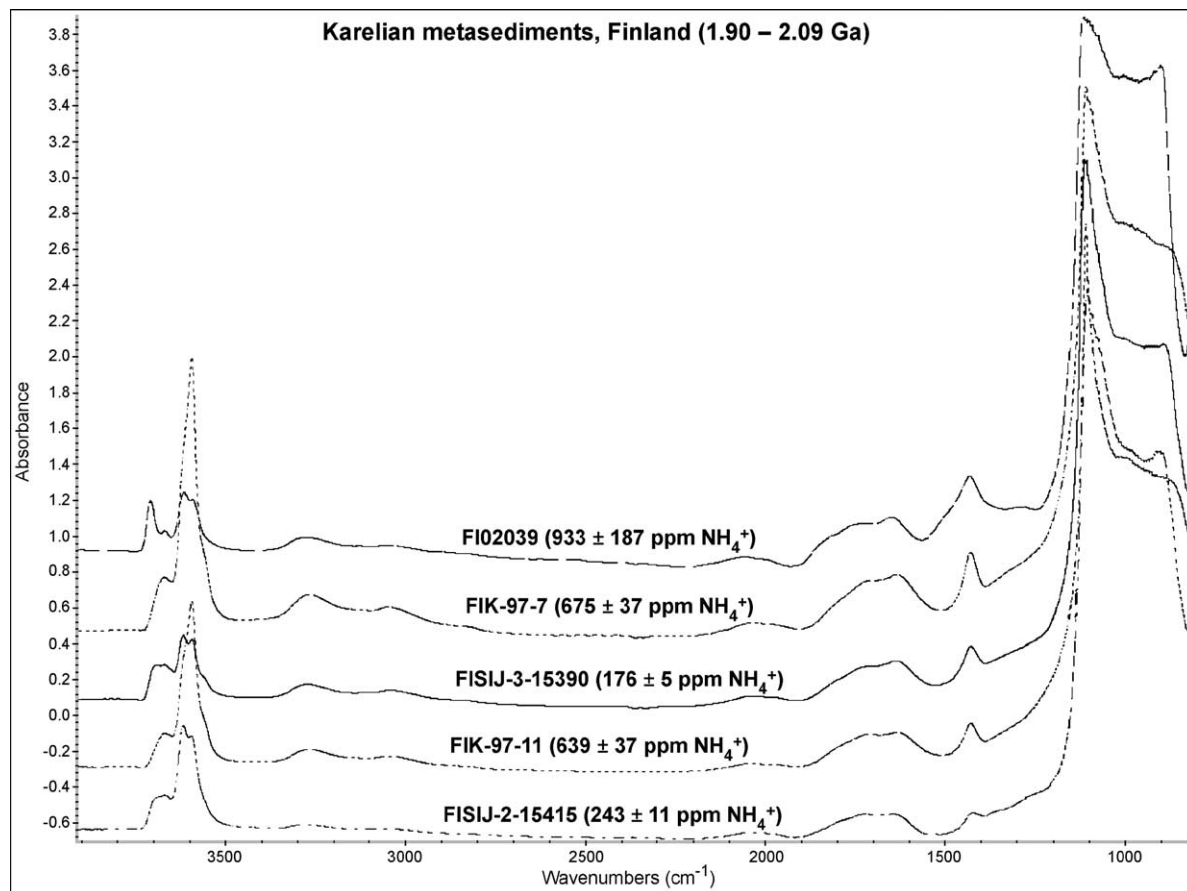


Fig. 3.  $\mu$ FTIR spectra of biotite grains from Finland metasediments showing the concentration, as determined by mass spectrometry. The spectrum for F102039 is from a grain of phlogopite. The absorbance scale is normalized for the spectrum of FISIJ-3-15390.

Yet, because hundreds of ppm of total N was detected during and after laser heating, contaminant nitrogen was an insignificant component of the total N. We find that the combination of the nitrogen concentrations in MS-2 and MS-4 and the nitrogen concentration measured in MS-15 (Table 1) yield  $[\text{NH}_4^+]_{\text{biotite}}$  that are in relatively good agreement with the values reported by Boyd and Philippot (1998). The minor difference with our results may be due to differences in the analytical methods or to concentration heterogeneities for small sample aliquots. We analyzed only small amounts of biotite (between 10 and 21  $\mu\text{g}$ ) in an ultrahigh vacuum ( $\sim 10^{-8}$  mbar) with very low blanks ( $< 1\%$  of the signal). The combined  $\delta^{15}\text{N}$  values of the two heating steps for MS-2 and MS-4 and the single

$\delta^{15}\text{N}$  measurements for MS-15 and MS-4 are in relatively close agreement, within the error, with prior studies (Boyd and Philippot, 1998). Thus, we interpret the data for the Moine samples to imply that individual grains of biotite contain minor heterogeneities both in concentration and nitrogen isotope composition.

### 3.2.1. Mica schists, Finland, 1.90–2.09 Ga

These diverse samples include 1.90–1.95 Ga upper Kalevian mica schists from the Kainuu Belt (eastern Finland), cores collected from interlayers in the  $2.062 \pm 0.002$  Ga volcanic Koivusaari formation of the Siilinjärvi region and the 2.06 to 2.09 Ga Rantamaa Formation of the Peräpohja Belt, northern Finland.

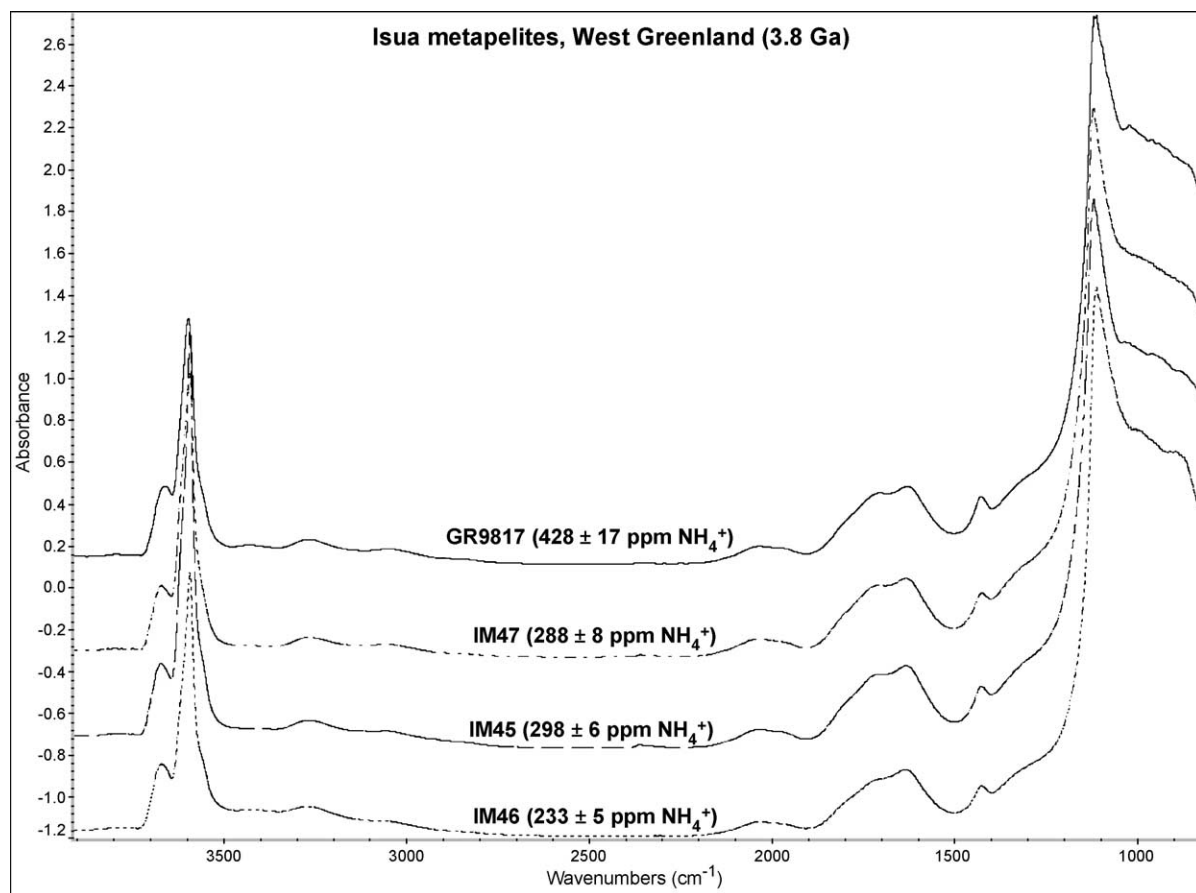


Fig. 4. Infrared spectra of biotite grains from ~3.8 Ga Isua metapelites showing the  $[\text{NH}_4^+]$  determined by mass spectrometry. The absorbance scale is normalized for the spectrum of GR9817.

3.2.1.1. *FIK-97-7* (sample location:  $N63^\circ 58' 20.0''$ ,  $E28^\circ 06' 16.2''$ ) and *FIK-97-11* (sample location:  $N63^\circ 58' 41.9''$ ,  $E28^\circ 05' 38.1''$ ) from the Kainuu belt. The samples were collected from the upper Kalevian

mica schists of the Talvivaara area in Kainuu. The mica schists represent turbiditic deep-water greywackes with intercalated black shales (Kousa et al., 2000). The content of  $\text{C}_{\text{org}}$  in the selected samples is

Table 1  
Ammonium concentration and nitrogen isotopic data for the single heating step experiment

Sample	Mass analyzed ( $\mu\text{g}$ ) <sup>a</sup>	$\delta^{15}\text{N}$ (‰) <sup>b</sup>	$[\text{N}_2]$ (ppm)	$[\text{NH}_4^+]_{\text{mica}}$ (ppm) <sup>b</sup>
MS-4	10 $\pm$ 1	+11.87 $\pm$ 1.27	1205 $\pm$ 121	1549 $\pm$ 155
MS-15	13 $\pm$ 1	+9.79 $\pm$ 1.50	810 $\pm$ 62	1041 $\pm$ 80
FIK-97-7	32 $\pm$ 1	+7.27 $\pm$ 1.34	522 $\pm$ 16	671 $\pm$ 21
FISIJ-3-15390	35 $\pm$ 2	+19.30 $\pm$ 1.55	137 $\pm$ 8	176 $\pm$ 5
FI02039 <sup>c</sup>	5 $\pm$ 1	+18.40 $\pm$ 1.78	726 $\pm$ 145	933 $\pm$ 187
GR9817	171 $\pm$ 5	+1.63 $\pm$ 1.52	398 $\pm$ 12	512 $\pm$ 15

<sup>a</sup> Uncertainties are estimates based on reproducibility of repeat weight measurements.

<sup>b</sup> This column was calculated from the  $[\text{N}_2]$  column using  $[\text{NH}_4^+] = 18/14 \cdot [\text{N}_2]$ .

<sup>c</sup> Single grain of phlogopite.



Table 2  
Ammonium concentration and nitrogen isotopic data for the double heating step experiment

Sample	Mass analyzed ( $\mu\text{g}$ ) <sup>a</sup>	Experiment	$\delta^{15}\text{N}$ (‰) <sup>b</sup>	$[\text{N}_2]$ (ppm)	$[\text{NH}_4^+]_{\text{biotite}}$ (ppm) <sup>c</sup>
MS-2	11 $\pm$ 1	700–800 °C	+14.02 $\pm$ 1.76	637 $\pm$ 58	
		Fusion	+20.38 $\pm$ 3.14	297 $\pm$ 27	
		Total	+16.04 $\pm$ 2.17	934 $\pm$ 64	1201 $\pm$ 82
MS-4	21 $\pm$ 1	700–800 °C	+7.05 $\pm$ 1.80	658 $\pm$ 31	
		Fusion	+14.47 $\pm$ 1.43	490 $\pm$ 23	
		Total	+10.22 $\pm$ 1.58	1148 $\pm$ 39	1476 $\pm$ 50
FIK-97-7	34 $\pm$ 2	700–800 °C	+3.28 $\pm$ 1.92	393 $\pm$ 23	
		Fusion	+9.82 $\pm$ 2.07	134 $\pm$ 8	
		Total	+4.94 $\pm$ 1.99	527 $\pm$ 24	678 $\pm$ 31
FIK-97-11	37 $\pm$ 3	700–800 °C	+4.15 $\pm$ 1.46	294 $\pm$ 24	
		Fusion	+13.03 $\pm$ 1.61	203 $\pm$ 16	
		Total	+7.78 $\pm$ 1.53	497 $\pm$ 29	639 $\pm$ 37
FISIJ-2-15415	74 $\pm$ 5	700–800 °C	+15.87 $\pm$ 1.00	90 $\pm$ 6	
		Fusion	+23.20 $\pm$ 0.96	99 $\pm$ 7	
		Total	+19.72 $\pm$ 0.98	189 $\pm$ 9	243 $\pm$ 11
GR97im45	325 $\pm$ 10	700–800 °C	+22.63 $\pm$ 1.87	79 $\pm$ 2	
		Fusion	+4.50 $\pm$ 1.43	153 $\pm$ 5	
		Total	+10.70 $\pm$ 1.61	232 $\pm$ 5	298 $\pm$ 6
GR97im46	605 $\pm$ 20	700–800 °C	+17.82 $\pm$ 1.44	103 $\pm$ 3	
		Fusion	+5.88 $\pm$ 1.64	78 $\pm$ 3	
		Total	+12.69 $\pm$ 1.53	181 $\pm$ 4	233 $\pm$ 5
GR97im47	375 $\pm$ 15	700–800 °C	+27.47 $\pm$ 1.43	89 $\pm$ 4	
		Fusion	+3.01 $\pm$ 1.47	135 $\pm$ 5	
		Total	+12.70 $\pm$ 1.45	224 $\pm$ 6	288 $\pm$ 8
GR9817	155 $\pm$ 5	700–800 °C	n.d.	157 $\pm$ 5	
		Fusion	−1.85 $\pm$ 1.32	110 $\pm$ 4	
		Total	n.d.	267 $\pm$ 6	343 $\pm$ 8

<sup>a</sup> Uncertainties on mass are estimates based on reproducibility of repeat weight measurements.

<sup>b</sup> The total is the calculated weighted mean  $\delta^{15}\text{N}$  of the two measurements; the error is the quadratic of the errors.

<sup>c</sup> This column was calculated from the  $[\text{N}_2]$  column using  $[\text{NH}_4^+] = 18/14 \cdot [\text{N}_2]$ .

low, ranging from 0.05% to 0.1%. The local geology of the Talvivaara area has been described by Loukola-Ruskeeniemi and Heino (1996). The upper Kalevian metaturbidites overlie lower Kalevian black shales, with elevated contents of C, S, Ni, Cu, Zn and Mn (Loukola-Ruskeeniemi and Heino, 1996). In a U–Pb study of Svecofennian detrital zircons, Claesson et al. (1993) investigated an upper Kalevian metagreywacke sample from the Jormua area, Kainuu Belt. Proterozoic zircons showed ages varying between 1.94 and 2.06 Ga, which was interpreted by Claesson et al. (1993) to provide a maximum depositional age of about 1.94 Ga for the upper Kalevian assemblage. The turbidites and black shales of the Kainuu Belt were subsequently deformed and metamorphosed to low-pressure amphibolite facies during the Svecokarelian orogeny at 1.9–1.8 Ga.

3.2.1.2. FISIJ-2-15415 (sample location:  $N63^\circ 04' 23.8''$ ,  $E27^\circ 32' 44.9''$ ; depth: 154.15 m) and FISIJ-3-15390 (sample location:  $N63^\circ 04' 24.1''$ ,  $E27^\circ 33' 03.5''$ ; depth: 153.90 m) from Siilinjärvi. The two mica schist core samples from the Siilinjärvi region in middle Finland were collected from sedimentary interlayers in the volcanic Koivusaari Formation, described briefly by Kousa et al. (2000). The Koivusaari succession comprises three basaltic members and one felsic pyroclastic member interpreted to have erupted at a subaqueous cratonic rift or rifted continental margin environment. Drill cores through the succession show minor intercalations of mica schist and sedimentary carbonate. The felsic member of the sequence has yielded a U–Pb zircon date of  $2062 \pm 2$  Ma (Pekkarinen and Lukkarinen, 1991), which is the best estimate for the depositional age of the sedimentary interlayers. The rocks of the region were meta-

morphosed to the PT conditions of amphibolite facies during the 1.9–1.8 Ga Svecokarelian orogeny.

3.2.1.3. *FI02039 (sample location: N65°57'34.6", E24°28'46.6", Rantamaa quarry) from the Peräpohja belt.* The Rantamaa Formation is a >400 m tidal flat succession of dolomitic carbonate with well-preserved stromatolitic and other supra- and intertidal structures (Kousa et al., 2000, and references therein). Locally present are interlayers of siliciclastic units, interpreted as tidal channel deposits and storm layers. Our sample FI02039 represents a single phlogopite grain extracted from a dolomite rock exposed at the Rantamaa quarry locality. The Rantamaa Formation overlies the sub-aerial lavas of the Jouttiaapa Formation dated to  $2090 \pm 70$  Ma by the Sm–Nd method (Huhma et al., 1990). The Rantamaa dolomites have highly positive  $\delta^{13}\text{C}$  values, varying from 2.9‰ to 11.4‰ (Karhu, 1993). This implies deposition during the Paleoproterozoic positive carbon isotope excursion, with a minimum age of about 2060 Ma (Karhu and Holland, 1996). During the Svecokarelian orogeny at 1.9–1.8 Ga, the rock sequence of the Peräpohja Belt was folded and metamorphosed under lower greenschist facies conditions.

Our nitrogen data from the double heating step experiments for the Finland micas show a trend that is similar to that of the Moine biotites (Table 2, Fig. 5). The preheating step for samples FIK-97-7, FIK-97-11 and FISIJ-2-15490 have  $\delta^{15}\text{N}$  values lower than the fusion step, compatible with the interpretation of preferential devolatilization of isotopically light structural  $\text{NH}_4^+$  during the first heating step. Comparison of the single heating step data (Table 1) with the total of the two heating steps (Table 2) for the biotites of sample FIK-97-7 displays homogeneity in  $[\text{NH}_4^+]$  and some minor heterogeneity in the nitrogen isotopes, perhaps showing small-scale  $\delta^{15}\text{N}$  heterogeneity analogous to our observations of the Moine biotites. Samples FISIJ-3-15390 (Table 1) and FISIJ-2-15415 (Table 2) have similar nitrogen isotopic compositions. Sample FI02039 is unique in our sample set in that it represents a single grain of phlogopite from a stromatolitic dolomite; its  $\delta^{15}\text{N}$  value is similar to the Siilinjärvi biotite.

### 3.2.2. Garnet-mica schists, Isua Supracrustal Belt (ISB), southern West Greenland ~3.8 Ga

An up-section sample series (sample location: N65°11'53.2, W49°48'14.8") was collected from a

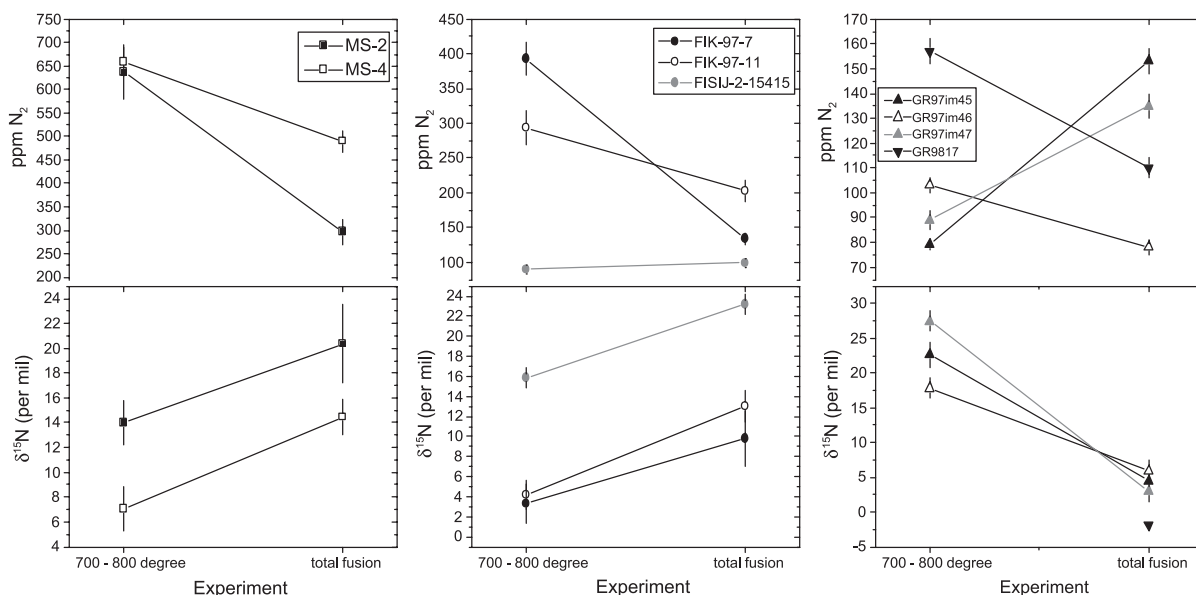


Fig. 5. Temperature evolution of the nitrogen concentration and  $\delta^{15}\text{N}$  values for the double heating step experiment on the selected samples (Table 2).

Fe–Mg-rich schist comprising a fine-grained unit of quartz+plagioclase+biotite+garnet±muscovite ±allanite (+minor sulfide) in the ‘Sequence B2’ mica schist unit (Nutman et al., 1984) of the Isua Supracrustal Belt, West Greenland. This amphibolite grade metasedimentary unit from the Itsaq Gneiss Complex has undergone a protracted metamorphic history, which may have included a 3.650 Ga tectonic collision, granitoid intrusions around 3.140 Ga and a few other metamorphic events during the Late Archean and Paleoproterozoic (Nutman et al., 1996). These rocks are predominantly garnet–biotite schists previously assigned a ferruginous metapelitic origin (Dymek and Klein, 1988; equivalent to Jacobsen and Dymek, 1988, sample #3; same rock unit as Mojzsis et al., 2003, sample GR97im43 and SJM/IM/43 of Pinti et al., 2001). Biotites are abundant (~10%) in hand sample and concentrated in bands oriented parallel to schistosity. Considering (i) the fine-grained texture of this rock, (ii) a mineral assemblage consistent with a pelitic protolith and (iii) sulfur isotopic ( $\Delta^{33}\text{S}$ ) compositions consistent with a marine origin (Mojzsis et al., 2003), the high  $[\text{NH}_4^+]_{\text{biotite}}$  seems consistent with nitrogen derived from the breakdown of marine microbial organic matter at time of deposition at ca. 3.8 Ga (e.g., Honma, 1996).

Nitrogen isotopic measurements from the Greenland samples show several characteristics different from the Proterozoic samples. Two nitrogen components are observed in our GR97im45–47 biotite samples; the preheating step is isotopically heavy compared with the fusion step, which has uniformly low  $\delta^{15}\text{N}$  values (Table 2). We tentatively interpret the nitrogen evolved from the preheating steps to represent a mixture of weakly bound structural ammonium affected by metamorphic fluids with a small quantity of structural  $\text{NH}_4^+$  of possible primary origin. Minor amounts of modern contaminants could have been measured in the preheating step (e.g., Pinti et al., 2001), but since we measured hundreds of ppm of total N, such contaminant nitrogen did not significantly contribute to the measurement. The second nitrogen component is most likely structural  $\text{NH}_4^+$ , representing the metamorphic alteration of primary ammonium. Metamorphic alterations are known to result in the volatilization of isotopically light structural  $\text{NH}_4^+$ , which can be modeled by a Rayleigh distillation process (Bebout and Fogel, 1992). The

residual  $\text{NH}_4^+$  in the biotite likely became more enriched in  $^{15}\text{N}$  with every metamorphic event affecting the ISB throughout its history and, therefore, the  $\delta^{15}\text{N}$  value of the fusion step probably represents a maximum for the nitrogen isotopic composition before metamorphism. Furthermore, the  $\delta^{15}\text{N}$  values of the fusion step must be considered maximum values of the structural  $\text{NH}_4^+$  before laser heating because of the preferential volatilization of  $^{14}\text{NH}_4^+$  during the preheating step. Our data show systematically low  $\delta^{15}\text{N}$  values characterizing the structural  $\text{NH}_4^+$  of the Greenland biotite samples and express the need for more laser mass spectrometric analyses of similar samples.

These results slightly differ from a previous nitrogen study (Pinti et al., 2001) on a different Isua sample (SJM/IM/43) collected from the same unit as GR97im45–47 and analyzed by quadrupole mass spectrometry. In that study, a total of 13 step-heating measurements resulted in generally increasing  $\delta^{15}\text{N}$  values and the release of two major nitrogen components: the first (low  $\delta^{15}\text{N}$  component) between 750 and 850 °C and the second (high  $\delta^{15}\text{N}$  component) between 1000 and 1100 °C. The difference between our nitrogen isotopic results for GR97im45–47 and those of Pinti et al. (2001) for SJM/IM/43 could be due to the nature of the experiment performed or to nitrogen isotopic heterogeneities at the outcrop scale (e.g., Sadofsky and Bebout, 2000). Their experiment consisted of multiple heating steps applied to the biotites, each lasting 30 min; such conditions may tend to favor the preferential devolatilization of isotopically light structural ammonium. The trend of heavier  $\delta^{15}\text{N}$  values measured at higher temperatures in long-duration heating steps (>30 min) was also observed by Boyd et al. (1993) and Sadofsky and Bebout (2000). Moreover, the concentration of nitrogen in our analyses of samples GR97im45–47 (Table 2) was a factor of 2 to 3 higher than that reported for SJM/IM/43 (Pinti et al., 2001). This difference is most easily explained by nitrogen concentration heterogeneities at the outcrop scale. Sadofsky and Bebout (2000) found considerable variation in the nitrogen concentration and isotopic composition of a single outcrop that experienced amphibolite grade metamorphism, such as that at Isua. Intrinsic isotopic and concentration heterogeneities at the scale of the outcrop probably

Table 3  
Data for the  $\mu$ FTIR estimation curve for  $[\text{NH}_4^+]_{\text{biotite}}$

Sample	Average A(N–H)/ A(Si–O)	S.D.	n	$[\text{NH}_4^+]$ (ppm)
MS-2	0.318	0.014	36	1201 $\pm$ 82
MS-4	0.413	0.022	43	1513 $\pm$ 162 <sup>a</sup>
MS-15	0.261	0.011	32	1041 $\pm$ 80
FIK-97-7	0.167	0.006	17	675 $\pm$ 37 <sup>a</sup>
FIK-97-11	0.148	0.009	15	639 $\pm$ 37
FISIJ-2-15415	0.059	0.009	15	243 $\pm$ 11
GR97im45	0.063	0.007	32	298 $\pm$ 6
GR97im46	0.055	0.003	22	233 $\pm$ 5
GR97im47	0.057	0.003	31	288 $\pm$ 8
GR9817	0.074	0.003	15	428 $\pm$ 17 <sup>a</sup>

A(N–H)/A(Si–O) is determined by  $\mu$ FTIR;  $n$ =number of spectra;  $[\text{NH}_4^+]$  is determined by mass spectrometry (see Tables 1 and 2).

<sup>a</sup> Average of the two experiments.

exist since multiple and protracted metamorphic events experienced by the ISB could have led to varying degrees of  $\text{NH}_4^+$  devolatilization. The starting isotopic composition and quantity of organic nitrogen in the protolith may also have resulted in heterogeneity at various scales, hence, the difference between the results presented here and those of Pinti et al. (2001) could be a real consequence of the early environment of formation.

As a test of this hypothesis, a single-step total fusion experiment was performed on biotites from sample GR9817 (Table 1), also from the “B2 mica schist” unit (Nutman et al., 1984) that lies  $\sim$ 1 km south and along strike of samples GR97im45–47. The

sample GR9817 yielded a  $\delta^{15}\text{N}$  value of +1.63‰. Two heating steps performed on this sample (Table 2) gave a  $\delta^{15}\text{N}$  value of  $-1.82$ ‰ for structural  $\text{NH}_4^+$  (fusion step), in accordance with the relatively light  $\delta^{15}\text{N}$  values for the other Isua biotites. Thus, it is likely that further sampling and analyses will be required to determine the extent of nitrogen heterogeneities at the outcrop scale and whether these could, in fact, reflect a faint record of intrinsic differences in nitrogen cycling during sedimentation in the early Archean.

### 3.3. Refinement of $\mu$ FTIR calibration for $[\text{NH}_4^+]_{\text{biotite}}$

We enhanced our preliminary  $\mu$ FTIR calibration curve of  $[\text{NH}_4^+]_{\text{biotite}}$  as determined by mass spectrometry (Table 3, Fig. 6). Each ratio of the absorbances A(Si–O)/A(N–H) used in the calibration was an average calculated from a minimum of 15 separate biotite spectra. Sample FISIJ-3-15390 was excluded from the calibration because its FTIR spectra were irregular and irreproducible. Sample FI02039 was a grain of phlogopite and, therefore, was not included in the calibration for  $[\text{NH}_4^+]_{\text{biotite}}$ . The small deviations in the calibration curve may be due to such variables as heterogeneities within and between each grain or, perhaps, some contribution from the interference of the  $\text{H}_2\text{O}$  bending mode at  $1630\text{cm}^{-1}$  with the Si–O combination around  $1645\text{cm}^{-1}$ .

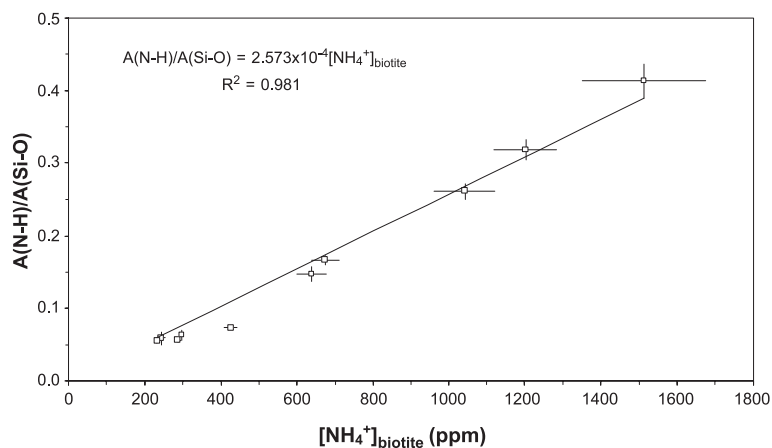


Fig. 6. Refined  $\mu$ FTIR estimation curve for  $[\text{NH}_4^+]_{\text{biotite}}$  with the mass spectrometric data presented (data shown in Table 3).

## 4. Discussion

### 4.1. Nitrogen of metamorphic origin

Sources of nitrogen to metamorphic fluids can be linked to the thermal decomposition, volatilization and dehydration of minerals containing  $\text{NH}_4^+$  (Hallam and Eugster, 1976) and to the subsequent oxidation of ammonium to  $\text{N}_2$  from within the rock. Nitrogen in fluids may also be derived via degassing of neighboring metasedimentary units (e.g., Haendel et al., 1986). Such fluids tend to rapidly escape from medium- to high-grade metamorphic terranes, except for those that accumulate as fluid inclusions (Bucher and Frey, 1994) or by cation exchange. Nitrogen in fluid inclusions is dominantly in the form of  $\text{N}_2$  (Duit et al., 1986; Darimont et al., 1988; Andersen et al., 1989), and the isotopic composition of nitrogen in such systems can give indications on its origin (Jia and Kerrich, 2000; Bebout et al., 1999; Mingram and Bräuer, 2001). Hanschmann (1981) calculated that nitrogen isotopic fractionation between  $\text{NH}_4^+$  in silicates and  $\text{NH}_3$  in fluids could be larger than between  $\text{NH}_4^+$  in silicates and fluid  $\text{N}_2$ . Volatilization reactions during prograde metamorphism can cause a decrease in whole rock  $\text{NH}_4^+$  concentrations (Duit et al., 1986; Haendel et al., 1986; Kol'tsov et al., 2001) and a gradual shift towards heavier nitrogen isotopic values through preferential loss of  $^{14}\text{N}$  (Haendel et al., 1986; Bebout and Fogel, 1992; Bebout, 1997). It was recently reported that the nitrogen isotopic composition does not increase during high-pressure, low-temperature metamorphic regimes characterizing subduction zones (Busigny et al., 2003b). Since nitrogen isotopes are affected differently in different metamorphic settings, we can only propose that  $\delta^{15}\text{N}$  values from metamorphic sediments should be considered maximum values of the premetamorphic nitrogen.

The isotopically heavy  $\delta^{15}\text{N}$  values (+17‰ to +27‰) evolved from the preheating step of the Greenland samples GR97im45–47 stand in marked contrast to the light  $\delta^{15}\text{N}$  values in the fusion step results and may point to interaction with externally derived metamorphic fluids. Subsequent crushing experiments performed on biotite grains from selected samples have shown that there is less than 0.1 ppm of unbound nitrogen in the ISB biotite (Table 4), which was too low to allow precise nitrogen isotope

Table 4

Nitrogen evolved during the crushing experiment

Sample	$\text{N}_2$ (mol/g)	+/-	$[\text{N}_2]$ (ppm)	+/-
GR97im45	2.23E-09	1.19E-10	0.063	0.003
GR97im47	2.18E-09	1.16E-10	0.061	0.003
SJM/IM/43	2.92E-09	1.56E-10	0.082	0.004

determination. These data demonstrate that the nitrogen analyzed in the two steps of samples GR97im45–47 is not in the form of unbound  $\text{N}_2$  and probably consists of structural  $\text{NH}_4^+$ . Jia and Kerrich (2000) reported data on quartz vein micas with a  $\delta^{15}\text{N}$  range from +11‰ to +24‰; we note that these are of similar metamorphic grade to that of the Greenland samples investigated here. They interpreted their results to be consistent with fluids derived from metamorphic dehydration reactions. Unless some unknown mechanism is responsible, we propose that the  $^{15}\text{N}$  enrichments observed in the preheating step of GR97im45–47 primarily reflect weakly bound ammonium affected by Rayleigh-type distillation during metamorphism, which should result in residual nitrogen with isotopically heavy values, as seen in many other samples (Bebout and Fogel, 1992; Bebout et al., 1999; Jia and Kerrich, 2000; Mingram and Bräuer, 2001). Additional detailed analyses are required to understand this characteristic of the Isua samples.

### 4.2. Implications for high $[\text{NH}_4^+]$ in precambrian biotites

It has been suggested that an igneous or metasedimentary protolith for metamorphosed rocks can be distinguished on the basis of ammonium concentration (especially in biotite; Itihara and Honma, 1979; Honma and Itihara, 1981; Itihara and Suwa, 1985; Duit et al., 1986; Tainosho and Itihara, 1988; Itihara and Tainosho, 1989; Honma, 1996). The elevated  $[\text{NH}_4^+]_{\text{biotite}}$  values measured in our samples from West Greenland may therefore be viewed as yet another diagnostic for a sedimentary protolith of these rocks. Multiple measurements of  $[\text{NH}_4^+]_{\text{biotite}}$  from a variety of rock types have been published, and Fig. 7 illustrates how our measurements for Isua and Finland biotites compare with this distribution. Igneous biotites generally contain  $\leq 100$  ppm  $\text{NH}_4^+$ , with only minor exceptions in the hundred  $\sim 150 \pm 50$  ppm range; these are generally interpreted to either have



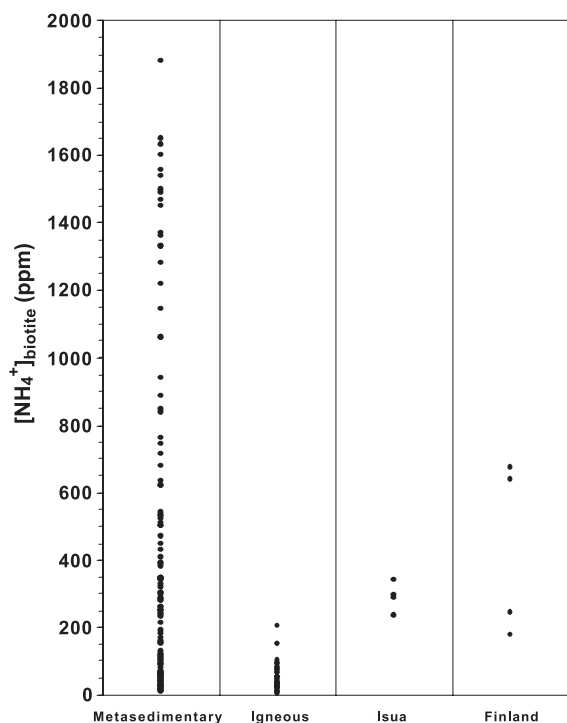


Fig. 7. Comparison of  $[\text{NH}_4^+]_{\text{biotite}}$  from our samples with other published data of biotites of sedimentary and igneous origin. Data for metasedimentary and igneous biotite are from the following: Itihara and Honma (1979), Honma and Itihara (1981), Itihara and Suwa (1985), Duit et al. (1986), Tainosho and Itihara (1988), Itihara and Tainosho (1989), Honma (1996), Boyd and Philippot (1998) and Pinti et al. (2001).

assimilated some  $\text{NH}_4^+$  from the surrounding metasediments or to have formed from a sedimentary protolith (Itihara and Honma, 1979; Itihara and Suwa, 1985; Hall, 1999). However, the process by which  $\text{NH}_4^+$  can devolatilize from micas, mix with hydrothermal fluids, diffuse into another unit and become structurally incorporated in the crystal lattices of other micas awaits study. The relatively high  $[\text{NH}_4^+]$  values found in some granites (~100 to 300 ppm) have been interpreted to stem from the anatexis of a sedimentary protolith with high amounts of organic nitrogen (Tainosho and Itihara, 1988; Boyd et al., 1993; Hall, 1999). Our sample biotites all contain more than 176 ppm of  $\text{NH}_4^+$ . High values for  $[\text{NH}_4^+]_{\text{biotite}}$  (>200 ppm), especially in metasediments, represent a potential biosignature, since there are few abiological reactions that form  $\text{NH}_4^+$  and none are known to yield  $\text{NH}_4^+$  in concentrations sufficient to explain those observed,

for example in the Greenland samples. Previous work has put forth  $[\text{NH}_4^+]_{\text{biotite}}$  as a biosignature for metapelites from Isua, where 234 and 253 ppm  $\text{NH}_4^+$  has been reported (Honma, 1996), from Finland, where up to 1558 ppm  $\text{NH}_4^+$  has been described (Itihara and Suwa, 1985), and from Scotland, where 845 to 1739 ppm has been measured (Boyd and Philippot, 1998). Future studies of the nitrogen isotopic composition of  $\text{NH}_4^+$  from metasedimentary phyllosilicates can potentially be used to investigate the biological fractionations of nitrogen associated with the original biomass.

#### 4.3. Phanerozoic nitrogen cycle

Conversion of  $\text{N}_2$  to ammonium is a highly endergonic reaction requiring the breakage of the  $\text{N}_2$  triple bond. The contemporary nitrogen cycle involves a complex interplay between neutral, reduced and oxidized nitrogen compounds (Fig. 8) flowing from the atmosphere (as free  $\text{N}\equiv\text{N}$ ) into biologically fixed nitrogen ( $\text{NH}_4^+$ ) via the nitrogenase enzyme complex. At present,  $\text{N}_2$  fixation is biologically advantageous only when oxidized or reduced nitrogen availability is strongly limited.

Biological nitrogen fixation discriminates against the heavy isotope  $^{15}\text{N}$  such that the resulting biomass is observed to be isotopically light in comparison with air  $\text{N}_2$  with a  $\delta^{15}\text{N}$  value of  $\sim -4\%$  (Delwiche and Steyn, 1970; Minagawa and Wada, 1986; Macko et al., 1987). The mineralization (degradation) of organic nitrogen releases  $\text{NH}_4^+$  cations that are incorporated into clays, aerobically oxidized by nitrifying bacteria or anaerobically oxidized by anammox bacteria (Dalsgaard et al., 2003; Kuypers et al., 2003). Significantly, nitrification by bacteria occurs only in oxygen-rich environments and leads to the release of  $\text{NO}_3^-$ , with a fractionation effect leaving the residual  $\text{NH}_4^+$  enriched in  $^{15}\text{N}$  by about 20‰ (Miyake and Wada, 1971). Anammox bacteria carry out the anaerobic oxidation of  $\text{NH}_4^+$  using  $\text{NO}_2^-$  in the production of  $\text{N}_2$  (Mulder et al., 1995). Inorganic nitrogen species, such as  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , assimilated by organisms can result in isotopic shifts in  $\delta^{15}\text{N}$  around  $-9\%$  and  $-5\%$ , respectively, for the organic nitrogen (Wada and Hattori, 1978). Nitrate can also be respired by denitrifying organisms and returned to the atmosphere as  $\text{N}_2$ . Bacterial denitrification strongly enriches residual  $\text{NO}_3^-$  in  $^{15}\text{N}$  by more

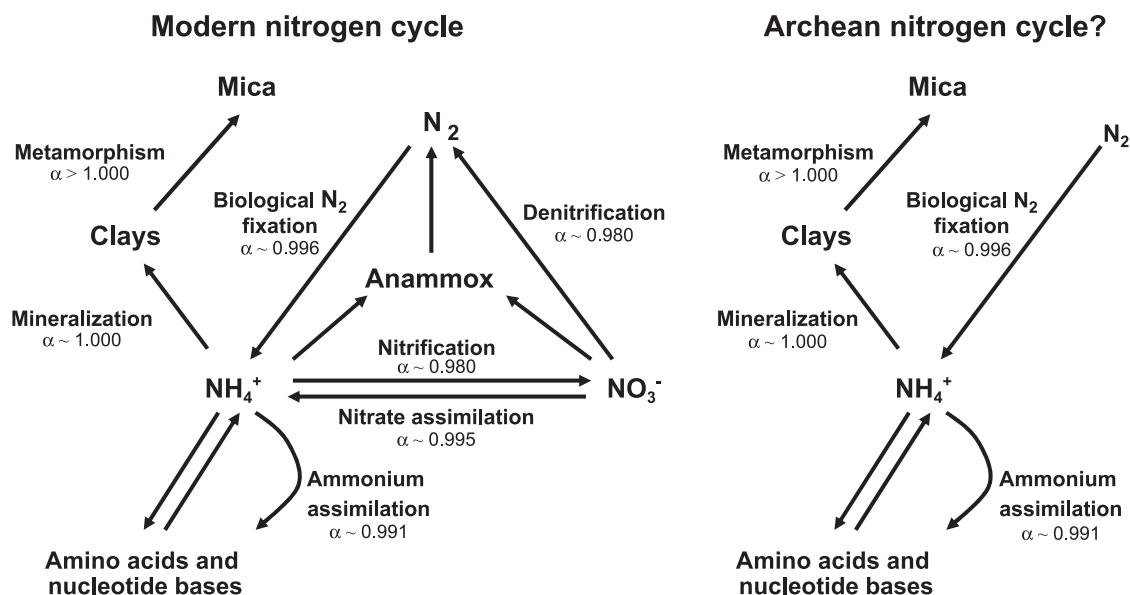


Fig. 8. Left: A simplified modern nitrogen cycle with known fraction effects for the nitrogenous reaction product. Right: The proposed nitrogen cycle operating during the Archean. Fractionation factors are from Delwiche and Steyn (1970), Miyake and Wada (1971), Wada and Hattori (1978), Haendel et al. (1986) and Williams et al. (1995).

than +20‰ (Miyake and Wada, 1971; Delwiche and Steyn, 1970; Wellman et al., 1968), and the remaining  $NO_3^-$  is then available for assimilation and reduction by organisms that carry the resultant heavy  $\delta^{15}N$  value. Modern sedimentary organic matter exhibits a range of  $\delta^{15}N$  values from +2‰ to +10‰, with an average around +6‰ or +7‰, consistent with the dominant role of denitrifying organisms supplying isotopically heavy  $NO_3^-$  to the oceans (Peters et al., 1978; Sweeney et al., 1978). If the Archean atmosphere–hydrosphere system was anoxic, a scarce pool of free  $O_2$  would have mitigated against widespread nitrification. Therefore, the expectation is that  $NO_3^-$  levels were too low to support denitrification as a dominant form of nitrogen metabolism before the rise of free oxygen [“The Great Oxygenation Event” (GOE); Holland, 1994] in the Paleoproterozoic.

#### 4.4. Precambrian nitrogen cycle and atmospheric oxygen

Multiple lines of evidence indicate that the Archean atmosphere was oxygen poor (Holland, 1984). The recent discovery of mass-independently fractionated sulfur isotopes in Precambrian sedimen-

tary rocks (Farquhar et al., 2000) provides an additional line of evidence strongly in support of this view (cf. Ohmoto, 1999). On the (generally) anoxic Archean Earth, it is therefore considered unlikely that the nitrogen cycle would have included a widespread biological cycling of abundant oxidized nitrogen compounds (Fig. 8). In a pioneering study (Beaumont and Robert, 1999),  $\delta^{15}N$  values of kerogens from Early Archean (3.5–3.4 Ga) cherts were found to range from about –6‰ to +13‰ and centered on 0‰. Positive  $\delta^{15}N$  values from Archean metasedimentary rocks are consistent with varying degrees of devolatilization during metamorphic events, which affected all Archean rocks. In samples spanning the time frame 2.1 to 1.6 Ga, it was observed that  $\delta^{15}N$  values shift towards those typical of the modern range of +2‰ to +10‰, which was interpreted to reflect changes to the global nitrogen cycle in response to an increase in atmospheric oxygen concentration. Boyd and Philippot (1998) studied a suite of Mesoproterozoic metasediments and found high concentrations of  $NH_4^+$  characterized by positive  $\delta^{15}N$  values, which was interpreted to reflect a dominance of denitrification in that environment, in line with the view that abundant free oxygen had stabilized  $NO_3^-$  in the

oceans by that time. However, such interpretations have recently been challenged. Jia and Kerrich (2004) propose that the delivery of extraterrestrial material over geologic time can explain secular variations in  $\delta^{15}\text{N}$  data through Earth's history (cf. Dauphas and Marty, 2004). A compilation of published  $\delta^{15}\text{N}$  data from various sedimentary rock types is shown in Fig. 9, which indicates that negative  $\delta^{15}\text{N}$  data were present relatively more often in Archean sediments as opposed to later times. The negative  $\delta^{15}\text{N}$  values found in Mesozoic (Jenkyns et al., 2001; Rigby and Batts, 1986) and Cenozoic (Rau et al., 1987; Sadofsky and Bebout, 2004) sediments are generally interpreted as a decrease in productivity (i.e., lower nitrate availability) in the ocean, temporarily enhancing the effects of nitrogen fixation to the marine cycle of nitrogen.

Despite the possibility of minor negative nitrogen isotopic fractionation from the microbial degradation of organic matter during diagenesis (Lehmann et al.,

2002), the observation that negative  $\delta^{15}\text{N}$  values often characterize Archean sediments is consistent with, and more easily explained by, biological nitrogen fixation (rather than denitrification) as the dominant nitrogen metabolism in the Archean. Fig. 9 also shows that the range of positive  $\delta^{15}\text{N}$  data does not seem to have varied much over time. However, we consider that most Archean sediments studied for nitrogen isotopes have been metamorphosed extensively, with some having experienced multiple high-grade metamorphic events, each of which can potentially increase the  $\delta^{15}\text{N}$  values of sediments. The nitrogen isotopic composition of the atmosphere probably did not vary much since the Hadean and may have been controlled by the recycling of sedimentary nitrogen in the mantle (Marty and Dauphas, 2003; cf. Jia and Kerrich, 2004). Nitrogen isotopic analyses of cherts of various ages seem to indicate that there has not been a significant variation of the atmospheric  $^{15}\text{N}/^{14}\text{N}$  ratio since  $\sim 3.2$  Ga (Sano and Pillinger, 1990). With this background,

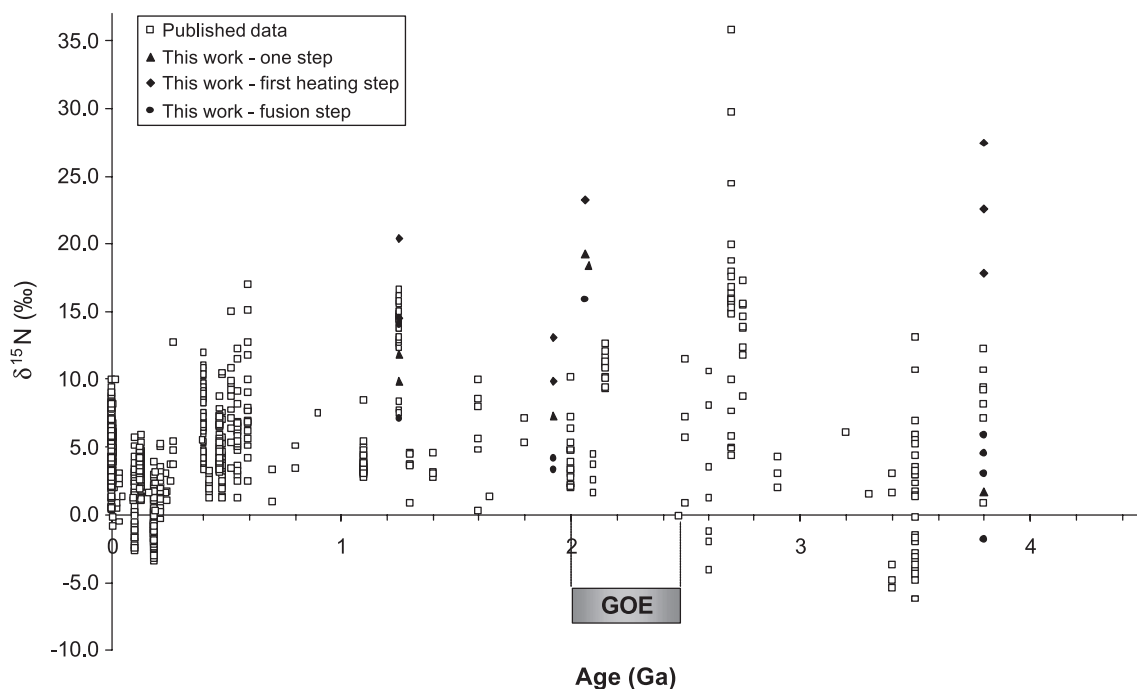


Fig. 9. Compilation of published  $\delta^{15}\text{N}$  data from various sedimentary rock types illustrating the evolution of nitrogen isotopes. For simplicity, the data do not show uncertainty on age estimates. Published data from Jia and Kerrich (2004), Sadofsky and Bebout (2004), Busigny et al. (2003b), Sephton et al. (2002), Mingram and Bräuer (2001), Pinti et al. (2001), Jenkyns et al. (2001), Jia and Kerrich (2000), Sadofsky and Bebout (2000), Beaumont and Robert (1999), Bebout et al. (1999), Boyd and Philippot (1998), Bebout and Fogel (1992), Imbus et al. (1992), Calvert et al. (1992), Sano and Pillinger (1990), Rau et al. (1987), Haendel et al. (1986), Rigby and Batts (1986), Hayes et al. (1983) and Peters et al. (1978). A complete list of data is available on request.

new insights into the nature and timing of the appearance of ancient nitrogen metabolisms may be feasible.

#### 4.4.1. Ancient nitrogen metabolisms

The availability of nitrogen compounds for the synthesis of biomolecules on the early Earth must have been an important factor in the evolution of the first nitrogen metabolisms. The ability to assimilate ammonium directly from the medium and incorporating it into amino acids and nucleotide bases is likely to be extremely ancient. This process is catalyzed by two enzymes, namely, glutamine synthetase (GS) and glutamine-2-oxoglutarate amino transferase (GOGAT), which coupled activities result in the synthesis of glutamine and glutamate, the precursors of all the nitrogenous compounds in the cell. These two key enzymes of the nitrogen cycle may have an evolutionary relationship with biological nitrogen fixation and are thought to be very ancient, perhaps predating the emergence of the Last Common

Ancestor (LCA; Kumada et al., 1993; Dincturk and Knaff, 2000).

#### 4.4.2. Origin of biological nitrogen fixation

Biological nitrogen fixation evolved early in the history of life. Could this metabolism have been operative on the early Earth, at Isua time? Phylogenetic trees based on comparative sequence analyses of small subunit ribosomal RNA (ssu rRNA; Fig. 10) reveal a distribution of nitrogen-fixing microorganisms in both the domains of Archaea and Bacteria (Young, 1992). There are no known Eucarya capable of nitrogen fixation. Archaeal nitrogen-fixing lineages have only been identified in Euryarchaeota, and current understanding of the metabolic diversity in the kingdom of Crenarchaeota is insufficient to conclude that nitrogen fixation is absent there. The existence of diverse nitrogen-fixing organisms in both domains suggests that nitrogen fixation could be a shared trait of the LCA (labeled Root in Fig. 10). Other phylogenetic analyses of amino acid sequences

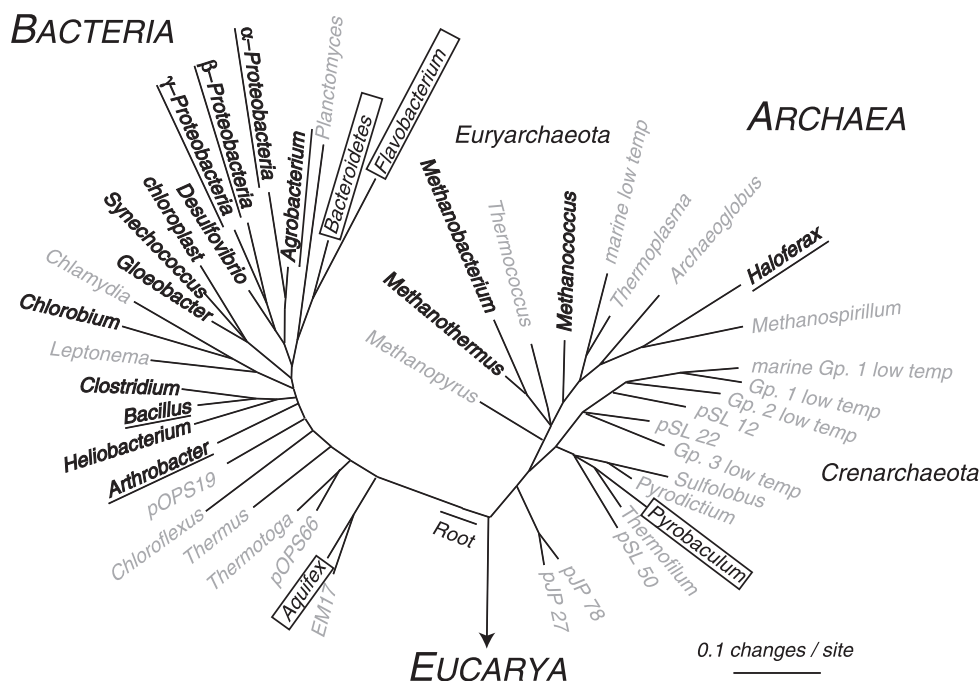


Fig. 10. Phylogenetic tree of organisms based on ssu rRNA (modified from Pace, 1997, with data from Zumft, 1997, and Young, 1992). The thick characters represent nitrogen-fixing lineages, the bold and framed characters show denitrifiers, the thick and underlined characters represent lineages where both denitrification and nitrogen fixation occur, and the light characters show lineages where neither of these two metabolisms has been observed.

of nitrogen-fixing genes (Leigh, 2000) and of paralogous genes central to nitrogen fixation (Fani et al., 2000) also suggest that this metabolism predated the divergence of Archaea from Bacteria.

Atmospheric nitrogen fractionated by biological nitrogen fixation or by ammonium assimilation results in isotopically light organic nitrogen. The nitrogen isotopic composition of ammonium released from the mineralization of organic matter can be modified by early diagenetic processes under different redox conditions (Lehmann et al., 2002). Subsequent metamorphic events can result in the preferential loss of  $^{14}\text{N}$ , leaving the residual  $\text{NH}_4^+$  slightly more enriched in  $^{15}\text{N}$  (Mingram and Bräuer, 2001; Bebout and Fogel, 1992; Haendel et al., 1986; cf. Busigny et al., 2003b). Such isotopic fractionations by thermal metamorphic processes can increase the  $\delta^{15}\text{N}$  value by 5‰ or more (Boyd et al., 1993). We interpret the nitrogen isotopic composition of the Isua biotite from samples GR97im45–47 and GR9817 ( $\delta^{15}\text{N}$  between  $-1.9\text{‰}$  and  $+5.9\text{‰}$  for the fusion step) as consistent with  $\text{NH}_4^+$  from ammonium assimilation and/or biological nitrogen fixation after subsequent enrichment in  $^{15}\text{N}$  through metamorphism. More nitrogen analyses of these sediments and similar samples could provide further support to this interpretation.

#### 4.4.3. Evolution of denitrification

Denitrification is the dominant metabolism modulating the contemporary nitrogen isotopic composition of oceanic  $\text{NO}_3^-$  and of modern sedimentary nitrogen (Wada et al., 1975; Peters et al., 1978; Sweeney et al., 1978). Denitrifying organisms are found in the domains of Bacteria and Archaea (Fig. 10), and this observation seem to support the view that denitrification is also a nitrogen metabolism of great antiquity (Zumft, 1997). The  $\delta^{15}\text{N}$  data of Paleoproterozoic metasedimentary biotites (1.90–2.09 Ga) from Finland reported in this study provide some support to the hypothesis that denitrification became the dominant nitrogen metabolism within only a few hundred million years after the GOE. However, our results do not exclude the possibility that metamorphic nitrogen losses significantly affected the  $\delta^{15}\text{N}$  value of biotites. More nitrogen isotopic analyses of Precambrian metasediments are warranted to reinforce this observation and to confirm that metasedi-

ments also record a Paleoproterozoic nitrogen isotopic shift.

#### 4.5. Abiological nitrogen fixation

A few common mechanisms considered operative on the early Earth, such as lightning, hydrothermal circulation/mixing or mineral catalysis, may have resulted in the abiotic fixation of nitrogen. Atmospheric physical models and experimental measurements demonstrate that lightning can synthesize reduced or oxidized nitrogen compounds, depending on  $p\text{H}_2$  of the atmosphere (Yung and McElroy, 1979). Thermodynamic calculations and experiments simulating reduced hydrothermal vent conditions (with  $\text{H}_2\text{S}$ ) have shown that  $\text{N}_2$  can be reduced to  $\text{NH}_4^+$ ; however, the yield for this reaction is low (in the nanomolar range; Schoonen and Xu, 2001). Mineral catalysis simulated using neutral and oxidized nitrogen species mixed with simple neutral or reduced compounds can produce  $\text{NH}_3$  (Summers and Chang, 1993; Summers, 1999; Summers and Lerner, 1998; Brandes et al., 1998). It was also shown that reduced nitrogen compounds can be synthesized photochemically on a Ti-oxide catalyst (Schrauzer and Guth, 1977; Bickley and Vishwanathan, 1979), but this contribution is considered to have been minor on the early Earth (Henderson-Sellers and Schwartz, 1980). The types of reactions noted here could perhaps have provided an essential prebiotic source of (endogenic)  $\text{NH}_3$  to the Hadean hydrosphere, but the contribution of these abiotic nitrogen-fixing processes for  $\text{NH}_4^+$  in the Isua metapelites is probably minimal, considering the high  $[\text{NH}_4^+]_{\text{biotite}}$  (233 to 512 ppm), comparable with concentrations in more recent metasedimentary biotites.

## 5. Conclusions

The objective of this study was to investigate portions of the Precambrian rock record for insights into ancient biogeochemical nitrogen cycling. We used  $\mu\text{FTIR}$  to evaluate the  $[\text{NH}_4^+]_{\text{biotite}}$ , which proved to be a useful tool for the preselection of samples for laser mass spectrometry. The results of these analyses were used to build a curve for a rapid estimation of  $[\text{NH}_4^+]_{\text{biotite}}$ . Analyzed biotites were found to contain



$\text{NH}_4^+$  concentrations between 176 and 1549 ppm, consistent with a sedimentary and biological origin. When combined with its nitrogen isotopic composition,  $\text{NH}_4^+$  can be used to evaluate the source of nitrogen in Archean sediments and to test hypotheses regarding the evolution of different nitrogen metabolisms in response to long-term chemical changes to the surface environment. We used laser mass spectrometry to perform two-step heating experiments as an attempt to distinguish potential sources of ammonium in biotite. We found that the nitrogen isotopic composition of the low-temperature step can be compatible with the preferential volatilization of  $^{14}\text{NH}_4^+$ , which leaves the residual structural ammonium, analyzed in the high-temperature fusion step, enriched in  $^{15}\text{N}$ . We determined that the 3.8-Ga-old Isua biotite schists carry two nitrogen components: The  $\text{NH}_4^+$  from the low temperature step may represent weakly bound ammonium of metamorphic origin, and the  $\text{NH}_4^+$  from the high-temperature step likely represents primary structural ammonium isotopically altered by metamorphism. The simplest explanation for the isotopic composition of the structural  $\text{NH}_4^+$  in Isua metapelites is biological nitrogen fixation and/or ammonium assimilation by 3.8 Ga. This result may therefore imply that the primary branching event of the phylogenetic tree based on ssu rRNA occurred before 3.8 Ga. Our results also suggest that isotopically heavy nitrogen from externally derived fluids occur in these sediments and that nitrogen heterogeneities may exist at the scale of the outcrop.

Our data are consistent with the trend in nitrogen isotopic compositions of Paleoproterozoic kerogens as reported by [Beaumont and Robert \(1999\)](#), but more analyses of more samples from single outcrops (as a test of heterogeneity) and diverse samples from well-characterized terranes spanning the GOE are warranted. Nitrogen isotopic compositions of  $\text{NH}_4^+$  from Paleo- and Mesoproterozoic metasedimentary biotites from Finland and Scotland are consistent with denitrification having become the dominant nitrogen metabolism after the oxidation of the Earth's surface. Further investigations of this system by the techniques described here are necessary to provide stronger support for the existence of the nitrogen isotopic shift in metasediments. These data hold the potential for providing a high-resolution

time evolution of the nitrogen cycle in a variety of samples from throughout the geological record. A few possible future applications of this work include the following: high temporal resolution analysis of samples spanning the GOE coupled with studies on possible connections with mass-independent fractionation of sulfur isotopes, nitrogen isotopic fractionation in the domain Archaea, nitrogen fixation in the Archaeal lineage of Crenarchaeota and nitrogen studies on Martian sediments returned from future sampling missions.

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