

1 **N₂ in deep subsurface fracture fluids of the Canadian Shield: Source and**
2 **possible recycling processes**

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15
16 **Key Words**

17 Nitrogen cycle, Canadian Shield, deep subsurface, abiotic nitrogen reduction, microbial ammonium
18 oxidation

19 **Abstract:** In addition to high concentrations of CH₄ and H₂, abundant dissolved N₂ is found in
20 subsurface fracture fluids in Precambrian cratons around the world. These fracture fluids have
21 hydrogeological isolation times on order of thousands to millions and even billions of years. Assessing
22 the sources and sinks of N₂ and related (bio)geochemical processes that drive the nitrogen cycle in these
23 long isolated systems can shed insights into the nitrogen cycles on early Earth with implications for other
24 planets and moons. In this study, we collected dissolved gas samples from deep subsurface fracture fluids
25 at seven sites (Kidd Creek, LaRonde, Nickel Rim, Fraser, Copper Cliff South, Thompson, and Birchtree)
26 in the Canadian Shield. Multiple gas components (e.g., H₂, O₂ and Ar) were integrated with δ¹⁵N_{N₂} values
27 to characterize the N₂ signatures. Results show that the dissolved N₂ in deep subsurface fracture fluids
28 from the Canadian Shield sites are more ¹⁵N-enriched than those from the Fennoscandian Shield and the
29 Witwatersrand Basin in the Kaapvaal Craton. The nitrogen isotopic signatures of the Canadian Shield
30 samples coupled with their hydrogeological framework indicate the N₂ was sourced from fixed
31 ammonium in silicate minerals in host rocks and was generated by metamorphic devolatilization.
32 Modeling of nitrogen devolatilization from host rocks supports this interpretation, but also suggests that a
33 second process, likely abiotic N₂ reduction, is required to account for the observed ¹⁵N enrichment in the
34 N₂ samples from the Canadian Shield. A 10-year monitoring study for one of the boreholes, at 2.4 km of
35 the Kidd Creek Observatory, shows a steady decrease in δ¹⁵N_{N₂} values with time, which coincides with
36 the temporal isotopic evolution of some other gas components in this borehole. Although it cannot be
37 confirmed at this time, this isotopic shift in N₂ may be potentially attributed to microbial processes (e.g.,
38 anaerobic oxidation of ammonium). Nevertheless, the large ¹⁵N enrichments for the majority of the
39 samples in this study suggest that the nitrogen cycle in the deep saline fracture fluids in the Canadian
40 Shield is dominated by abiotic processes. This is in contrast to the nitrogen cycles in the subsurface
41 fracture fluids in the Fennoscandian Shield and the Witwatersrand Basin, which have been shown to be
42 strongly affected by extant microbial ecosystems discovered in those fracture waters.

43 **1. Introduction**

44 Precambrian cratons occupy the majority of the Earth's continental crust (> 70% by
45 surface area; Goodwin, 1996). These remnants of early Earth's crust are dominated by
46 (meta-)igneous rocks varying from ultramafic to felsic in composition (e.g., Hoffmann, 1989).
47 Abundant fractures developed over the history of these igneous rocks host a large terrestrial
48 subsurface water reservoir of up to 30% of the planet's total groundwater inventory (Warr et al.,
49 2018). Geochemical signatures (e.g., salinity, major and trace elemental compositions, dissolved
50 gas contents, redox condition, $\delta^{18}\text{O}$ and $\delta^2\text{H}$) of these fluids indicate that they have been strongly
51 influenced by water-rock reactions, and, in some cases, affected by mixing with varying amounts
52 of secondary (paleo-)meteoric water (e.g., Ward et al., 2004; Onstott et al., 2006; Li et al., 2016;
53 Heard et al., 2018; Warr et al., 2021a and references therein). Most of these deep subsurface
54 fracture water systems have been hydrogeologically isolated over geological time scales, e.g., up
55 to hundreds of million years to billions of years in the Canadian Shield (Holland et al., 2013;
56 Warr et al., 2018), up to tens to hundreds of million years in the Fennoscandian Shield
57 (Kietäväinen et al., 2014) and the Witwatersrand Basin in the Kaapvaal Craton, South Africa
58 (Heard et al., 2018; Lippmann et al., 2003). Closed-system water-rock interactions over these
59 extended time periods has progressively produced chemicals (e.g., H_2 , hydrocarbons, sulfate) and
60 highly reducing habitable environments favorable for chemo(litho)trophic microbes (Lin et al.,
61 2005, 2006; Li et al., 2016; Magnabosco et al., 2018; Lollar et al., 2019).

62 Studies of the subsurface fracture fluids from the Kaapvaal Craton, the Canadian Shield
63 and the Fennoscandian Shield have identified some general quasi-depth-controlled variations in
64 geochemistry and microbial community attributed to the degree of penetration of
65 (paleo-)meteoric water into the subsurface (Warr et al., 2021a and references therein). Based on

66 noble gas studies, the shallower fracture fluids generally have shorter residence times, whereas
67 the deeper, more saline fracture fluids have longer residence times (Lippmann et al., 2003;
68 Lippmann-Pipke et al., 2011; Heard et al., 2018; Warr et al., 2018; 2021a). The shallow fracture
69 fluids are mostly highly oxidizing fresh to brackish waters, and contain abundant biomass with
70 diverse microbial communities; in contrast, the deep fracture fluids are mostly highly reducing
71 saline to brine waters, and contain low-abundance biomass with less microbial diversity (e.g.,
72 Ward et al., 2004; Onstott et al., 2006; Lin et al., 2006; Magnabosco et al., 2015; Lollar et al.,
73 2019).

74 These geochemical and microbiological differences can directly impact the isotopic
75 signatures of the dissolved gaseous components in the fracture fluids. For example, different
76 carbon recycling pathways have been clearly identified in these fracture fluids based on the
77 carbon and hydrogen isotopic signatures of CH₄ and other light n-alkanes (e.g., Sherwood Lollar
78 et al., 1993a, b, 2002, 2006, 2008; Ward et al., 2004; Warr et al., 2021b) and clumped isotopic
79 signatures of CH₄ and N₂ (Young et al., 2017; Labidi et al., 2020; Warr et al., 2021b). These
80 studies have reported consistent observations from both the Canadian Shield and the Kaapvaal
81 Craton suggesting that CH₄ in the shallower fracture fluids is dominantly microbial, whereas
82 CH₄ in the deeper, more saline fracture fluids with longer residence times can show
83 predominantly abiogenic signatures (Sherwood Lollar et al., 2006; 2008, 2021; Warr et al.,
84 2021b;). More recently, formate and acetate in fluids at 2.4 to 3 km below surface (kmbs) at one
85 of the key deep subsurface sites of the Canadian Shield, the Kidd Creek (KC) Observatory, have
86 been demonstrated to be produced by abiotic organic synthesis (Sherwood Lollar et al., 2021).
87 Multiple sulfur isotopic signatures of dissolved sulfate in fluids from 2.4 kmbs of the KC

88 Observatory also indicate that the dissolved sulfate in these fluids was derived from abiotic
89 process by indirect radiolytic oxidation of sulfide minerals in host rocks (Li et al., 2016).

90 Despite numerous studies on the carbon and sulfur cycles in the subsurface fracture fluids
91 in Precambrian cratons, to date, the cycle of another life-constituting element, nitrogen, has not
92 been well examined, particularly in the Canadian Shield. N₂ is typically the second largest
93 dissolved gas component by volume in subsurface fracture fluids in Precambrian cratons around
94 the world. In an earlier study, Sherwood Lollar et al. (1993a) reported the $\delta^{15}\text{N}$ values of N₂
95 collected from fracture fluids in the Fennoscandian Shield and the Canadian Shield. Thirteen N₂
96 samples from the Fennoscandian Shield show a $\delta^{15}\text{N}$ range from 0.2‰ to 5.1‰ (with one high
97 value of 10.0‰), whereas four N₂ samples from the Canadian Shield mostly show higher $\delta^{15}\text{N}$
98 values (Thompson: 1.4‰; Matagami: 8.6‰; Norita: 11.8‰; Val d'Or: 9.1‰). Labidi et al.
99 (2020) also reported two $\delta^{15}\text{N}$ data from Sudbury (2.6‰ and 2.8‰) and two $\delta^{15}\text{N}$ data from the
100 KC Observatory (6.6‰ and 6.8‰). Dissolved N₂ in subsurface fracture fluids from the
101 Witwatersrand Basin in the Kaapvaal Craton was studied by Silver et al. (2012). After correction
102 for air contamination using the N₂/O₂ ratio as an index, a $\delta^{15}\text{N}_{\text{N}_2}$ range from -1.3‰ to 5.8‰ was
103 yielded and considered to result from complicated geochemical and, more importantly,
104 biological recycling processes (Silver et al., 2012).

105 The relatively few $\delta^{15}\text{N}$ data from the Canadian Shield make it difficult to compare with
106 those from the Fennoscandian Shield and the Kaapvaal Craton for the understanding of nitrogen
107 cycle in subsurface fracture fluids in Precambrian cratons. In this context, it is important to carry
108 out a more comprehensive investigation of N₂ in deep fracture fluids in the Canadian Shield,
109 particularly those from the KC Observatory. The KC Observatory located in Timmins (Ontario)
110 is an iconic site for the study of subsurface fracture fluids, given the tremendous knowledge

111 about this site accumulated from studies over nearly three decades (e.g., Sherwood Lollar et al.,
112 1993a, b, 2002, 2021; Doig et al. 1995; Holland et al., 2013; Li et al., 2016; Lollar et al., 2019).
113 In particular, long-term monitoring has been possible at an observatory location 2.4 kmbs since
114 2007, commencing shortly after the exploratory boreholes were drilled. Noble gas studies
115 indicate that the mean residence times of the fluids at KC were extremely long (e.g., 1.0 – 2.2 Ga
116 at 2.9 kmbs; Warr et al., 2018), while hydrogeological draining of the fluids may result in some
117 boreholes decreasing in age over time (e.g., at 2.4 kmbs, initially 1.1 – 1.7 Ga but has decreased
118 to 0.2 – 0.6 Ga recently; Holland et al., 2013, Warr et al., 2018). To date, these fluids show the
119 presence of chemolithotrophic organisms (sulfate-reducing organisms in particular) but with low
120 biomass and low rates of activity (Li et al., 2016; Lollar et al., 2019; Sherwood Lollar et al.,
121 2021; Warr et al., 2021b). The geochemical and isotopic signatures of these fluids (including
122 dissolved components) may represent the closest abiotic end-members available in the terrestrial
123 deep subsurface (e.g., Sherwood Lollar et al., 2021; Warr et al., 2021b). Such abiotically
124 dominated sites provide a valuable opportunity to assess the long-term abiotic nitrogen recycling
125 processes occurring in these highly reducing waters. This knowledge may further provide crucial
126 insights into the geochemical processes that could have influenced the origin and early evolution
127 of life on the early Earth, and inform models of possible habitability on other planets and moons
128 (NASEM, 2019).

129 Here we report the gas concentrations and $\delta^{15}\text{N}_{\text{N}_2}$ values of 45 samples from 7 sites in the
130 Canadian Shield (Fig. 1). In particular, 25 samples were collected from the KC Observatory from
131 2.1 to 2.9 kmbs, and at the 2.4 kmbs sampling location, over a temporal span of 10 years (2007-
132 2017). This sample suite provides a high-density dataset to evaluate the spatial and temporal
133 changes of the nitrogen cycle in these fracture water systems. Aiming to better constrain the

134 nitrogen source and recycling processes in the fracture fluids in the Canadian Shield, bulk-rock
135 nitrogen concentrations and isotope compositions were also analyzed on 23 host rock samples
136 from KC, Sudbury, and Thompson. For comparison, nitrogen isotope compositions were also
137 measured on refractory carbon component in 6 graphite-bearing samples from KC.

138

139 **2. Geological background and samples**

140 The samples in this study were collected from two sites in the Abitibi greenstone belt
141 (i.e., KC and LaRonde), three sites in the Sudbury Basin (i.e., Copper Cliff South, Nickel Rim,
142 and Fraser), and two neighboring sites (Birchtree and Thompson) in the Thompson nickel belt in
143 the western Superior boundary zone, near Thompson, Manitoba (Fig. 1).

144 The KC Observatory in Ontario is geologically located in the volcanic successions of the
145 Kidd-Munro assemblage in the Abitibi. The host rocks at KC include 2.71 – 2.72 Ga ultramafic,
146 mafic (gabbros and basalts) to felsic (mainly rhyolites) rocks with graphite-bearing lenses
147 formed in a rifting sub-proximal seafloor setting (Bleeker and Parrish, 1996; Hannington et al.,
148 1999; Prior et al., 1999). The rocks experienced regional metamorphism which started at 2.69
149 Ga, reached peak metamorphism of greenschist facies (~ 400 °C) at 2.64 Ga, and ended as late as
150 2.60 Ga locally (Barrie and Davis, 1990; Smith et al., 1993; Davis et al., 1994; Bleeker et al.,
151 1999; Powell et al., 2011).

152 LaRonde (LR) in Quebec is geologically located in the Bousquet formation of the 2.7 Ga
153 Blake River group in the Abitibi. The host rocks are composed of a continuous magmatic series
154 of tholeiitic – transitional mafic to felsic rocks in the lower member, and transitional – calc-
155 alkaline intermediate to felsic rock in the upper member (Mercier-Langevin et al., 2007). The

156 rocks experienced a prograde metamorphism to upper greenschist – lower amphibolite facies and
157 a subsequent greenschist-facies retrograde metamorphism (Dimroth et al., 1983).

158 Copper Cliff South (CCS), Nickel Rim (NR) and Fraser (FR) are all in the Sudbury
159 impact basin (Ontario). The dominant rock assemblage in the region is the Sudbury Impact
160 Complex (SIC) which was formed by meteoritic impact-induced melting of the Archean
161 basement (Faggart et al., 1985) in ~1.85 Ga (Krogh et al., 1982; Davis, 2008). FR is in the
162 middle of the North Range and NR is in the east of the South Range of the SIC. Both sites
163 geologically lie in the footwall of the SIC that is dominated by 2.64 Ga granitic rocks (Meldrum
164 et al., 1997) and Archean meta-volcanic and metasedimentary rocks that were metamorphosed to
165 upper greenschist – lower amphibolite facies at 2.69 Ga with some reaching granulite facies at
166 2.65 Ga (Krogh et al., 1984). CCS is located in a radial offset quartz-diorite dyke in the South
167 Range with brecciated country rocks and abundant sulfide mineralization (Lightfoot and Farrow,
168 2002) related to the ~1.85 Ga bolide impact. Post-impact thermal influences by multiple
169 orogenies, such as the Yavapai-Mazatzal (1.7-1.6 Ga), the Chieflakian-Pinwarian (1.5-1.4 Ga)
170 and the Grenville (1.2-1.0 Ga), have been found at various degrees across the Sudbury region,
171 e.g., mid- to upper-greenschist facies in the South Range (Fleet et al., 1987) and sub-greenschist
172 facies (< 150 °C) in the North Range (Thompson et al., 1998).

173 The Thompson nickel belt (near Thompson, Manitoba) is a northeast-southwest trending
174 belt formed in an early Proterozoic continental margin. It is constituted by reworked Archean
175 basement and meta-supracrustal rocks (Hulbert et al., 2005). In the western part of the belt where
176 BT and TH are located, the rocks are dominated by 2.2 – 2.0 Ga serpentinized ultramafic rocks,
177 metagabbros, amphibolites, gneisses, schists, skarns, and quartzites (Hulbert et al., 2005;

178 Zwanzig et al., 2007). The rocks experienced a series of metamorphic events during 1850 – 1750
179 Ma (Couëslan et al., 2013) with peak metamorphic temperatures of ~ 700 °C in the Thompson
180 area (Paktunç, 1984). Later (not yet dated) low-temperature (250 – 300 °C) alteration of rocks at
181 Thompson by chloride-rich fluid is evidenced by the PGE-Au-As mineralization (Chen et al.,
182 1993).

183 Gas samples were collected following the method described by Ward et al. (2004) and
184 Holland et al. (2013). In brief, a packer was used to seal the borehole and funnel the water and
185 gas flow from the borehole through a sterilized Tygon tube into a bucket. After a period of
186 flushing to ensure representative sampling, gases were introduced into an inverted beaker
187 submerged into the fracture fluids collected in a bucket. The gases were then transferred through
188 a 22-g syringe needle on a Luer attachment at the top of the beaker into 160 ml borosilicate vials
189 with blue butyl stoppers which had been sterilized, prefixed with 50 µL saturated HgCl₂ solution,
190 and pre-evacuated.

191 Rocks samples were collected from exploration drill cores provided by the mines. A total
192 of 23 samples were selected from representative lithologies of the host rocks from KC, Sudbury,
193 and Thompson.

194

195 **3. Methods**

196 Gas concentrations and nitrogen isotope compositions of N₂ were analyzed at University
197 of Toronto. Gas concentrations were generally measured within days after sample collection to
198 avoid possible shifts in gas (e.g., H₂ and O₂) concentrations due to diffusion and/or reaction,
199 although the results of re-analyses in 2007 on a batch of samples collected in 2000 show no

200 significant difference in H₂ and O₂ concentrations in comparison with the results obtained in
201 2000. Gas concentrations were measured by a Varian gas chromatography equipped with Varian
202 Molecular Sieve 5A PLOT fused silica column and a micro-thermal conductivity detector
203 (μ TCD) for H₂, He, N₂, CO₂, O₂, Ar and a flame ionization detector for alkanes following the
204 published protocols (e.g., Ward et al., 2004). NH₃ is not specially examined here because it
205 requires high pH condition to drive NH₃ from fluids to gas phases (e.g., Deng et al., 2018),
206 whereas the modeling based on the pH and temperature conditions of the studied fluid samples
207 suggests that NH₃ mostly concentrates in fluids and barely occurs in the gas phases (Li et al.,
208 2012). The 2 σ analytical error is < 5% of the absolute concentrations based on repeated analyses
209 of lab standards and natural samples. The nitrogen isotopic ratio of N₂ was measured by gas
210 chromatography-isotope ratio mass spectrometry. An aliquot of a gas sample was taken by gas-
211 tight syringe from a sample bottle and injected into a Varian gas chromatography, in which a
212 slow flow rate of 1.2 ml/min was used for the carrier gas (ultrahigh-purity helium) to ensure the
213 separation of N₂ from other gas components such as H₂ and O₂. CO₂ and CO are not significant
214 interferences in this analysis because their natural abundances in the gas samples are below the
215 detection limit (< 0.01 vol%). A time window was set to allow N₂ but no other interference gases
216 to be carried into a Finnigan 252 isotope ratio mass spectrometer for isotopic measurements. The
217 isotopic ratios are reported using the δ notation ($\delta^{15}\text{N} = (^{15}\text{N}/^{14}\text{N})_{\text{sample}} / (^{15}\text{N}/^{14}\text{N})_{\text{standard}} - 1$), in
218 which “standard” is atmospheric N₂. All samples were measured in duplicate. Repeat analyses of
219 these samples and laboratory working standards cross-calibrated against international IAEA
220 nitrogen reference materials gave 2 σ analytical errors better than 0.2%.

221 Nitrogen isotopic ratios of rocks were measured at University of Alberta following the
222 method described by Li et al. (2021a). In brief, after removal of the surface by sawing, rock

223 chips from drill cores were ground into fine powders (< 200 mesh). For the graphite-bearing lens
224 samples, nitrogen was measured on both bulk-rock samples and refractory reduced carbon
225 residues following the extraction protocol of Eigenbrode and Freeman (2006). The powder
226 samples were weighted and loaded into pre-cleaned one end-sealed quartz tube together with
227 CuO reagents, pumped in a metal manifold, sealed under high vacuum, and combusted first at
228 1200 °C for 30 min to 60 min (depending on lithology; see details in Li et al., 2021a) and then at
229 900 °C overnight to completely release the fixed nitrogen in minerals. The sample tube was then
230 cracked under high vacuum to release N₂, which was further cryogenically purified, quantified
231 by a capacitance manometer, and carried by an ultrahigh-purity helium gas to a Thermo Finnigan
232 MAT 253 for nitrogen isotopic measurements. The $\delta^{15}\text{N}$ values are reported relative to the
233 atmospheric N₂. Repeat analyses of two standards (Low Organic Content Soil and High Organic
234 Content Soil) from Elemental Microanalysis Ltd gave 2 σ analytical errors better than 0.2‰ (Li
235 et al., 2021a).

236

237 **4. Results**

238 **4.1. Gas concentrations and $\delta^{15}\text{N}_{\text{N}_2}$ values**

239 The major gas concentrations and $\delta^{15}\text{N}$ values of N₂ are reported in Table 1. The results
240 are briefly summarized below.

241 Twenty-five gas samples from KC are dominated by CH₄ (64 – 83 vol%) and other light
242 n-alkanes (e.g., ethane, propane, butane; in total < 9 vol%). N₂ is the second most abundant gas
243 component with concentrations varying from 4.5 vol% to 16.9 vol% and $\delta^{15}\text{N}$ values from 3.2‰
244 to 8.0‰ (Table 1). These isotopic values fall in the $\delta^{15}\text{N}$ range of 2.8‰ – 8.8‰ from a limited

245 set of KC samples in Montgomery (1994) and Labidi et al. (2020). H₂ gas was detected in all KC
246 samples except one, with variable concentrations from 0.3 vol% to 10.7 vol%.

247 Two gas samples from LR are also dominated by CH₄ (66 – 72 vol%) and N₂ (9 – 15
248 vol%) with $\delta^{15}\text{N}$ values of 6.5‰ and 7.4‰, respectively. Both samples contain H₂ (1.8 vol% and
249 3.6 vol%, respectively). All these features are similar to those of the KC samples.

250 Six gas samples from CCS (on the south rim of the Sudbury Basin) are characterized by
251 extremely high H₂ concentrations (10 – 58 vol%). CH₄ is the other major gas component in these
252 samples (14 – 70 vol%). N₂ concentrations are generally low in the samples studied at this site
253 (Table 1). The $\delta^{15}\text{N}_{\text{N}_2}$ values (0.6‰ to 4.2‰) of these samples are also much lower than those of
254 the KC and LR samples. It is worth noting that variable amounts of O₂ (0.4 – 5.5 vol%) are
255 detected in these samples, which suggests significant air contamination (see Section 4.2). In a
256 previous unpublished study by Montgomery (1994), much higher N₂ concentrations of up to 65
257 vol% and $\delta^{15}\text{N}$ values up to 17.5‰ were reported from CCS. The other samples from the
258 Sudbury Basin show significantly higher N₂ concentrations. The five NR gas samples are
259 dominated by N₂ (47 – 53 vol%), He (24 – 32 vol%) and CH₄ (15 – 24 vol%). H₂ occurs all
260 samples in varying amounts (0.01 – 2.7 vol%). The $\delta^{15}\text{N}_{\text{N}_2}$ values display a small range from
261 3.5‰ to 5.0‰. The only gas sample available from FR is also dominated by N₂ (66 vol%), He
262 (17 vol%) and CH₄ (20 vol%). It also contains much higher Ar concentration (5.2 vol%) than all
263 the other studied gas samples from the Canadian Shield (< 2 vol%). The reason for this is not
264 fully understood at this time. The FR sample has a $\delta^{15}\text{N}_{\text{N}_2}$ value of 5.0‰, similar to those of the
265 NR samples.

266 Three gas samples from BT and four gas samples from TH are all dominated by CH₄ and
267 N₂. The CH₄ concentrations (63 – 69 vol%) are higher than N₂ concentrations (29 – 31 vol%) in
268 the BT samples, whereas the N₂ concentrations (46 – 66 vol%) are higher than the CH₄
269 concentrations (35 – 48 vol%) in the TH samples. Despite the difference in N₂ concentration, the
270 $\delta^{15}\text{N}_{\text{N}_2}$ values are consistent between BT and TH, ranging from 7.4‰ to 8.7‰. All of the BT and
271 TH gas samples except one contain detectable H₂, generally at levels between 0.02 – 2.75 vol%.

272

273 **4.2. Distinguishing sampling-related air contamination**

274 A characteristic feature of the studied gas samples from the Canadian Shield is the
275 ubiquitous occurrence of H₂ gas (Table 1). This indicates that highly reducing conditions have
276 been established and maintained in the host fracture fluids of these gas samples, which is
277 consistent with all past studies. These highly reduced fracture fluids should not contain any *in-*
278 *situ* O₂ gas (Holland et al., 2013; Warr et al., 2018, 2021a). Therefore, any O₂ detected in the gas
279 samples should be indicative of recent air contamination, mostly likely during sampling.

280 To assess the air contamination in the studied samples, $\delta^{15}\text{N}_{\text{N}_2}$ values are compared with
281 N₂/O₂ and N₂/Ar concentration ratios (Fig. 2). Fig. 2A illustrates a decreasing trend of $\delta^{15}\text{N}_{\text{N}_2}$
282 values following the decrease in N₂/O₂ ratios toward the air end-member, particularly seen in
283 some of the CCS and KC samples. The data comparison (Fig. 2A) shows that more consistent
284 ranges of $\delta^{15}\text{N}_{\text{N}_2}$ values can be observed for samples with N₂/O₂ ratios > 15 at each site. The
285 $\delta^{15}\text{N}_{\text{N}_2}$ values of samples with N₂/O₂ < 15 are more or less altered by air contamination.

286 Unlike O₂, which can be readily identified as a result of air contamination in these highly
287 reducing fluids, ⁴⁰Ar (the dominant isotope of Ar) can be produced by subsurface processes and
288 hence cannot be used to evaluate air contamination (Fig. 2B). ⁴⁰Ar, a product of the radioactive

289 decay of ^{40}K , can accumulate in the fracture waters over time and vary significantly depending
290 on the lithology of local host rocks and isolation time of the fracture fluids (Warr et al., 2019).
291 N_2 concentrations may also vary significantly, depending on the thermal history, nitrogen source,
292 abiotic reactions, and biological activities (if any) in the fracture waters (Silver et al., 2012).
293 These two factors can result in highly heterogeneous N_2/Ar ratios in the deep subsurface of the
294 Canadian Shield (Fig. 2B), which may be a result of *in-situ* processes and not necessarily reflect
295 air contamination. The data compilation of less contaminated samples (i.e., $\text{N}_2/\text{O}_2 > 15$) on Fig.
296 2C illustrates that the N_2/Ar ratios increase in order from LR, FR, NR, KC, to BT and TH.
297 Therefore, without knowing the N_2/Ar values of uncontaminated gases, N_2/Ar ratios alone cannot
298 be used to estimate the fractions of air components in gas samples from Precambrian cratons.

299 Accordingly, the discussion below on the $\delta^{15}\text{N}_{\text{N}_2}$ values of the Canadian Shield samples
300 will focus only on those with $\text{N}_2/\text{O}_2 > 15$.

301

302 **4.3. $\delta^{15}\text{N}$ values of rock samples**

303 The nitrogen concentrations and $\delta^{15}\text{N}$ values of rock samples are listed in Table 2.

304 Six silicate host rock samples from KC, including three rhyolites, two silicified cherty
305 breccia and one quartz feldspar porphyry, show a $\delta^{15}\text{N}$ range from 6.0‰ to 10.4‰. Six samples
306 of the graphite-bearing lenses from KC give a $\delta^{15}\text{N}$ range from 6.7‰ to 15.6‰ for bulk-rock
307 nitrogen, but a lower $\delta^{15}\text{N}$ range from 0.4‰ to 10.4‰ for extracted refractory reduced carbon
308 residues.

309 Sudbury host rock samples comprise of two samples (a meta-gabbro and a breccia
310 matrix) from the Creighton mine, which has similar wall rock to the CCS mine, and six samples
311 from NR (two felsic gneisses, two felsic norites, one granodiorite, and one breccia). All the

312 samples give a small $\delta^{15}\text{N}$ range from 1.3‰ to 3.4‰ except one felsic norite sample with a $\delta^{15}\text{N}$
313 value of -5.6‰.

314 Three host rock samples from TH, including one gneiss, one schist, and one skarn, show
315 relatively high $\delta^{15}\text{N}$ values from 7.0‰ to 10.2‰.

316

317 **5. Discussion**

318 **5.1. $\delta^{15}\text{N}_{\text{N}_2}$ variability across sites in the Canadian Shield**

319 After discarding all the samples with $\text{N}_2/\text{O}_2 < 15$, the $\delta^{15}\text{N}_{\text{N}_2}$ distribution (Fig. 2C) shows
320 a general trend with the lowest values in NR and FR, and steadily increasing values from LR,
321 KC, to BT and TH. However, this isotopic pattern is not confirmative at the moment given that
322 only a small number of samples were collected from a specific depth in each of the sites (except
323 KC). A larger dataset from multiple depths at KC give much larger $\delta^{15}\text{N}_{\text{N}_2}$ variation than those of
324 the other sites (Fig. 2C; see Section 5.4 for discussion). Two samples from different depths at
325 CCS also show large $\delta^{15}\text{N}_{\text{N}_2}$ variation (Montgomery, 1994; Fig. 2A). Therefore, more data from
326 each site are needed in future studies to examine the inter-site $\delta^{15}\text{N}_{\text{N}_2}$ variability and its
327 controlling factors in the Canadian Shield.

328

329 **5.2. Comparison with the Fennoscandian Shield and the Kaapvaal Craton**

330 The $\delta^{15}\text{N}$ values of N_2 from subsurface fracture fluids have been reported from some sites
331 on other Precambrian cratons, e.g., the Fennoscandian Shield (Sherwood Lollar et al., 1993a) and
332 the Kaapvaal Craton (Silver et al., 2012). As mentioned above, the thirteen published gas
333 samples from the Fennoscandian Shield (Sherwood Lollar et al., 1993a) have $\delta^{15}\text{N}_{\text{N}_2}$ values from
334 0.2‰ to 5.1‰ (except one high value of 10.0‰) with a peak at the bin of 0-1‰ (Fig. 3A). Silver

335 et al. (2012) reported the $\delta^{15}\text{N}_{\text{N}_2}$ values of eighteen gas samples from Beatrix (BE116, 325, 327),
336 Driefontein (Dr938), Evander (EV219, 522, 818), Kloof (KL441, 739), Masimong (MM5),
337 Merriespruit (MS151), Mponeng (MP104) in the Witwatersrand Basin in the Kaapvaal Craton.
338 These fracture systems have mean residence times varying from 0.7 Ma (EV522) to 20 Ma
339 (KL739 and MP104) with one sample (Dr938) having a ^{14}C -derived age of 1022 years
340 (Lippmann et al., 2003; Lin et al., 2006; Silver et al., 2012 and reference therein). The hydrogen
341 and oxygen isotope compositions of these fracture fluids spread from on (or close to) the global
342 meteoric water line (GMWL) to above the GMWL (Lippmann et al., 2003; Ward et al., 2004;
343 Lin et al., 2006; Onstott et al., 2006; Warr et al., 2021a). Their $\delta^{15}\text{N}_{\text{N}_2}$ values fall in the range of
344 -1.3‰ to 5.8‰ (Fig. 3B). The $\delta^{15}\text{N}_{\text{N}_2}$ values do not correlate with either the residence times or
345 the magnitude of isotopic deviation above the GMWL, indicating the major control on the $\delta^{15}\text{N}_{\text{N}_2}$
346 values of the samples in the Kaapvaal Craton may not be geological processes (e.g., fluid-rock
347 interaction) but biological processes.

348 In contrast, the Canadian Shield samples show consistently higher $\delta^{15}\text{N}_{\text{N}_2}$ values (Fig.
349 3C). A total of thirty-five samples studied here cluster in a $\delta^{15}\text{N}_{\text{N}_2}$ range of 3.0‰ to 8.0‰ with a
350 peak at the bin of $7\text{--}8\text{‰}$ (Fig. 3C), while three historical samples from Montgomery (1994) and
351 Sherwood Lollar (1993a) give more variable $\delta^{15}\text{N}_{\text{N}_2}$ values of 1.4‰ , 1.5‰ and 17.5‰ ,
352 respectively (Fig. 2A). The $\delta^{15}\text{N}_{\text{N}_2}$ discrepancy between the Canadian Shield samples and the
353 samples from the Fennoscandian Shield and the Kaapvaal Craton could be attributed to the
354 differences in nitrogen source and abiotic and biological processes among these deep subsurface
355 fracture waters, which are discussed in detail below.

356

357 **5.3. Source of N_2 in the Canadian Shield**

358 Several sources could potentially contribute to the N₂ in the terrestrial subsurface fracture
359 water systems, such as ancient atmospheric N₂, dissolved inorganic nitrogen species (nitrate,
360 nitrite, ammonium) in pore water and/or fluid inclusions in host rocks, and fixed nitrogen in host
361 rocks.

362 In the studied samples from the Canadian Shield, although noble gas studies imply that
363 these fracture fluids contain a minor initial component of noble gas (e.g., Xe) derived from
364 ancient air (Holland et al., 2013; Warr et al., 2018), a ballpark estimation based on the
365 concentration data of gases and rocks in Li et al. (2016) and typical N₂ concentration of seawater
366 (Kipfer et al., 2002) suggests that the ancient air contributed at most 0.1 – 6% of the gas
367 components. Therefore, ancient atmospheric N₂ can be excluded as the main source. In addition,
368 a recent study discovered extreme ¹⁵N¹⁵N enrichment of atmospheric N₂ (Yeung et al., 2017).
369 Assuming the ancient atmospheric N₂ had comparable ¹⁵N¹⁵N enrichment, and little re-ordering
370 of the internal bonding of N₂ molecules in these low-temperature fracture systems, a ¹⁵N¹⁵N
371 enrichment would be expected if significant amount of ancient atmospheric N₂ contributed to the
372 fluids. However, Labidi et al. (2020) found no ¹⁵N¹⁵N enrichment in the KC samples. This
373 suggests that the amounts of atmospheric N₂, from both ancient air and modern air
374 contamination, are relatively small in the total N₂ of the samples.

375 Dissolved inorganic nitrogen species in pore water and fluid inclusions in host rock have
376 been proposed to account for the abundant nitrate, N₂ and ammonium in the subsurface fracture
377 waters in the Witwatersrand Basin in the Kaapvaal Craton, South Africa (Silver et al., 2012). In
378 this model, it requires (1) a high NH₃-NH₄⁺ pool in fluid inclusions in the first place, (2) efficient
379 migration of nitrogen in fluid inclusions to fracture fluids, and (3) abiotic and biological
380 recycling processes to convert NH₃/NH₄⁺ to other nitrogen species (e.g., N₂ in this case). These

381 conditions are relatively difficult to reach in the Canadian Shield where the igneous-rock
382 dominated host rocks contain small volumes of primary fluid inclusions. Furthermore,
383 geochemical and hydrogeological analyses have shown that contribution of young surface fluids
384 is little in these fracture systems (e.g., Warr et al., 2021a), ruling out later infiltration of dissolved
385 inorganic nitrogen from surface.

386 Sedimentary rocks are a commonly considered source for N₂ in basinal environments.
387 However, in the Canadian Shield, host rocks are dominated by crystalline rocks. At KC where
388 host rock lithology has been well characterized from drill cores, graphite-bearing lenses occupy a
389 very small volume in host rocks. The nitrogen concentrations of the graphite-bearing lenses (40-
390 99 ppm; Table 2) are significantly smaller than those of typical deep-sea sediments and their
391 metamorphosed equivalents (hundreds to thousands of ppm; e.g., Bebout and Fogel, 1992;
392 Busigny et al., 2003; Sadofsky and Bebout, 2004; Li and Bebout, 2005). Therefore, the graphite-
393 bearing lenses can less likely account for the widely occurring gases in the highly confined and
394 locally controlled fracture water compartments (Warr et al., 2018). This conclusion is further
395 supported by the carbon and hydrogen isotopic signatures of n-alkanes in the gas samples. The
396 $\delta^{13}\text{C}$ and $\delta^2\text{H}$ patterns of n-alkanes are very different to that of conventional thermogenic gases
397 (Sherwood Lollar et al., 1993b, 2002, 2008; Warr et al., 2021b). Overall, both isotopic
398 fingerprints and hydrogeological frameworks do not favor organics as the main source of the
399 gases.

400 Significant amounts of nitrogen (in form of ammonium) can be assimilated into the
401 crystal structures of secondary phyllosilicate minerals (e.g., clay and serpentine) formed during
402 the alteration of igneous rocks by seawater and/or hydrothermal fluids (Busigny et al., 2005; Li

403 et al., 2007). The nitrogen measurement on six representative silicate samples from KC give a
404 nitrogen concentration range from 12 ppm to 34 ppm (average: ~ 24 ppm; Table 2) and a $\delta^{15}\text{N}$
405 range from 6.0‰ to 10.3‰ (average: 8.0‰). Given the large volume of the silicate host rocks in
406 Precambrian cratons, such high nitrogen concentrations of the igneous host rocks represent a
407 huge nitrogen reservoir, with an inventory orders in magnitude higher than that in the small
408 volume of fluids. Mobilization by devolatilization of a small proportion of this reservoir is able
409 to account for the N_2 in the subsurface fracture waters.

410 To devolatilize the ammonium fixed in the igneous host rocks, it requires relatively high
411 temperatures. In light of previous laboratory and field studies (e.g., Haendel et al., 1986; Bebout
412 and Fogel, 1992), nitrogen devolatilization from minerals and rocks could have easily taken
413 place under the peak (and retrograde) metamorphic conditions in the study sites, e.g., 400 °C at
414 KC, 700 °C at TH, and variable temperatures (depending on the distance to the crater; Abramov
415 and Kring, 2004) during the impact event at Sudbury. At crustal conditions, high temperatures
416 facilitate N_2 as the devolatilization product (e.g., Bebout and Fogel, 1992; Li et al., 2009). The
417 lower temperature limit for N_2 devolatilization from minerals and rocks is however
418 unconstrained. Even taking the smectite-illite transition temperature of 60 °C (Freed and Peacor,
419 1989) as the lower limit, it is still higher than the current temperatures of the studied fracture
420 fluid systems. In particular, the Canadian Shield has been relatively stable and maintained a low
421 thermal gradient since at least 2 Ga (Slack, 1974). Temperature estimation using the method of
422 Li et al. (2016) suggests that the temperature at KC should have been < 60 °C since at latest 1.3
423 Ga for the 2.1 kmbs site and 0.9 Ga for the 2.9 kmbs site. Therefore, the observed N_2 was mostly
424 likely produced during earlier time periods. N_2 production would have been most efficient during
425 the peak metamorphism at each site, and diminish exponentially with the decrease in temperature

426 with time. Given that the analyzed host rocks consistently show high nitrogen concentrations
427 (Table 2), it only requires a very small (almost negligible) fraction of the total nitrogen in the
428 rocks to account for the observed N₂ in the fluids, which can be easily satisfied.

429 **5.4. Possible N recycling processes in subsurface fracture fluids**

430 **5.4.1. Abiotic N₂ reduction**

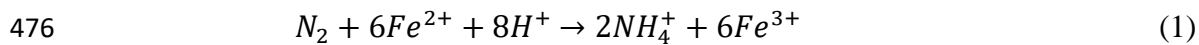
431 Based on above discussions, the N₂ in the deep subsurface fracture fluids in the Canadian
432 Shield most likely originated by devolatilization of fixed ammonium in their silicate host rocks.
433 Nitrogen devolatilization from rocks is associated with an isotopic effect with preferential uptake
434 of ¹⁴N by N₂ and preferential retention of ¹⁵N in host rock (e.g., Bebout and Fogel, 1992;
435 Haendel et al., 1986; Li et al., 2021b, c). The magnitude of ¹⁵N depletion in the N₂ product is
436 dependent on two factors, i.e., the temperature-dependent isotope fractionation factor and the
437 fraction of ammonium in rock that is converted into N₂. The nitrogen isotope fractionation
438 factors between N₂ and clay minerals can be derived from Li et al. (2021b, c) to be -13.2‰ at 60
439 °C, -10.9‰ at 100 °C, -6.2‰ at 200 °C, -4.1‰ at 400 °C, -2.3‰ at 700 °C and -1.5‰ at 1000
440 °C. Based on these fractionation factors, the theoretical δ¹⁵N differences between N₂ product and
441 remaining ammonium in host rocks along the progress of metamorphic devolatilization are
442 illustrated by a Rayleigh fractionation model in Fig. 4A-C for KC, Sudbury and Thompson,
443 respectively. Comparison of average δ¹⁵N values between N₂ and host rocks yield Δ¹⁵N_{N₂-rock}
444 values of -0.3‰ for the 2.1 kmbs site, -1.1‰ for the 2.4 kmbs site, and -3.1‰ for the 2.9 kmbs
445 site at KC, +2.5‰ at NR, +4.0‰ at FR, +0.3‰ at TH and -0.4‰ at BT. All these values are
446 higher than the expected values for N₂ devolatilization at their peak metamorphic conditions
447 (Fig. 4A-C). Any N₂ generated from retrograde metamorphism would make the isotope
448 discrepancy ever larger. At CCS, although all the samples in this study have been severely

449 contaminated by air during sampling, their values are still higher than the predicted values (Fig.
450 4B), not even mention the extremely high $\delta^{15}\text{N}$ values (up to 17.5‰) observed on less air-
451 contaminated samples (Montgomery, 1994). These results indicate, while the magnitude and
452 direction of isotope fractionation are indeed consistent with N_2 production via devolatilization of
453 ammonium in host rocks, the extents of observed isotopic discrepancy between N_2 and host
454 rocks require an additional process to explain the ^{15}N enrichments in N_2 .

455 Diffusive loss of N_2 and abiotic N_2 reduction are two known processes that can induce
456 ^{15}N enrichments in remaining N_2 . Extensive studies of the noble gas concentrations (in the KC
457 system in particular) have demonstrated that diffusion cannot be a major factor due to the
458 concordance in the ages of the fluids derived from both light (helium and neon) and heavy (argon
459 and xenon) noble gases. In systems where diffusion is a significant process, preferential loss of
460 light noble gases would produce an artifact of significantly younger apparent ages for helium and
461 neon – which is notably not observed in any of the KC studies (Holland et al., 2013; Warr et al.,
462 2018). Accordingly, diffusion process is less likely to significantly impact the $\delta^{15}\text{N}_{\text{N}_2}$. Rather
463 than diffusion, abiotic N_2 reduction may play an important role. Following the cooling of the
464 fluids, N_2 input from metamorphic devolatilization diminished to negligible level. Meanwhile,
465 the nonstop interaction between water and Fe^{2+} -bearing minerals (e.g., olivine, pyroxene, biotite,
466 pyrite) in host rocks can produce secondary minerals to seal the fracture system and subsequently
467 establish and maintain a highly reducing environment enriched in H_2 and dissolved Fe^{2+} (Li et
468 al., 2016) inside the fracture system. These reducing agents in the fluids, as well as abundant
469 Fe^{2+} -bearing minerals in host rocks, would facilitate abiotic reduction reactions. This is
470 supported by the observation that abiogenic products (e.g., alkanes, acetate, and formate)
471 dominate the gaseous and/or dissolved organic compounds in the KC fracture fluids (Sherwood

472 Lollar et al., 2021) and also widely occur (if not dominate) in other sites in the Canadian Shield
473 (Sherwood Lollar et al., 2006).

474 Abiotic N₂ reduction in a geological setting generally refers to the mineral catalyzed
475 reduction of N₂ to produced NH₃ or NH₄⁺ in fluids, via reactions such as equation (1):



477 Laboratory experiments demonstrate that abiotic N₂ reduction is most efficient at 500 °C
478 (Brandes et al., 1998), but can also occur at temperatures as low as 22 °C (Smirnov et al., 2008).

479 Abiotic N₂ reduction generally produces a kinetic nitrogen isotopic effect due to the difficulty in
480 breaking the strong N≡N bond of N₂. Based on the nitrogen isotopic measurements of altered
481 igneous rocks, Li et al. (2007, 2014) yielded nitrogen isotopic enrichment factors of –15‰ to –
482 11‰ for abiotic N₂ reduction at medium- to high-temperature hydrothermal conditions (>300
483 °C). The magnitude of kinetic nitrogen isotopic effect could be larger at lower temperatures (Li
484 et al., 2009), but no data are available yet. Using the average value of -13‰ (Li et al., 2007,
485 2014) as the lower limit and a Rayleigh fractionation model (Fig. 4D-F), a ballpark estimate can
486 be made for the fractions of N₂ required to be reduced in order to match the observed δ¹⁵N value
487 in the studied samples. The results suggest that the fractions of the initial N₂ that has been
488 abiotically reduced is 25 – 63% at 2.1 kmbs, 21 – 61% at 2.4 kmbs, and 7 – 54% at 2.9 kmbs,
489 respectively (Fig. 4D). This decreasing trend along depth is self-consistent with the temperature
490 and time framework that 2.1 kmbs has the shortest time for N₂ production but longest time and
491 lowest temperature for abiotic N₂ reduction, whereas the 2.9 kmbs has the longest time for N₂
492 production but shortest time and highest temperature for abiotic N₂ reduction. Similar extents of
493 abiotic N₂ reduction are also obtained from NR (27 – 70%), FR (35 – 74%), TH (18 – 65%), and

494 BT (14 – 63%) (Fig. 4 E, F). Future examinations on concentrations and isotope compositions of
495 ammonium in the fluids may help to test this possibility.

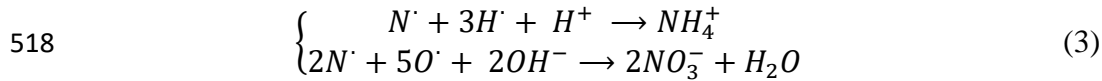
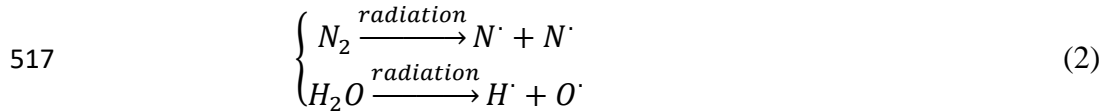
496 It is worth noting that, based on mass balance and isotopic comparison, Silver et al.
497 (2012) also proposed that abiotic N₂ reduction could have occurred in the subsurface fracture
498 fluids in the Kaapvaal Craton. This implies that abiotic N₂ reduction might occur widely in
499 Precambrian cratons.

500

501 **5.4.2. The role of radiolysis?**

502 Another process that is worth briefly discussing is radiolysis. Energy released from the
503 decay of radioactive isotopes of K, U, Th in host rocks has been shown to play an important role
504 in the long-term production of deep subsurface hydrogen (e.g., Lin et al., 2005; Sherwood Lollar
505 et al., 2014), carbon (Sherwood Lollar et al., 2021), and sulfur (Lin et al., 2006; Li et al., 2016)
506 in deep fracture fluids in the Precambrian cratons around the world. An intriguing question is
507 how much this process may contribute to the nitrogen cycle in these deep saline fracture fluids.

508 Silver et al. (2012) carried out laboratory experiments to irradiate NH₃/NH₄⁺ in anaerobic
509 solutions and observed production of nitrite and nitrate. Consequently, radiolysis has been
510 proposed to be part of the multiple abiotic and biotic mechanisms to drive the nitrogen cycle in
511 the subsurface fluids in the Kaapvaal Craton (Silver et al., 2012). Radiolytic impact on fixed
512 ammonium in minerals and rocks is poorly examined by far. It is unclear whether radiolysis in
513 the Precambrian cratonic environments facilitate N₂ production. But laboratory experiments on
514 the N₂-H₂-H₂O system (e.g., Etoh et al., 1987; Karasawa et al., 1991) have demonstrated that N₂
515 can be consumed by radiolysis to form not only NH₄⁺ as the dominant product (Karasawa et al.,
516 1991) but also NO₃⁻ (Etoh et al., 1987), via overall reactions described in equations (2)-(3).



519 This differs from the mineral catalyzed abiotic N₂ reduction process in equation (1). However,
 520 the nitrogen isotope fractionation during radiolytic decomposition of N₂ has not been
 521 constrained, although a kinetic isotopic effect likely occurs. Future studies integrating laboratory
 522 examination of nitrogen isotope fractionations during radiolysis of the N₂-H₂-H₂O system and
 523 the analyses of concentrations and isotope compositions of ammonium and nitrate (if there is
 524 any) in fracture fluids are necessary to further assess these processes in the deep subsurface of
 525 Precambrian cratons.

526

527 **5.4.3. Microbial nitrogen recycling?**

528 The samples studied here contain a set of N₂ samples from one of the long-term
 529 monitoring boreholes (BH12299 at 2.4 kmbs) at the KC Observatory collected over nearly 10
 530 years since the completion of the borehole drilling in May 2007. Interestingly, the δ¹⁵N_{N₂} values
 531 of this set of samples (Table 1) display a trend of steady decrease (for >1‰) over time (Fig. 5).
 532 Since significant addition of an air component over time can be ruled out based on the ¹⁵N¹⁵N
 533 results (Labidi et al., 2020), this trend suggests that another *in-situ* source of ¹⁵N-depleted N₂ has
 534 been progressively added into the original N₂ reservoir in this borehole over the monitoring
 535 period. Although the N₂ in the deeper fracture fluids (e.g., 2.9 kmbs) at the KC Observatory is
 536 relatively ¹⁵N depleted, it cannot be the source for the 2.4 kmbs fracture fluids because current
 537 evidence supports hydrogeologic isolation between these fracture systems (Warr et al., 2018,
 538 2021b). One potential *in-situ* source for a ¹⁵N-depleted N₂ source in the 2.4 kmbs fracture system

539 is dissolved ammonium. The temperature and pH conditions of the KC fluids favor the
540 preservation of ammonium as the net product from abiotic N₂ reduction (Li et al., 2012). This
541 may therefore provide an ideal ¹⁵N-depleted source for this secondary N₂.

542 The abiotic conversion of NH₄⁺/NH₃ to N₂ requires either oxic condition and/or relatively
543 high temperature (>500 °C) without catalyst (Li et al., 2009). Even with efficient catalyst (e.g.,
544 Fe), the conversion rate is very slow at temperatures < 300 °C (Li et al., 2021d). In addition, no
545 evidence to show an oxic environment in the studied fracture systems. Thus, abiotic conversion
546 of NH₄⁺/NH₃ at the low-temperature environment (24 °C at 2.4 kmbs) may not contribute
547 detectable N₂ to BH12299 fluid at a decadal time scale. Microbially-driven anaerobic oxidation
548 of ammonium (e.g., Van De Graaf et al., 1995; Byrne et al., 2008) is a more efficient mechanism
549 to convert ammonium to N₂. Microbial recycling of nitrogen has been suggested to occur in
550 subsurface fracture fluids in the Kaapvaal Craton (Silver et al., 2012; Lau et al 2016) and
551 elsewhere in the Canadian Shield (Sheik et al., 2021), but cannot be explored further for the KC
552 samples due to the lack of biogeochemical data and isotopic compositions of other nitrogen
553 species (ammonium, nitrite or nitrate, if there is any) in these fracture fluids. It is worth
554 highlighting though that recent studies using cell counts and culture-based methods have
555 identified low abundance of H₂-utilizing, alkane-oxidizing sulfate-reducing bacteria in the KC
556 fracture fluids (Lollar et al., 2019; Wilpiseski et al., 2020). This directly supports the prevailing
557 hypothesis that microbial activities could have been sustained over geological timescale in the
558 long isolated KC fracture fluids based on sulfur isotopic studies (Li et al., 2016). Microbial
559 activities related to methanogenesis and methanotrophy in the 2.4 kmbs KC fracture fluids have
560 been further inferred from the isotopic signatures of CH₄ (Sherwood Lollar et al., 2002; Young et
561 al., 2017, Warr et al., 2021b). In particular, a potential ongoing addition of biogenic CH₄ to the

562 same fracture fluid system (BH12299) has been suggested based on a temporal decrease in the
563 mass-18 isotopologues of CH₄ over the last decade (Warr et al., 2021b). A speculation is that the
564 KC fracture fluids could have been or is being influenced by microbial activities involving
565 multiple metabolic pathways including H₂ oxidation, sulfate reduction, methanogenesis,
566 anaerobic oxidation of methane, and anaerobic oxidation of ammonium. This hypothesis will
567 need to be tested by future microbial studies with detailed constraints on metabolic pathways and
568 their geochemical fingerprints.

569

570 **6. Conclusions**

571 N₂ in the highly reducing saline fracture waters of the Canadian Shield provides a novel
572 opportunity to look into the (bio)geochemical nitrogen recycling processes in deep subsurface
573 systems that have been long investigated in the context of the deep carbon cycle and subsurface
574 life, but to date with very little characterization and quantification of the deep nitrogen cycle.
575 Here we observed that the N₂ in subsurface fracture waters with residence times from hundreds
576 of million years to billions of years in the Canadian Shield were more enriched in ¹⁵N than the N₂
577 samples from fracture waters with residence times of hundreds of thousands to tens of millions
578 of years in the Fennoscandian Shield and the Kaapvaal Craton. Detailed isotopic comparison
579 between the Canadian Shield N₂ samples and their potential sources excludes air, dissolved
580 inorganic nitrogen, and organic compounds in host rocks as their sources. Instead, fixed
581 ammonium in igneous host rocks is the most likely source. Modeling of nitrogen devolatilization
582 of igneous rocks supports this interpretation, but also suggests that a second process is required
583 to account for the observed ¹⁵N enrichments in the N₂ samples from the Canadian Shield. Long-
584 term abiotic N₂ reduction in the closed fracture water systems is the most likely process. The

585 data in this study imply a common occurrence of abiotic N₂ reduction in the long isolated, highly
586 reducing fracture water systems in the Canadian Shield, and possibly in similar environments on
587 the early Earth and other planets. The accumulation of the ammonium product can not only
588 provide a critical reagent for abiotic synthesis of amino acid (an essential building block for life)
589 toward the origin of life but also supply a necessary nutrient to support potential ammonium
590 assimilating or anaerobic ammonium oxidizing microbes in these environments.

591

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600

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Figure captions

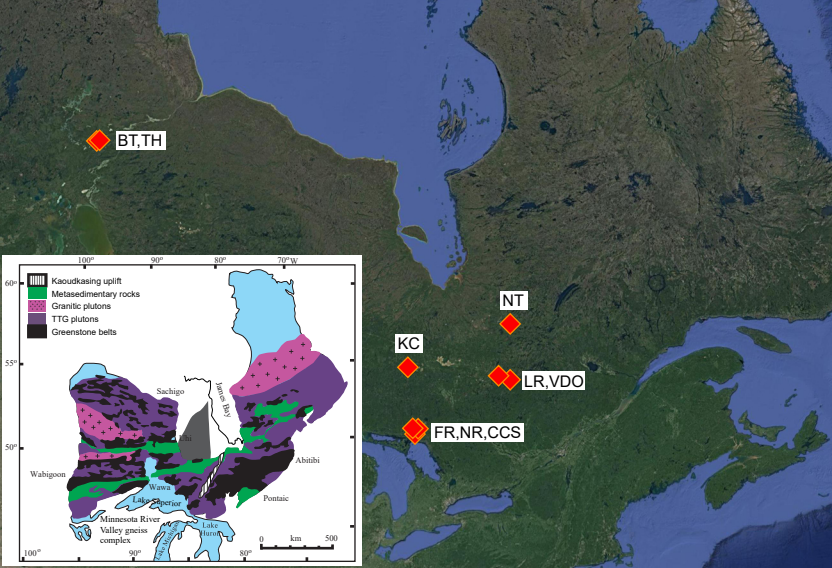
Fig. 1. Location map of sample sites for this study together with the sites with $\delta^{15}\text{N}_{\text{N}_2}$ values reported in Sherwood Lollar et al. (1993a). The general geology of the Canadian Shield is shown in the inserted panel (revised from Hoffmann, 1989; Li et al., 2016; Warr et al., 2019). BT = Birchtree; TH = Thompson; KC = Kidd Creek; FR = Fraser; NR = Nickel Rim; CCS = Copper Cliff South; LR = LaRonde; VDO = Val D'Or; NT = Norita

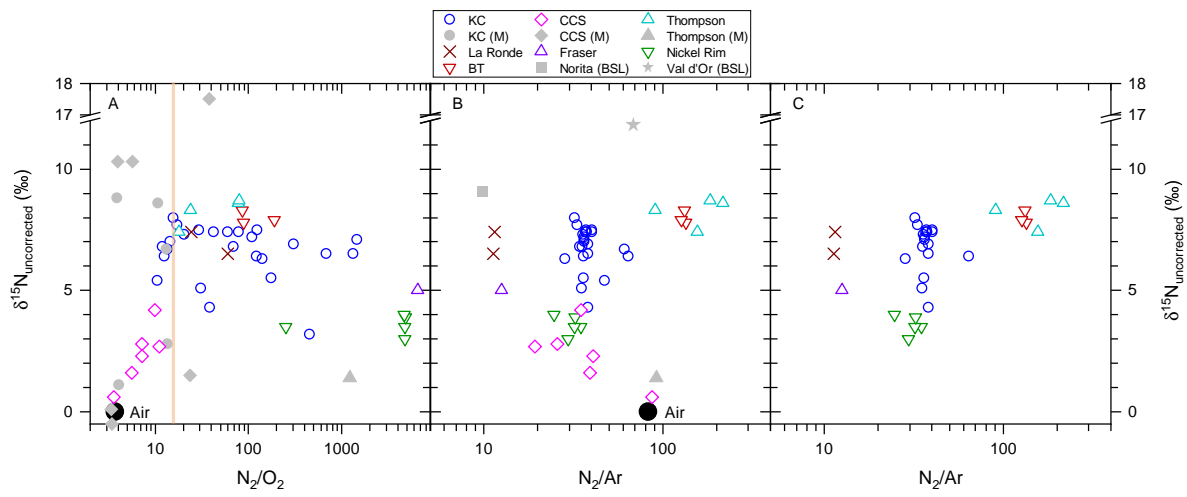
Fig. 2. Comparison of $\delta^{15}\text{N}$ values of N_2 with N_2/O_2 ratio (A) and N_2/Ar ratio (B, C) for subsurface fracture waters from the Canadian Shield. Error bars are smaller than data symbols. These highly reducing fracture waters produce H_2 and do not contain O_2 , thus the N_2/O_2 ratio can be used as a robust index for air contamination. The vertical line ($\text{N}_2/\text{O}_2 = 15$) in panel A divides significantly contaminated samples to the left and less contaminated samples to the right. In contrast, the fracture waters contain various amounts of ^{40}Ar (and N_2/Ar ratio) from the decay of ^{40}K in host rocks. Thus N_2/Ar ratios cannot be used to quantify the air component. All samples with known N_2/Ar ratios $\delta^{15}\text{N}$ values are compiled in Panel B. Whereas only samples with known N_2/O_2 values of >15 are plotted in panel C. Historical samples marked by “(M)” are unpublished thesis data from Montgomery (1994); samples marked by “(BSL)” are from Sherwood Lollar et al. (1993a). Both are shown as filled grey symbols.

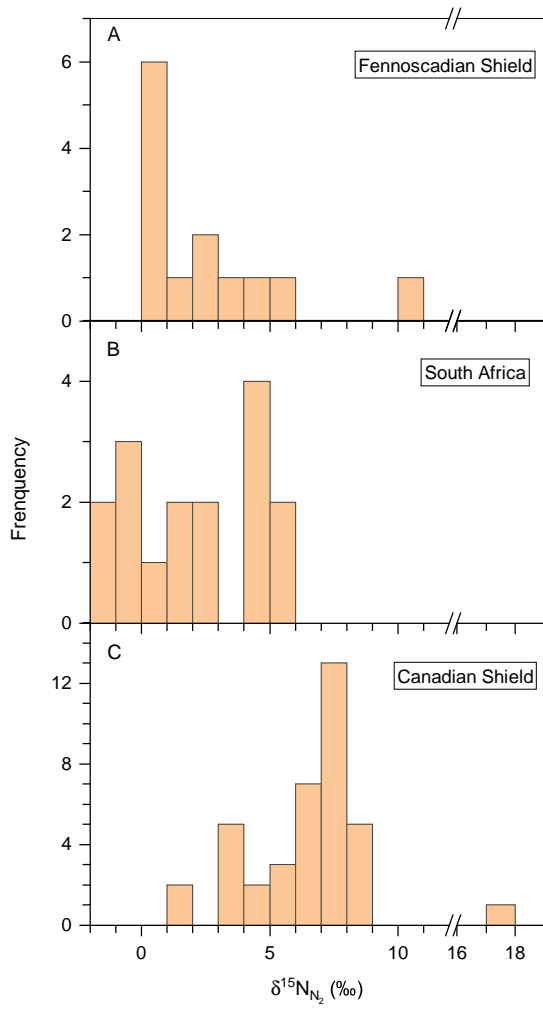
Fig. 3. Comparison of $\delta^{15}\text{N}$ values of N_2 in subsurface fracture waters from the Kaapvaal Craton (A), the Fennoscandian Shield (B), and the Canadian Shield (C). The Canadian Shield samples are consistently more ^{15}N enriched than those from the other two cratons. See text for discussion.

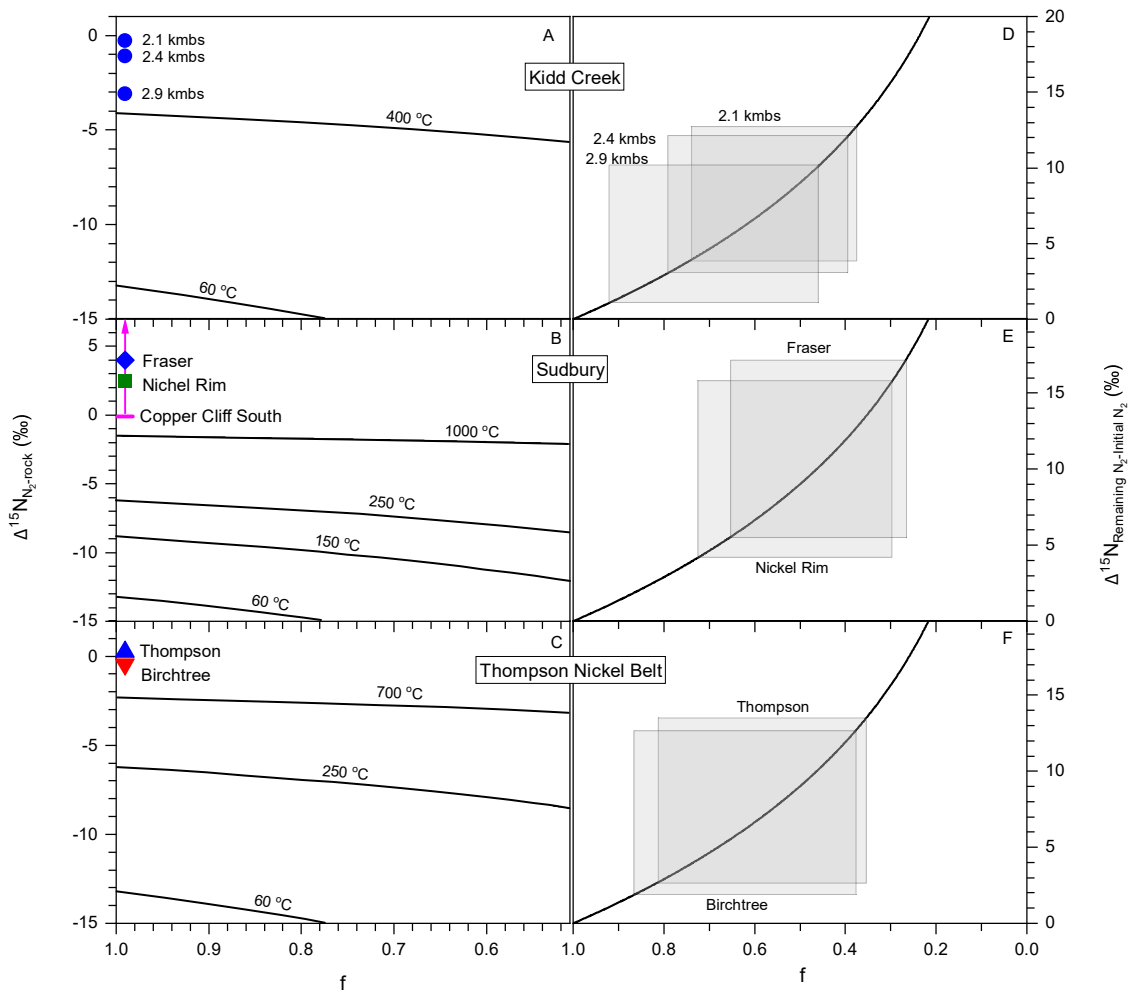
Fig. 4. Comparison of $\Delta^{15}\text{N}_{\text{N}_2\text{-rock}}$ between observed values and theoretical values of metamorphic devolatilization calculated by Rayleigh fractionation modeling (A-C), and increase in $\delta^{15}\text{N}$ of N_2 (expressed as $\Delta^{15}\text{N}_{\text{Remaining N}_2\text{-initial N}_2}$) after abiotic nitrogen reduction (D-F). The f in both panels denote the fraction of remaining nitrogen in the reactant. See text for discussion.

Fig. 5. Diagram showing steady decrease in the $\delta^{15}\text{N}$ value of N_2 from one single borehole at 2.4 km below surface of the Kidd Creek mine over a 10-year period.









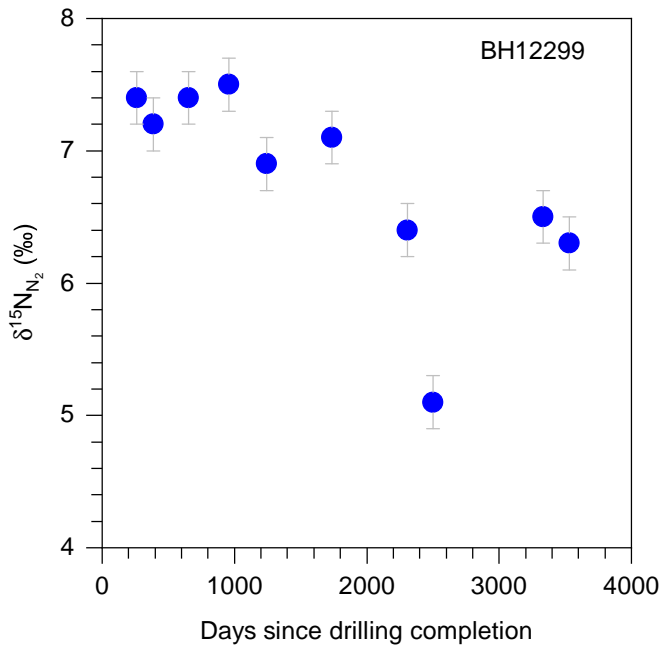


Table1. Major components^s and $\delta^{15}\text{N}_{\text{N}_2}$ value of gas samples from deep subsurface fracture fluids in the Canadian Shield.

Location	Sample ID	H ₂ (vol%)	He (vol%)	Ar (vol%)	O ₂ (vol%)	CH ₄ (vol%)	N ₂ (vol%)	N ₂ /O ₂	N ₂ /Ar	$\delta^{15}\text{N}_{\text{N}_2}$ (‰)
Kidd Creek	10.1.2000-KCL6900-1F	0.33	n.d.	0.23	0.66	75.05	7.93	12.0	34.5	6.8
	10-1-2000-KCL6900-2D	1.95	n.d.	0.29	0.60	73.60	9.32	15.5	32.1	8.0
	10-1-2000-KCL6900-3A	2.47	n.d.	0.34	0.66	72.48	11.31	17.1	33.3	7.7
	10-1-2000-KCL6900-4D	1.32	n.d.	0.35	0.88	70.48	12.69	14.4	36.3	7.0
	10-1-2000-KCL6900-5D	2.03	n.d.	0.39	0.69	70.70	14.01	20.3	35.9	7.3
	10-1-2000-KCL6900-6B	1.40	n.d.	0.33	0.95	75.08	11.90	12.5	36.1	6.4
	12.2.2008-KC7850-12261	3.16	2.45	0.39	0.20	72.15	13.84	69.2	35.5	6.8
	10.5.2007-KC7850-12262	8.37	2.91	0.35	0.48	69.10	14.03	29.2	40.1	7.5
	22.10.2015-KC7850-12262	2.7	2.00	0.43	1.96	56.70	26.40	13.5	61.4	6.7
	27.8.2007-KC7850-12287A	10.67	2.47	0.34	1.54	72.17	16.10	10.5	47.4	5.4
	20.6.2008-KC7850-12287A	3.61	2.45	0.36	0.24	70.10	14.45	60.2	40.1	7.4
	12.2.2008-KC7850-12299	6.52	2.15	0.45	0.40	71.98	16.87	42.2	37.5	7.4
	19.6.2008-KC7850-12299	3.32	2.42	0.42	0.14	67.70	15.35	109.6	36.5	7.2
	31.3.2009-KC7850-12299	4.82	2.87	0.40	0.19	64.37	14.81	77.9	37.0	7.4
	12.01.2010-KC7850-12299	4.62	2.54	0.40	0.12	71.49	14.94	124.5	37.4	7.5
	21.10.2010-KC7850-12299	3.97	2.62	n.d.	<0.05	70.32	15.32	> 306.4		6.9
	29.2.2012-KC7850-12299	3.19	2.39	n.d.	<0.01	71.92	14.60	> 1460.0		7.1
	20.09.2013-KC7850-12299	5.58	2.69	0.21	0.11	70.98	13.50	122.7	64.3	6.4
	02.04.2014-KC7850-12299	2.52	1.60	0.28	0.32	74.6	9.88	30.9	35.3	5.1
	12.07.2016-KC7850-12299*	3.60	2.41	0.35	<0.01	71.3	13.36	> 1336.0	38.2	6.5
	25.01.2017-KC7850-12299*	4.80	3.70	0.54	0.11	71.6	15.40	140.0	28.5	6.3
	08.2.2012-KC9500-13675	<0.01	1.63	n.d.	<0.01	78.70	4.53	> 453.0		3.2
	1.3.2012-KC9500-13684	5.30	2.10	n.d.	<0.01	75.83	6.80	> 680.0		6.5
	29.11.2012-KC9500-BH2	16.6	0.43	0.29	0.06	71.0	10.50	175.0	36.2	5.5
	13.07.2016-KC9500-bubA	0.70	1.77	0.27	0.27	71.6	10.34	38.3	38.3	4.3
	LaRonde	24.03.2016-LR6480-L2900-LR-290-	1.83	5.09	1.33	0.63	66.11	15.25	24.2	11.5
24.03.2016-LR6480-L2900-LR-290-		3.63	7.20	0.79	0.15	72.10	8.92	59.5	11.3	6.5

Location	Sample ID	H ₂ (vol%)	He (vol%)	Ar (vol%)	O ₂ (vol%)	CH ₄ (vol%)	N ₂ (vol%)	N ₂ /O ₂	N ₂ /Ar	δ ¹⁵ N _{N₂} (‰)
Copper Cliff South	CCSM-GH-11-7-2001-1E	54.00	3.46	0.07	0.40	33.15	2.87	7.2	41.0	2.3
	CCSM-GH-11-7-2001-2E	43.00	3.38	0.23	5.54	14.68	20.02	3.6	87.0	0.6
	CCSM-GH-11-7-2001-3D	9.94	4.37	0.16	0.57	70.19	5.61	9.8	35.1	4.2
	CCSM-GH-11-7-2001-5E	57.59	2.62	0.07	0.49	23.96	2.74	5.6	39.1	1.6
	CCSM-GH-11-7-2001-6E	51.00	6.42	0.12	0.43	31.79	3.10	7.2	25.8	2.8
	CCSM-GH-11-7-2001-7E	19.7	2.52	0.28	0.49	57.97	5.41	11.0	19.3	2.7
Nickel Rim	29.11.2013-NR1730m_NR170128	0.01	32.23	1.51	0.21	15.40	52.65	250.7	34.9	3.5
	05.03.2014-NR1730m_NR170128	0.03	29.90	1.50	<0.01	19.38	48.09	> 4809.0	32.1	3.5
	05.03.2014-NR1730m_NR170182	1.64	27.73	1.51	<0.01	n.a.	48.87	> 4887.0	32.4	3.9
	05.03.2014-NR1730m_NR170183	2.67	26.19	1.91	<0.01	24.29	47.35	> 4735.0	24.8	4.0
	22.10.2014-NR1730m_NR170128	0.01	24.12	1.61	<0.01	24.34	47.73	> 4773.0	29.6	3.0
Fraser	06.03.2014_FML4700_FR47774	<0.01	16.68	5.24	<0.01	19.82	65.76	> 6576.0	12.5	5.0
Birchtree	28.5.2007-BT3950L-gas stopper BH	0.02	1.35	0.22	0.34	62.9	29.26	86.1	133.0	8.3
	6-11-2007-BT3900-9167N-BH (Floor#2)	<0.005	1.38	0.23	0.35	68.7	30.92	88.3	134.4	7.8
	27-3-2008-BT3900-9167N-BT1	0.08	1.33	0.24	0.16	62.9	30.54	190.9	127.3	7.9
Thompson	1065750 13.06.06 (3-403-2)	2.75	2.40	0.51	1.92	34.7	46.26	24.1	90.7	8.3
	1065760 14.06.06 (1-401-2)	0.79	2.70	0.42	3.63	36.5	65.51	18.0	156.0	7.4
	1163630 13.06.06 (7-399-5)	0.30	2.99	0.28	0.77	36.6	60.77	78.9	217.0	8.6
	1065800 13.06.06 (4-405-3)	0.03	2.80	0.28	0.65	45.5	51.78	79.7	184.9	8.7

[§] Major Gas contents of these samples have been reported by Sherwood Lollar et al., 2006; Li et al. (2016); Telling et al. (2018); Warr et al. (2019). The contents of N₂, Ar, and O₂ reported here, if slightly different to those in the literature, were re-analyzed results on the same gas bottle used for δ¹⁵N_{N₂} analysis.

* Labidi et al. (2020) reported δ¹⁵N values of 6.6‰ for sample 12.07.2016-KC7850-12299 and 6.7‰ for sample 25.01.2017-KC7850-12299.

Table 2. $\delta^{15}\text{N}$ values of rock samples from Kidd Creek, Sudbury, and Thompson

Location	Sample ID	Lithology	Bulk-rock N (ppm)	Bulk-rock $\delta^{15}\text{N}$ (‰)	Reduced carbon component $\delta^{15}\text{N}$ (‰)	
Kidd Creek	BH12612A (465.30-465.62 m)	Graphite-bearing lens	68.9	7.0	7.0	
	BH12612A (470.50-470.96 m)	Graphite-bearing lens	67.6	6.7	6.7	
	BH12612A (392.80-393.15 m)	Graphite-bearing lens	98.6	9.3		
	BH12612A (413.38-413.75 m)	Graphite-bearing lens	40.5	8.7	1.3	
	BH12299 (369.5 m)	Graphite-bearing lens	54.9	15.6	10.4	
	BH12299 (472.4 m)	Graphite-bearing lens	89.8	10.2	0.4	
	Average			70.1	9.6	5.2
	BH12612A (232.10-232.26 m)	Rhyolite	30.9	7.8		
	BH12612A (243.30-243.07 m)	Rhyolite	22.7	7.3		
	BH12612A (248.80-249.35 m)	Rhyolite	34.3	6.0		
	BH12612A (292.70-293.15 m)	Silicified cherty breccia	23.0	10.3		
	BH12612A (353.20-353.38 m)	Silicified cherty breccia	11.9	10.4		
	BH12612A (475.85-476.02 m)	Quartz feldspar porphyry	19.3	6.3		
	Average			23.7	8.0	
Sudbury	Creighton Mine BH131106 (395.50-396.60 m)	Meta-gabbro	14.1	2.6		
	Creighton Mine BH131106 (395.50-396.60 m)	Breccia matrix	14.9	2.3		
	Average			14.5	2.4	
	Nickel Rim BH130075 (319.96-320.14m)	Felsic gneiss	12.7	1.3		
	Nickel Rim BH170143 (75.87-76.07m)	Felsic norite	13.6	3.4		
	Nickel Rim BH170143 (109.98-110.16 m)	Felsic norite	49.3	-5.6		
	Nickel Rim BH170143 (178.00-178.20 m)	Granodiorite	9.7	2.2		
	Nickel Rim BH170143 (283.91-284.04 m)	Felsic gneiss	12.3	3.1		
	Nickel Rim BH170143 (349.90-364.11)	Breccia	10.0	1.8		
Average			17.9	1.0		
Thompson	Thompson mine assorted core	Archean gneiss	30.2	7.0		
	Thompson mine assorted core	Thompson Formation skarn	50.9	8.1		
	Thompson mine assorted core	schist	50.2	10.2		
	Average			43.8	8.4	