Correction

EARTH, ATMOSPHERIC, AND PLANETARY SCIENCES

The authors note that the MSL Science Team group author list published incorrectly. The group author information in the SI Appendix, including the list of authors in the MSL Science Team as well as their affiliations, has been corrected.

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Evidence for indigenous nitrogen in sedimentary and aeolian deposits from the Curiosity rover investigations at Gale crater, Mars

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The Sample Analysis at Mars (SAM) investigation on the Mars Science Laboratory (MSL) Curiosity rover has detected oxidized nitrogen-bearing compounds during pyrolysis of scoured aeolian sediments and drilled sedimentary deposits within Gale crater. Total N concentrations ranged from 20 to 250 nmol N per sample. After subtraction of known N sources in SAM, our results support the equivalent of 110–300 ppm of nitrate in the Rocknest (RN) aeolian samples, and 70–260 and 330–1,100 ppm nitrate in John Klein (JK) and Cumberland (CB) mudstone deposits, respectively. Discovery of indigenous martian nitrogen in Mars surface materials has important implications for habitability and, specifically, for the potential evolution of a nitrogen cycle at some point in martian history. The detection of nitrate in both wind-drifted fines (RN) and in mudstone (JK, CB) is likely a result of N2 fixation to nitrite generated by thermal shock from impact or volcanic plume lightning on ancient Mars. Fixed nitrogen could have facilitated the development of a primitive nitrogen cycle on the surface of ancient Mars, potentially providing a biochemically accessible source of nitrogen.

Mars | nitrogen | astrobiology | nitrates | Curiosity

Terrestrial life requires a fixed form of nitrogen for incorporation into biomolecules such as nucleobases and amino acids that serve as building blocks for DNA, RNA, and proteins. Nitrogen in the form of N2 comprises ~2% of the martian atmosphere (1–3), but little is known about other possible N reservoirs on Mars, including those that may contain fixed forms of N (i.e., NH3, NH4+, or NO3−) in the mantle, crust, and sediments. Although Mars currently has ~0.15 mbar N2 in its atmosphere (1), estimates of the primordial N inventory of the martian atmosphere range from 5 to 300 mbar N2 (ref. 4, and references therein). Loss of N to impact erosion and stripping by solar wind have greatly reduced the N content of the martian atmosphere over time, the latter process resulting in a 15N/14N ratio of N2 on Mars of 173 ± 11 measured by Mars Science Laboratory (MSL) (5), in agreement with Viking lander measurements (2, 3), and reflecting an enrichment of the heavier isotope of 15N due to preferential escape of 14N (6). This enrichment in the heavier stable isotope is consistent with the behavior of other volatiles in Mars’ atmosphere (1) and consistent with the estimated 50–90% loss of volatile species to space (7).

In addition to N2 escape, the inventory of N on Mars must include potential N sequestration on the martian surface. Any N cycling on Mars requires breaking the N2 triple bond through “fixation” via oxidation or reduction of atmospheric N2. Nitrogen fixation on Earth is predominantly biological and occurs by conversion of N2 to ammonia via enzyme-catalyzed reactions, although abiotic N2 fixation also occurs at much lower levels, mostly by lightning and volcanism (e.g., ref. 8), and photolysis of marine organic N to NO3−, which has been hypothesized as a source of Atacama Desert NO3− (9). Several abiotic mechanisms have been proposed for N2 fixation on Mars (10, 11) and subsequent incorporation of N into the sedimentary record (12, 13). The presence of N species in Mars surface sediments and shallow subsurface is predicted (e.g., ref. 14) and is additionally supported by the presence of

Significance

We present data supporting the presence of an indigenous source of fixed nitrogen on the surface of Mars in the form of nitrate. This fixed nitrogen may indicate the first stage in development of a primitive nitrogen cycle on the surface of ancient Mars and would have provided a biochemically accessible source of nitrogen.


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2A complete list of the MSL Science Team is available in Supporting Information.

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nitrates in martian meteorites (15, 16), as well as other possible N phases (17, 18).

Assigning a martian source to any nitrogen measured by Sample Analysis at Mars (SAM) in samples from Gale crater (19, 20) requires rigorous analysis of N contributions from all possible sources, including terrestrial contamination from the SAM instrument itself, which we have done for this work. Here, we will (i) describe and quantify N species in scooped samples from an aeolian deposit and sedimentary materials from two drill sites in the Sheepbed mudstone as detected by SAM evolved gas analysis (EGA) and GCMS, (ii) evaluate and quantify the contributions of terrestrial N, and (iii) discuss possible N sources and implications of detection of martian N in Gale crater solid samples.

Methods

During SAM’s nominal solid-sample analysis mode, EGA is performed, in which the quadrupole mass spectrometer (QMS) directly analyzes the gases released from samples heated from Mars ambient to ~870 °C at 35 °C/min under a ~0.8-sccm He flow and a pressure of ~25 mbar in the pyrolysis oven (21). Before analysis, a sieved (<150 μm) and portioned (<76 mm³) sample is delivered to the Sample Manipulation System and into one of SAM’s quartz-glass cups, which has been preconditioned before the experiment by heating to >800 °C under a flow of He and active pumping by SAM’s wide-range pumps. Gases evolved during pyrolysis are continuously monitored using the QMS, and a portion of the gases are sent to the hydrocarbon trap (Tenax) and then to the GCMS. The SIMS instrument has been described in detail elsewhere (21, 22), and individual experimental parameters are presented in SI Text.

Data are presented for the three solid sample sites analyzed by SAM—Rocknest (RN), John Klein (JK), and Cumberland (CB). RN is an aeolian deposit consisting of fine-grained material with a bulk composition similar to martian fines previously characterized at other locations on Mars, and as such, the RN deposit is thought to be representative of both local soil and global dust on Mars (23, 24). JK and CB are both drilled samples from mudstone taken ~1.5 m from one another, and part of the Sheepbed member of the Yellowknife Bay formation in Gale crater. These deposits are inferred to be ancient fluviolacustrine deposits (25), and their mineralogy (26) suggests an ancient habitable environment at this location on Mars, as well as the possibility of indigenous organic carbon preserved in this mudstone (20). Although RN contains some component of globally distributed martian dust, the JK and CB locations, drilled ~3 m apart, represent a restricted time interval on a local scale, with a K-Ar age of 4.21 ± 0.35 billion years measured for these mudstones (27). Based on ground tests and models, the mass of the portion delivered to SAM was estimated to be 50 ± 8 mg (28) for the RN analyses (28), 45 ± 18 mg for the single portions at JK and CB, and 135 ± 31 mg for the triple portions at JK and CB (20). Multiple portions of the same sample from each site were analyzed.

Results

SAM Experiments on Mars. EGA (Fig. 1) and GCMS (Fig. 2) detected N-containing compounds including nitric oxide (NO) (base peak m/z 30), hydrogen cyanide (HCN) (base peak m/z 27), and trifluoro-N-methyl-acetamide (TFMA) (diagnostic peak m/z 127). Abundances are shown in Table 1, and mass spectra for each compound are shown in SI Text. Fig. 2 shows two reconstructed GC chromatograms from the CB-5 sample with m/z corresponding to the base peaks for each species (solid lines) compared with CB-6 residue, which can be considered a blank for CB-5 (dotted lines). TFMA is a compound produced when N-methyl-N-tert-butyldimethylsilyl trifluoroacetamide (MTBSTFA) reacts with molecules containing labile hydrogen atoms, such as water. MBSTFA, carried for one of SAM’s wet chemistry experiments, is a terrestrial N-containing compound in SAM that contributes to the background (29, 30). Cyanogen chloride (ClCN) (base peak m/z 61) and trifluoroacetoniitrite (TFA) (base peak m/z 69) were also identified at low abundance (~2–3 nmol) in GC chromatograms. The m/z 27 peak was identified as hydrogen cyanide (HCN) by matching m/z ratios of 26/27 from the National Institute of Standards and Technology database; however, definitive identification is complicated by mass interferences that can come from hydrocarbon fragments, and the HCN is poorly retained on the GC column. N₂ (m/z 28), N₂O (m/z 44), and NO₂ (m/z 46) could not be identified or quantified due to mass spectral interferences with CO, CO₂, and ClCN (respectively) from other species also evolving during EGA.

The reduced N species TFMA, ClCN, TFA, and HCN were detected at all sites (Fig. 1). All reduced species evolve at low temperature and peak before 300 °C. Generally, HCN is twice more abundant at JK and CB than at RN. HCN is present in all experiments above blank levels except for in CB-1. ClCN and TFA coevolve with HCN in all experiments in small amounts (2–3 nmol) but are more abundant at JK and CB than RN, where both are only present at subnanomole levels (Table 1).

The most abundant and variable species across the three samples is NO (Table 1 and Fig. 1), which generally evolves coincident with the onset of the oxygen release associated with thermal decomposition of chlorinated oxidants (20, 29). The average abundance of NO in RN1-4 is 212 ± 46 nmol and the release can be deconvolved into at least three NO evolutions: (i) a broad peak starting at 150 °C, centered at ~275 °C; (ii) a similarly broad release between 300 and 400 °C overlapping with the first release; and (iii) a sharper peak centered at ~475 °C. For JK, NO evolves in three major peaks centered at ~250, 300, and 400 °C, with total NO abundance similar to RN (JK single portion average of 202 ± 43 nmol). Unlike RN, there was no large evolution of NO >400 °C at JK or CB. CB shows the highest NO abundances of 462 ± 62 nmol
for the average of CB single portions. The NO evolution from CB starts <200 °C, just before the onset of oxygen, followed by large peak at (275 °C) with shoulders at 350 and 425 °C (Fig. 1.)

Blanks and Instrument Background. The terrestrial derivatization reagent MTBSTFA contributed to the SAM background in both the blanks and in samples (29, 30). Masses associated with all of the N compounds detected by SAM EGA are also detected in laboratory experiments performing EGA on fused silica doped with MTBSTFA and 1 wt % perchlorate (SI Text). MTBSTFA has one N atom and is therefore a potential source of N that could react with other species from the sample. Therefore, terrestrial N sources must be quantified to verify that the N detected is martian. The method to quantify MTBSTFA-derived nitrogen is modified from the method to quantify MTBSTFA-derived carbon from Ming et al. (20) and is detailed in SI Text. Estimates for MTBSTFA-derived N are compared with total detected N and used to adjust NO abundances (Table 1).

Discussion
Constraining MTBSTFA Sources of N. Total N detected in SAM EGA is more abundant than N estimates from MTBSTFA contributions

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Table 1. Abundances of N compounds at RN, JK, and CB

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total detected N, nmol</th>
<th>HCN, nmol</th>
<th>NO, nmol</th>
<th>CICN, nmol</th>
<th>TFA, nmol</th>
<th>TFMA, nmol</th>
<th>MTBSTFA-derived N, nmol*</th>
<th>Residual N, nmol†</th>
<th>Maximum % NO from MTBSTFA, %</th>
<th>Corrected NO, nmol‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>RN-Blank</td>
<td>15 ± 3</td>
<td>2.5 ± 0.5</td>
<td>12 ± 2</td>
<td>ND</td>
<td>ND</td>
<td>&lt;1</td>
<td>49 ± 10</td>
<td>46 ± 9</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>RN-1</td>
<td>215 ± 43</td>
<td>28 ± 6</td>
<td>186 ± 37</td>
<td>ND</td>
<td>ND</td>
<td>&lt;1</td>
<td>54 ± 11</td>
<td>50 ± 10</td>
<td>27 ± 8</td>
<td>135 ± 27</td>
</tr>
<tr>
<td>RN-2</td>
<td>321 ± 64</td>
<td>40 ± 8</td>
<td>280 ± 56</td>
<td>ND</td>
<td>ND</td>
<td>1.3 ± 0.3</td>
<td>79 ± 16</td>
<td>76 ± 15</td>
<td>27 ± 8</td>
<td>204 ± 41</td>
</tr>
<tr>
<td>RN-3</td>
<td>231 ± 46</td>
<td>26 ± 5</td>
<td>201 ± 41</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.9 ± 0.4</td>
<td>70 ± 14</td>
<td>67 ± 13</td>
<td>33 ± 9</td>
<td>136 ± 27</td>
</tr>
<tr>
<td>RN-4</td>
<td>211 ± 42</td>
<td>25 ± 5</td>
<td>180 ± 36</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>5.0 ± 1.0</td>
<td>79 ± 16</td>
<td>75 ± 15</td>
<td>42 ± 12</td>
<td>104 ± 21</td>
</tr>
<tr>
<td>JK-Blank</td>
<td>30 ± 5</td>
<td>1.1 ± 0.2</td>
<td>26 ± 5</td>
<td>ND</td>
<td>2.1 ± 0.4</td>
<td>1.5 ± 0.3</td>
<td>86 ± 17</td>
<td>84 ± 17</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>JK-1</td>
<td>291 ± 58</td>
<td>53 ± 11</td>
<td>230 ± 46</td>
<td>2.1 ± 0.4</td>
<td>1.5 ± 0.3</td>
<td>4.1 ± 0.8</td>
<td>127 ± 25</td>
<td>124 ± 25</td>
<td>54 ± 15</td>
<td>106 ± 21</td>
</tr>
<tr>
<td>JK-2</td>
<td>277 ± 55</td>
<td>46 ± 9</td>
<td>223 ± 45</td>
<td>2.9 ± 0.6</td>
<td>1.5 ± 0.3</td>
<td>3.9 ± 0.8</td>
<td>118 ± 24</td>
<td>115 ± 23</td>
<td>52 ± 15</td>
<td>108 ± 22</td>
</tr>
<tr>
<td>JK-3 (3× portion)</td>
<td>680 ± 136</td>
<td>83 ± 17</td>
<td>579 ± 116</td>
<td>2.0 ± 0.4</td>
<td>4.2 ± 0.8</td>
<td>11 ± 2</td>
<td>223 ± 45</td>
<td>221 ± 44</td>
<td>38 ± 11</td>
<td>359 ± 72</td>
</tr>
<tr>
<td>JK-4</td>
<td>213 ± 43</td>
<td>54 ± 11</td>
<td>152 ± 31</td>
<td>1.7 ± 0.3</td>
<td>1.8 ± 0.4</td>
<td>3.5 ± 0.7</td>
<td>80 ± 16</td>
<td>78 ± 16</td>
<td>51 ± 14</td>
<td>75 ± 15</td>
</tr>
<tr>
<td>CB-Blank1</td>
<td>90 ± 18</td>
<td>24 ± 5</td>
<td>62 ± 13</td>
<td>&lt;1</td>
<td>ND</td>
<td>3.1 ± 0.6</td>
<td>116 ± 23</td>
<td>88 ± 18</td>
<td>100</td>
<td>NA</td>
</tr>
<tr>
<td>CB-1</td>
<td>272 ± 54</td>
<td>15 ± 3</td>
<td>246 ± 49</td>
<td>1.4 ± 0.3</td>
<td>2.7 ± 0.5</td>
<td>6.9 ± 1.4</td>
<td>99 ± 20</td>
<td>71 ± 14</td>
<td>29 ± 8</td>
<td>175 ± 35</td>
</tr>
<tr>
<td>CB-2</td>
<td>447 ± 89</td>
<td>47 ± 10</td>
<td>391 ± 78</td>
<td>1.6 ± 0.3</td>
<td>3.5 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>85 ± 17</td>
<td>57 ± 11</td>
<td>15 ± 4</td>
<td>334 ± 67</td>
</tr>
<tr>
<td>CB-3</td>
<td>551 ± 110</td>
<td>48 ± 10</td>
<td>494 ± 99</td>
<td>2.3 ± 0.5</td>
<td>2.6 ± 0.5</td>
<td>4.5 ± 0.9</td>
<td>68 ± 14</td>
<td>40 ± 8</td>
<td>8 ± 2</td>
<td>454 ± 91</td>
</tr>
<tr>
<td>CB-5</td>
<td>560 ± 112</td>
<td>53 ± 11</td>
<td>502 ± 100</td>
<td>2.7 ± 0.5</td>
<td>&lt;1</td>
<td>2.0 ± 0.4</td>
<td>41 ± 8</td>
<td>13 ± 3</td>
<td>3 ± 1</td>
<td>490 ± 98</td>
</tr>
<tr>
<td>CB-6 (3× portion)§</td>
<td>466 ± 93</td>
<td>16 ± 3</td>
<td>448 ± 90</td>
<td>1.2 ± 0.3</td>
<td>&lt;1</td>
<td>35 ± 7</td>
<td>6 ± 1</td>
<td>1 ± 0</td>
<td>443 ± 89</td>
<td></td>
</tr>
<tr>
<td>CB-6 (residue)</td>
<td>88 ± 18</td>
<td>25 ± 5</td>
<td>60 ± 12</td>
<td>ND</td>
<td>2.7 ± 0.5</td>
<td>81 ± 16</td>
<td>52 ± 10</td>
<td>86 ± 24</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CB-7 (3× portion)§</td>
<td>357 ± 71</td>
<td>11 ± 2</td>
<td>340 ± 68</td>
<td>2.0 ± 0.4</td>
<td>2.8 ± 0.6</td>
<td>&lt;1</td>
<td>43 ± 9</td>
<td>13 ± 3</td>
<td>4 ± 1</td>
<td>327 ± 65</td>
</tr>
<tr>
<td>CB-Blank2</td>
<td>25 ± 5</td>
<td>ND</td>
<td>25 ± 5</td>
<td>ND</td>
<td>ND</td>
<td>25 ± 5</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Contributions of N from MTBSTFA can be used to correct for the maximum terrestrial contribution of N to NO in a worst-case scenario assumption that all MTBSTFA-N forms NO.

*Total MTBSTFA-derived N in nanomoles determined from the sum of the EGA-QMS quantification of abundances of silylated products. See SI Text for details.
†Residual N is the N in nanomoles resulting from subtraction of reduced N species detected in the blank for each set of experiments (RN-Blank, 2.7 ± 0.5 nmol; JK-Blank, 4.7 ± 0.9 nmol; CB-Blank1, 28 ± 6 nmol). This reduced N is assumed to be derived from MTBSTFA.
‡Corrected NO is derived by subtracting the nanomoles of residual N from the nanomoles of NO to take into account MTBSTFA-derived N.
§In CB-6 and -7, three portions of sample were deposited into a hot cup (200 °C), just before the onset of oxygen, followed by large peak at (275 °C) with shoulders at 350 and 425 °C (Fig. 1.)
trace for EGA of three nitrate species, calcium nitrate, may have been reported in 2005 (37). This suggests that N is currently and NO has been noted in the literature (32) and is characterized as a triple bond through photodissociation in the martian thermosphere by or reduction of atmospheric N. Alkali (e.g., Na, K) and alkaline earth (e.g., Mg, Ca) nitrates generally decompose into nitric oxide at elevated temperatures can decrease in the presence of perchlorates and metal nitrates is possible at the temperatures observed in SAM EGA data (31). Thermal decomposition of nitrates present in the samples (31). Thermal decomposition of N species detected (NO, RN, JK, and CB samples, and is more abundant than our estimates for total MTBSTFA-derived N. The main source of NO is produced by thermal decomposition of martian sediments. This is further supported by the consistent evolution temperature of HCN (and CICN) in RN, JK, and CB EGA pyrograms (Fig. 1), whereas there is considerable variation in the NO release profile across the three sites.

Nevertheless, to demonstrate the strength of the evidence for a martian NO source in these samples, we assume a worst-case scenario in which all MTBSTFA-derived N (minus reduced species present in SAM experimental blanks) is combusted to NO, even though laboratory experiments indicate that reduced species HCN, TFA, and CICN are also formed, and our CB-6 reheat experiment supports an MTBSTFA source for these reduced species. Based on our calculations, MTBSTFA contributed 27–42% of the NO at RN, 38–54% at JK, and 1–29% at CB (Table 1). If we take a slightly less conservative but more realistic approach and assume that the reduced species observed are predominantly products of MTBSTFA decomposition, then MTBSTFA contributed 13–27% of the NO at RN, 14–30% at JK, and 1–10% at CB.

**N-Bearing Species Distribution.** NO is the dominant N-bearing species in the RN, JK, and CB samples, and is more abundant than our estimates for total MTBSTFA-derived N. The main source of NO in these samples has been suggested to be thermal decomposition of nitrates present in the samples (31). Thermal decomposition of nitrates is possible at the temperatures observed in SAM EGA data (decomposition begins at ~200 °C), particularly if iron nitrates are present (32). Alkali (e.g., Na, K) and alkaline earth (e.g., Mg, Ca) metal nitrates generally decompose into nitric oxide at elevated temperatures (>560 °C); however, their decomposition temperatures can decrease in the presence of perchlorates and metal oxides (31, 33). Alternative or additional sources of NO include combustion of labile organic nitrogen-bearing compounds (other than MTBSTFA) under oxidizing conditions (34). However, this would represent a large amount of organic N, and as no organic compounds of martian origin have been definitively identified, an inorganic source of N is the more plausible origin for the NO.

Additional laboratory experiments to characterize the thermal decomposition temperatures of calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O; Sigma], iron(III) nitrate nonahydrate [Fe(NO₃)₃·9H₂O; Sigma], and magnesium nitrate hexahydrate [Mg(NO₃)₂·6H₂O; Sigma] for comparison with SAM EGA data were performed using a custom-built SAM-like laboratory system (SI Text). Onset of m/z 30 evolution of each species can be diagnostic of the source of NO in martian solid samples: ~100 °C for Fe(NO₃)₃, ~300 °C for Mg(NO₃)₂, and ~500 °C for Ca(NO₃)₂. Both JK and CB have their primary peak maximums ~300 °C, whereas the release profile at RN includes a peak maximum ~300 °C as well as a second one ~450 °C, consistent with the double peak release shown by Fe(NO₃)₃ (Fig. 4). The two-step decomposition of Fe(NO₃)₃ has been noted in the literature (32) and is characterized by a lower temperature release occurring over a broader temperature range, followed by a second, sharper release over a narrower range of temperature. This two-step release is thought to be a result of dehydration accompanied by hydrolysis, in which iron nitrate melts in its own water of crystallization followed by the evaporation and precipitation of Fe(OH)NO₃, ultimately resulting in the formation of Fe₂O₃ as the final product.

Although HCN is generally present at higher abundances in samples than in blanks, at present it is difficult to assign a non-terrestrial origin to this compound. However, it is not unreasonable to assume that pyrolysis of martian surface materials may produce HCN from macromolecular material of meteoritic, cometary, and interplanetary dust particle origin (35, 36).

**Significance of Results.** Detection of indigenous, fixed N in martian rocks and sediments has significant implications for the past habitability potential of Mars, as life on Earth requires bioavailability of N for synthesis of key biomolecules. Several hypotheses exist for mechanisms of cycling of N on Mars and these depend upon breaking the N≡N triple bond through “fixation” or reduction of atmospheric N₂. Detection of NO formed from products of O₂, CO₂, and N₂ photodissociation in the martian thermosphere by Mars Express was reported in 2005 (37). This suggests that N is currently being fixed in the martian thermosphere, although it is unknown how much, if any, is transported to the lower atmosphere and surface. Thermal shock, either from lightning or impact, has been the favored mechanism by which the N₂ bond could have been broken in the early martian (and terrestrial) atmosphere, resulting in HNO³, which reacts to form NO₂⁻ and NO₃⁻ in the presence of liquid water (4, 12, 38, 39). If NO detected by SAM EGA is attributable to the decomposition of nitrates, as laboratory

Fig. 3. The total nanomoles of N in the sum of all N species detected (NO, HCN, CICN, TFA, TFMA; Table 1) in blue, compared with the worst-case scenario prediction of nanomoles of N contributed by MTBSTFA (Table 1 and SI Text) in light blue. In blanks, except for CB-Blank2, the estimate of MTBSTFA-derived N is greater than actual N detected. CB samples had the largest abundance of detected N species, and the lowest percentage N contribution from MTBSTFA (Table 1).

Fig. 4. The m/z 30 trace for EGA of three nitrate species, calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O; Sigma], iron(III) nitrate nonahydrate [Fe(NO₃)₃·9H₂O; Sigma], and magnesium nitrate hexahydrate [Mg(NO₃)₂·6H₂O; Sigma] on a laboratory SAM-like system compared with RN, JK, and CB SAM experiments. The release profile at RN-4 is most consistent with the two-step decomposition of Fe(NO₃)₃ suggesting that Fe-nitrate may be a component of the RN sample.
experiments suggest, this would imply that (i) N fixation has occurred on Mars, and (ii) a biochemically available source of N is present on Mars and likely was in the past.

Based on estimates of sample mass and corrections for terrestrial N contribution, nitrogen present as NO in Gale crater is equivalent to 20–250 ppm N, with the highest concentrations in the CB mudstone. Converted to the equivalent amount of nitrate, this gives 110–300 ppm of nitrate in the RN aeolian samples, 70–200 ppm nitrate in JK mudstone samples, and 330–1,100 ppm nitrate in CB mudstone samples. Manning et al. (40) calculated an impact generated nitrate reservoir of \(-5 \times 10^{15}\) mol on Mars that is unlikely to have experienced significant decomposition. This translates into \(-0.11\) wt % NO3 using a 950-m impact veneer depth (e.g., ref. 40) and a soil density of 2 g/cm3, and is consistent with the upper limit of our detection of \(-0.11\) wt % NO3 at CB. Our detections also fall within the estimates of nitrate concentration from meteorites, which vary widely from recent detection of 4,800 \pm 60 ppm nitrate in the martian meteorite EETA79001 (15) to previous estimates of \(>1\) ppm (16).

The detection of nitrate at one site representing the martian global dust/local soil reservoir and two representing a potential refi lakebed (25) may suggest widespread atmospheric deposition. The hyperarid Atacama Desert provides a terrestrial analog for abiogenic N fixation and deposition, as biological inputs are severely limited, and oxidized N is stored in soils as nitrate instead of being removed via denitrification or leaching by water (9). Understanding whether similar processes are currently fixing N2 to NO3 and subsequently depositing nitrate on the martian surface is compelling for the habitability of Mars in the present and recent past. The abundances detected in all three Mars samples \((-0.01–0.1\) wt % NO3) are an order of magnitude lower than those in the Atacama Desert and Dry Valleys of Antarctica, which range from \(-0.1\) to \(>1\) wt % NO3 (e.g., ref. 41) and can be as high as 36% in Atacama Desert nitrate ore deposits (42). The low nitrate abundance in martian samples is consistent with models of the impact-generated nitrate reservoir (e.g., ref. 40), suggesting that we are detecting the products of ancient N-fixation processes under different atmospheric conditions, with no additional inputs, and negligible decomposition of the original reservoir. The difference in abundance between JK mudstone \((0.01–0.03\) wt % NO3) and CB mudstone \((up to \sim 0.1\) wt % NO3), two mudstone samples taken \(-1.5\) km apart, points to nitrate fixation in the martian geologic record. We do not detect nitrate in the RN samples, with a low abundance of \(\sim 0.03\) wt % NO3, which shows evidence of more alteration than CB (26). Alternatively, increased nitrate abundance at CB could reflect additional past input via some in situ fixation mechanism, biological or not. Detection of indigenous reduced N (e.g., ammonia) would support biological fixation; however, not only is ammonia difficult to detect by SAM (SI Text), but it is readily destroyed by photochemistry, and thus not likely to have been preserved unless structurally incorporated into minerals. One interpretation for the persistence of oxidized N and the apparent lack of reduced N in a lacustrine environment is the absence of development of chemolithoautotrophic N fixation. The presence of fixed N on Mars suggests that, at some point, the first half of a nitrogen cycle was established. On Earth, N is recycled back into the atmosphere by denitrification via biological activity, but on Mars, the probable absence of near-surface life in post-Noachian times would result in fixed N accumulating as nitrate in the martian geologic record. Nevertheless, if pre-Noachian life had arisen on Mars, it is reasonable to assume that it exploited the available nitrate, and our results suggest that the search for stratigraphic evidence of an ancient martian N cycle should continue.

**Conclusion**

Nitrogen species were detected in pyrolysis of one sample scooped from an aeolian deposit and two samples drilled from the same sedimentary rock unit in Yellowknife Bay. The molar abundance of N in these samples exceeds that predicted to be present due to all known terrestrial instrumental sources. The bulk of the N detected is the form of nitric oxide (NO) and may indicate a mineralogical sink for atmospheric N2 in the form of nitrate, with estimated abundance of up to \(\sim 1,100\) ppm NO3 in martian sedimentary deposits. The detection of NO in both wind-drifted fines (RN) and in mudstone (JK, CB) likely reflects ancient impact and/or volcanic plume lightning generated N2 fixation to NO3 (10–12). Fixed N is essential to terrestrial life, and this requirement has driven the evolution of metabolism on Earth. The presence of fixed N on Mars suggests that, at some point, the first half of a nitrogen cycle was established. On Earth, N is recycled back into the atmosphere by denitrification via biological activity, but on Mars, the probable absence of near-surface life in post-Noachian times would result in fixed N accumulating as nitrate in the martian geologic record. Nevertheless, if pre-Noachian life had arisen on Mars, it is reasonable to assume that it exploited the available nitrate, and our results suggest that the search for stratigraphic evidence of an ancient martian N cycle should continue.

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22. Franz HB, et al. (2014) Analytical techniques for retrieval of atmospheric composition from these SAM experiments are archived in the Planetary Data System (pds.nasa.gov).