

Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica

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Nitrous oxide is a potent atmospheric greenhouse gas¹ that contributes to ozone destruction². Biological processes such as nitrification and denitrification are thought to drive nitrous oxide production in soils, which comprise the largest source of nitrous oxide to the atmosphere¹. Here we present measurements of the concentration and isotopic composition of nitrous oxide in soil pore spaces in samples taken near Don Juan Pond, a metabolically dormant hypersaline pond in Southern Victoria Land, Antarctica in 2006, 2007 and 2008, together with *in situ* fluxes of nitrous oxide from the soil to the atmosphere. We find fluxes of nitrous oxide that rival those measured in fertilized tropical soils³. Laboratory experiments—in which nitrite-rich brine was reacted with a variety of minerals containing Fe(II)—reveal a new mechanism of abiotic water-rock reaction that could support nitrous oxide fluxes at Don Juan Pond. Our findings illustrate a dynamic and unexpected link between the geosphere and atmosphere.

Don Juan Pond (DJP), a shallow hypersaline playa lake located in the south fork of the Wright Valley (Southern Victoria Land) Antarctica (Supplementary Fig. S1), contains a groundwater-derived calcium-chloride-rich eutectic brine⁴ (413 g CaCl₂ and 29 g NaCl kg⁻¹; up to 671‰ or 40.2% salt) and is the most saline water body on Earth. Despite extremely low temperatures (Supplementary Fig. S2), the brine has no significant ice cover, even during winter, and is the only ice-free body of water in the Antarctic McMurdo Dry Valleys. DJP is bounded to the north and south by steep mountains of Beacon sandstone through which cut numerous intrusive dykes of igneous Ferrar dolerite. Ferrar dolerite contains pyroxenes (augite, pigeonite), phyllosilicates (biotite) and feldspars (plagioclase, labradorite, alkaline feldspars), is up to nine per cent weight iron(II) (ref. 5) and has a similar composition to basaltic rocks on Mars⁶. The geological setting of the Wright Valley, subzero temperatures and brine geochemistry make DJP an ideal Mars analogue environment⁴.

In November of 2006, 2007 and 2008, we measured the isotopic composition and concentration of N₂O in the soil pore space (aqueous and gas phases) near the DJP shore (see the Methods section) and determined *in situ* soil-atmosphere N₂O fluxes. Soil pore space N₂O concentrations varied from 1.16 to 2.03 μmol l⁻¹ of bulk soil (approximately 3.9 to 6.8 μmol l⁻¹ of pore fluid assuming 30% porosity), and were up to 500 times higher than expected for soils in equilibrium with atmospheric N₂O. Soil-atmosphere N₂O fluxes (0.7 to 55 μmol m⁻² h⁻¹) varied inversely with water table height (Supplementary Table S1). DJP N₂O fluxes were comparable to those in urea-fertilized tropical soils

(1–176 μmol m⁻² h⁻¹; ref. 3) and were significantly higher than those in temperate estuaries (<1 μmol m⁻² h⁻¹; ref. 7) or in other Antarctic environments (0.02–6 μmol m⁻² h⁻¹; ref. 8), except Lake Bonney moat, where predicted gradient-driven N₂O fluxes (191 μmol m⁻² h⁻¹) are high⁹.

DJP brine contained elevated concentrations of nitrate (5,533 ± 626 μM), nitrite (25.3 ± 9.2 μM) and ammonium (490.3 ± 82.6 μM), but N₂O concentrations were below detection, which was not surprising given the expected concentration of N₂O (<2 nM) at *in situ* conditions (salinity ~665 ppt, temperature = 8 °C) and the equilibrium with South Pole air. The lack of measurable dissolved N₂O in the brine implies that any N₂O produced would be released quickly to the atmosphere. Incubation of DJP brine at 8 or 20 °C showed no detectable N₂O production in the absence of the mineral dolerite, illustrating that components of the brine itself (for example, dissolved organic carbon¹⁰) were not involved in N₂O production.

Although biological processes dominate N₂O production in most environments, all attempts to measure biological activity in DJP brine and soils have failed thus far (J. C. Priscu, manuscript in preparation). An alternative mechanism of N₂O production is chemodenitrification, an abiotic process wherein inorganic nitrogen species are reduced to gaseous species, either nitric oxide or N₂O, by means of coupled oxidation of ferrous iron¹¹ or humic acids¹⁰. As the rocky till soils surrounding DJP derive from weathering of Fe(II)-rich Ferrar dolerite (Supplementary Fig. S1), we postulated that reaction between the nitrate/nitrite-rich brine and soil Fe(II)-containing minerals was responsible for the observed N₂O production.

This hypothesis was tested in laboratory experiments. First, sterile solutions of DJP brine were mixed with autoclaved Ferrar dolerite grains or with pure mineral phases, augite and olivine, in sealed serum vials. Second, sterile solutions of DJP brine or artificial brine (a sterile 650‰ calcium chloride solution amended with nitrate or nitrite at concentrations comparable to DJP brine) were percolated through a column containing sterile Ferrar dolerite pellets collected from the edge of DJP. Third, sterile DJP brine was incubated in flasks with sterile dolerite or sterile dune sand that was collected above DJP and was not in contact with the brine (see the Methods section for details).

Immediate production of N₂O and molecular hydrogen (H₂) occurred following the reaction of dolerite, augite or olivine with sterile-filtered (0.1 μm) DJP brine at room temperature (Fig. 1a). In flask experiments, a magnetic mineral also accumulated on the stir bar during the experiment. Magnetite is often produced

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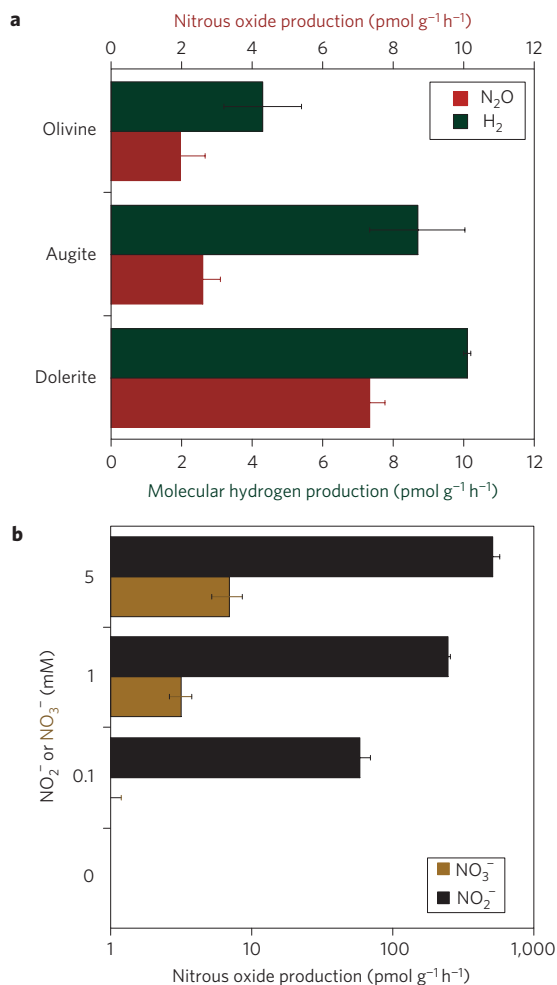
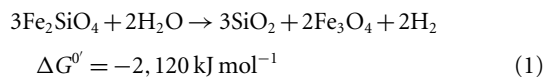


Figure 1 | Production of N₂O in laboratory experiments. **a**, Production of nitrous oxide and molecular hydrogen during incubation of olivine, augite, and dolerite with DJP brine at room temperature. **b**, Production of nitrous oxide with dolerite incubated with sterile artificial brine amended with different concentrations of nitrite or nitrate. No nitrous oxide production was observed in the nitrate- and nitrite-free controls. Error bars denote standard deviation of the mean ($n = 3$ replicates). See the Methods section for experimental details.

during serpentinization¹² and may have been the magnetic mineral we observed. The production of magnetite and H₂ during serpentinization is described by equation (1):



During serpentinization, igneous rocks containing ferromagnesian minerals react with water and are converted to more stable secondary serpentine minerals¹². The contemporaneous N₂O and H₂ formation observed here is probably the result of a reaction between dolerite-minerals and brine, albeit at significantly lower temperatures than those usually associated with serpentinization¹³. Hydrogen production also seems to occur *in situ*, as the soils around DJP were enriched with H₂ (21.7–27.7 μmol l⁻¹ of bulk soil) by about 700 times relative to soil in equilibrium with South Polar air (~3 nM).

Experiments carried out with DJP dolerite as the reductant, and sterile artificial brine containing either nitrate or nitrite as the oxidant, showed that N₂O production was one hundred times

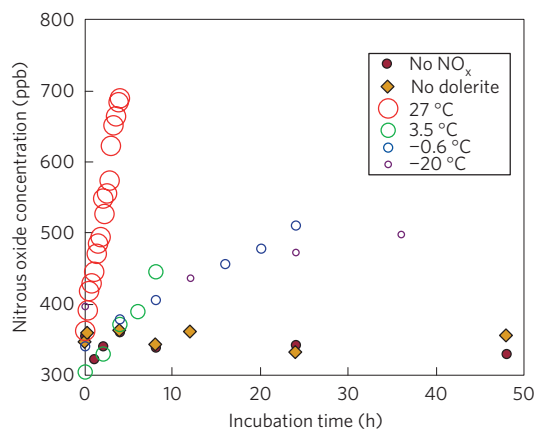


Figure 2 | N₂O production as a function of temperature. Nitrous oxide production in controls (lacking nitrite/nitrate or dolerite) and as a function of temperature.

higher with nitrite as the oxidant (Fig. 1b), which is not surprising as the reduction of nitrate to nitrite would be the rate-limiting step¹⁴. Anoxic incubation of dolerite and DJP brine, amended with an extra 3 mM of 99.5 atom% ¹⁵N–NO₃⁻ (the *in situ* NO₃⁻ concentration was 5 mM NO₃⁻), showed substantial ¹⁵N enrichment in N₂O (34 ± 0.9 atom% ¹⁵N) but no ¹⁵N enrichment in molecular nitrogen (N₂), clearly showing that N₂O is the primary reaction product.

Instantaneous N₂O production also occurred when DJP brine and dolerite were reacted in flasks or in column percolation experiments across a range of temperatures (Fig. 2, Supplementary Table S2). Percolation of dolerite with sterile artificial brine lacking nitrate or nitrite did not generate N₂O (Fig. 2). Production of N₂O occurred at the lowest temperature tested, –20 °C, and the rate of production increased with temperature (Fig. 2, Supplementary Table S2). During incubation with dolerite and DJP brine, neither substrate (nitrate or nitrite in solution and Fe(II) in the solid phase) limited the reaction, allowing use of the Arrhenius equation to calculate the activation energy of the process. The activation energy (E_a) was 18.4 kJ mol⁻¹, demonstrating a diffusion-controlled reaction mechanism and indicating that N₂O production would be enhanced by brine fluid advection through geologic materials, relative to brines pooled in a pond-like setting.

The ¹⁵N site preference in N₂O is defined as the difference between the nitrogen isotope ratio in the central nitrogen (N^α) and the terminal nitrogen (N^β) atoms: $SP = \delta^{15}\text{N}^\alpha - \delta^{15}\text{N}^\beta$ (ref. 15). This intramolecular distribution of ¹⁵N within the linear N₂O molecule is thought to reflect the production mechanism^{16–18} rather than the substrates. As a result of this, the site preference has been used to identify the biological source(s) of N₂O (for example, nitrification or denitrification) in a variety of soil systems^{6,19,20}. Here, we analysed the site preference of N₂O from DJP field samples and in samples from laboratory chemo-denitrification experiments, to compare them with others reported from pure culture experiments, abiotic reactions and emissions from more temperate terrestrial ecosystems.

Despite a fairly consistent bulk $\delta^{15}\text{N}$ –N₂O, DJP soils exhibited a wide range of site preference values (–45‰ to +4‰; Table 1), suggesting a complex series of production, and possibly consumption, processes. Biological processes such as nitrification and denitrification produce N₂O with a wide variety of isotopic values, including some negative site preference values. For example, whereas N₂O produced from nitrification has a positive site preference ranging from +9.2‰ to +21.1‰ (ref. 17), the site preference of N₂O produced from the same organisms by means of denitrification ranged from –8.5‰ to +8.5‰ (ref. 17). The site preference in N₂O from heterotrophic denitrification ranged from

Table 1 | Isotopic composition of N and O in dissolved inorganic N species and nitrous oxide.

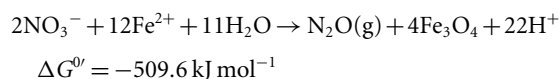
Inorganic N	$\delta^{15}\text{N}$	$\delta^{18}\text{O}$	<i>n</i>			
NO_2^- 2007	-106.6 ± 0.35	-6.55 ± 0.25	2			
NO_3^- 2007	1.9 ± 0.2	62.6 ± 0.4	2			
NH_4^+ 2007	7.59 ± 0.61	-	2			
Nitrous oxide	$\delta^{15}\text{N}^{\text{bulk}}$	$\delta^{18}\text{O}$	$\delta^{15}\text{N}^{\alpha}$	$\delta^{15}\text{N}^{\beta}$	SP	<i>n</i>
Flux 2007	-44.6 ± 2	50.6 ± 0.4	-42.5 ± 3.1	-46.6 ± 1.3	4.1 ± 2.5	3
Flux 2008	-45.4 ± 0	50.5 ± 1.4	-44.8 ± 1	-46.1 ± 1.1	1.3 ± 2.1	2
Soil pore 2008	-42.9	50.6	-42.3	-43.9	1.2	1
Soil pore 2008	-43	52.4	-43.5	-43.9	1.9	1
Soil pore 2007	-34.5 ± 0.2	76.7 ± 0.9	-57.1 ± 1.1	-11.9 ± 0.7	-45.2 ± 1.9	2
Soil pore 2007	-35.4 ± 0	61.9 ± 0.3	-39.2 ± 0.2	-30.9 ± 0.1	-9 ± 0.3	2
Lab expt 2008	-36.3 ± 0	64.4 ± 0.2	-43 ± 0.6	-29.6 ± 0.6	-13.3 ± 1.2	2
N_2O Beacon val.	-16.2 ± 0.3	119 ± 0.8	-5.9 ± 0.2	-26.6 ± 0.3	20.7 ± 0	2
N_2O Beacon val.	-16.3 ± 0.4	119.1 ± 1	-6 ± 0.6	-26.6 ± 0.2	20.6 ± 0.4	2

Isotopic ratios in per mil (‰) relative to atmospheric N_2 (for $\delta^{15}\text{N}$) or Standard Mean Ocean Water ($\delta^{18}\text{O}$). Isotopic data reflect the average (\pm standard deviation) for *n* measurements on the isotope-ratio-monitoring mass spectrometer. $\delta^{15}\text{N}^{\alpha}$ = the isotopic composition of the central nitrogen atom in N_2O ; $\delta^{15}\text{N}^{\beta}$ = the isotopic composition of the terminal nitrogen atom in N_2O ; SP = site preference; flux = sample from *in situ* flux incubation; soil pore = degassed soil sample; year of sample collection is noted. Beacon val. = ice-cemented permafrost sample collected in front of the Taylor Glacier.

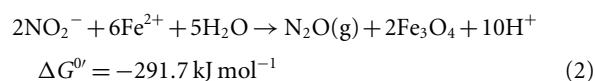
-5‰ to $+22\text{‰}$, depending on the microorganism responsible²¹, and N_2O consumption by means of denitrification further increases the site preference as a result of the preferential cleavage of $^{14}\text{N}^{\alpha}\text{-O}$ bonds²². Thus, we note that although a wide range of N_2O site preference values can be attributed to biological processes, no biological process has been documented to produce N_2O with a site preference as low as -45‰ .

Abiotic N_2O production mechanisms also show a wide range of isotopic signatures. Automobile fuel combustion produced N_2O with site preference values between -20‰ and $+50\text{‰}$ (ref. 23). Reaction of nitrite with trimethylamine borane or hydroxylamine with MnO_2 produced N_2O with a more constant site preference value near $+30\text{‰}$ (ref. 21). Our chemodenitrification experiments with sterile brine and Fe(II) minerals yielded a range of site preference values from -13.3‰ to $+22.6\text{‰}$ (Table 1). Given that even these reactions did not produce N_2O with a site preference as low as we observed in DJP soil, we hypothesize that kinetic isotope effects associated with the abiotic reaction may be responsible for the production of N_2O with low and varying site preference under different conditions¹⁷. Furthermore, if more than one pool of nitrogen oxides is involved in the reaction, the site preference may reflect variations in the $\delta^{15}\text{N}$ signatures of these substrate molecules (Table 1).

Our laboratory results demonstrate conclusively that significant N_2O production results from a previously unknown abiotic reaction where nitrate and nitrite reduction are coupled to the oxidation of dolerite-derived Fe(II). Several mineral components of dolerite could react with nitrate or nitrite, and we propose the following reactions to explain our observations (after ref. 14; equation (2)):



or



Previously documented abiotic reactions between nitrate and Fe(II)-bearing minerals produced mainly nitrite and ammonium

(for example, Fe(II)-silicates, wüstite, Fe^0 ; refs 11,14); N_2O was the dominant product only when nitrite and siderite were reacted²⁴. The new process we document occurs in a unique hypersaline habitat, at low temperature (down to at least -20 °C), and generates N_2O , rather than N_2 , as the dominant product.

The complex suite of brine-rock reactions documented here generates a variety of products, including N_2O , H_2 and potentially magnetite, in an extremely cold, hypersaline environment on Earth over a range of temperatures. In addition to DJP, this unusual mechanism may occur in other environments on Earth, and could serve as a component of the martian nitrogen cycle and a source of fuel (H_2) to support microbial chemosynthesis²⁵. Abiotic reactions and fixation by impacts have concentrated nitrate in martian soils, regoliths and brines²⁵⁻²⁷, and such processes transfer nitrogen from the atmosphere to the geosphere with limited possibilities (for example, impact decomposition²⁷) for returning flux to the atmosphere. Our results suggest that an unrealized mechanism—reaction of brine-derived nitrate with basaltic rock—offers a means for mobilizing nitrate from surface soils and regoliths and returning it to the martian atmosphere as N_2O .

Methods

Nitrous oxide fluxes were measured in a closed plexiglass chamber (20 cm tall, 24.5 cm inner diameter and with an interior fan²⁸) connected by means of gas-tight PVC tubing to an infrared photo-acoustic multi-gas monitor (TGA 1312, Europe Scientific) operated in circulation mode. During sampling, the air temperature was 4.2 °C ; the brine temperature was 8.7 °C . The flux chamber was inserted into the soil to a depth of 5 cm and the chamber height above the soil was measured to calculate the volume. N_2O concentrations were measured in 15–30 min increments for one to two hours and emission rates were calculated from the concentration increase over time using linear regression analysis. The gas analyser was calibrated with certified N_2O standard gas (101 ppm N_2O in He) before each use; the response to N_2O was linear over four orders of magnitude.

To determine soil N_2O and H_2 concentration, three replicate samples (30 cm³) were collected into cut-end 60 ml disposable plastic syringes and closed with rubber stoppers. Soil was transferred to a 600 ml argon-purged glass bottle that was closed with a rubber septum and screw cap and vigorously shaken (1 min) to equilibrate soil gas with the headspace. The nitrous oxide concentration was measured by connecting the gas analyser to the bottle by means of PVC tubing, as described above. The nitrous oxide mixing ratios were calculated from the N_2O concentration, headspace and sediment volume, and the porosity (30%). The gas analyser was calibrated by injecting a known volume of N_2O standard (above) into the same bottles. The variation of N_2O concentration in three subsequent measurements was ± 20 ppb. The H_2 concentration was measured

using a reactive mercury bed reduction gas analyser that was calibrated with certified standards (1 ppm H₂ in He).

Samples for N₂O isotopic analyses were collected in 500 ml wide-mouth glass bottles by inserting the bottle in an inverted position into unsaturated sediment for 4 h, after which the bottles were closed with a butyl rubber septum and screw cap. The gas from the bottle headspace was then transferred into an evacuated 250 ml glass serum vial that was crimp-sealed with a butyl rubber stopper.

N₂O isotopic measurements ($\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{18}\text{O}^{\text{bulk}}$, $\delta^{15}\text{N}^{\alpha}$, $\delta^{15}\text{N}^{\beta}$) were carried out on a Finnigan DeltaPLUS XP isotope-ratio-monitoring mass spectrometer. Online purification of N₂O was achieved by a custom-built cryogenic trapping system²⁹. Individual measurements were referenced against a laboratory N₂O reference tank, which had been calibrated versus air for $\delta^{15}\text{N}^{\text{bulk}}$, $\delta^{15}\text{N}^{\alpha}$ and $\delta^{15}\text{N}^{\beta}$ and versus Vienna Standard Mean Ocean Water for $\delta^{18}\text{O}$ (S. Toyoda; Tokyo Tech). Ion source scrambling coefficients were determined for the mass spectrometer by parallel analyses of three independently calibrated N₂O reference gases using standard equations¹⁵. This calibration yields a site preference of 15–16‰ for tropospheric N₂O.

In the laboratory, freshly crushed dolerite (8 g, >3 mm) was placed into 70 ml serum vials that were sealed with a crimped butyl rubber septum and purged with ultrapure argon. Before use, the vials and dolerite were sterilized by autoclaving (121 °C, 40 min). After cooling, 20 ml of double-filtered (= successive filtration through a 0.1 µm filter) DJP brine or artificial CaCl₂ brine with different nitrate and nitrite concentrations was added using a syringe. Vials were placed into a shaking incubator (200 rpm) at 20 °C for 6 days, after which the N₂O and H₂ concentrations were measured by means of gas chromatography–electron capture detection and a reactive mercury bed reduction gas analyser, respectively.

For flask experiments, N₂O production rates were determined by placing 20–30 g of freshly crushed dolerite (>3 mm), olivine or augite (the last two supplied by Giverny) and a Teflon-coated magnetic stir bar into a 600 ml glass flask along with 50 ml of DJP brine. The flask was closed with a butyl rubber septum and screw cap, placed onto a magnetic stir plate inside a temperature-controlled incubator and connected to the gas analyser with copper tubing. Nitrous oxide production was comparable under ambient air and an Ar atmosphere. Three replicate concentration readings were taken at each time point and N₂O production rates were calculated from linear regression analysis of concentration versus time.

For column percolation experiments, a column (4.5 cm diameter, 50 cm long) was filled with crushed dolerite (1,540 g; >3 mm). Brine (500 ml) was recirculated through the column at a rate of 5 ml min⁻¹ using a peristaltic pump. The gas analyser was connected in-line. The system was placed in an incubation chamber at various temperatures from -20 to +20 °C. Nitrous oxide concentrations and production rates were calculated as described above.

For ¹⁵NO₃⁻ addition experiments, 3 mM Na ¹⁵NO₃⁻ was added to DJP brine and incubated with dolerite in sealed serum vials (with shaking) for 1 week at 20 °C. The headspace from each incubation vial was transferred into an evacuated sterile 60 ml sealed serum vial and ¹⁵N enrichment in N₂O and N₂ was determined using isotope-ratio-monitoring mass spectrometry.

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References

- Forster, P. *et al.* in *IPCC Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon, S. *et al.*) (Cambridge Univ. Press, 2007).
- Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. Nitrous oxide (N₂O): The dominant ozone-depleting substance emitted in the 21st century. *Science* **329**, 123–125 (2009).
- Perez, T. *et al.* Identifying the agricultural imprint on the global N₂O budget using stable isotopes. *J. Geophys. Res.* **106**, 9869–9878 (2001).
- Burt, D. M. & Knauth, L. P. Electrically conducting, Ca-rich brines, rather than water, expected in the Martian subsurface. *J. Geophys. Res.* **108**, doi:10.1029/2002JE001862 (2003).
- Clarkson, P. D. Geology of the Shackleton Range: IV. The Dolerite Dykes. *Br. Antarct. Surv. Bull.* **53**, 210–212 (1981).
- Harvey, R. P. The Ferrar dolerite: An Antarctic analog for Martian basaltic lithologies and weathering process. Workshop on the Martian Highlands and Mojave Desert Analogs, 4012.pdf (2001). <http://www.lpi.usra.edu/meetings/martianhighlands2001/pdf/4012.pdf>.
- Middelburg, J. J. *et al.* Nitrous oxide emissions from estuarine intertidal sediments. *Hydrobiologia* **311**, 43–55 (1995).
- Gregorich, E. G. *et al.* Emission of CO₂, CH₄ and N₂O from lakeshore soils in an Antarctic Dry Valley. *Soil Biol. Biochem.* **38**, 3120–3129 (2006).
- Priscu, J. C., Downes, M. T. & McKay, C. P. Extreme supersaturation of nitrous oxide in a poorly ventilated Antarctic lake. *Limnol. Oceanogr.* **41**, 1544–1551 (1996).
- Thorn, K. A. & Mikita, M. A. Nitrite fixation by humic substances: Nitrogen-15 nuclear magnetic resonance evidence for potential intermediates in chemodenitrification. *Soil Sci. Soc. Am. J.* **64**, 568–582 (2000).
- Postma, D. Kinetics of nitrate reduction by detrital Fe(II)-silicates. *Geochim. Cosmochim. Acta* **54**, 903–908 (1990).
- Evans, B. W. Control of the products of serpentinization by the Fe²⁺–Mg⁺ exchange potential of olivine and orthopyroxene. *J. Petrol.* **49**, 1873–1887 (2008).
- Kelley, D. S., Baross, J. A. & Delaney, J. R. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annu. Rev. Earth Planet. Sci.* **30**, 385–491 (2002).
- Sørensen, J. & Thorling, L. Stimulation by lepidocrocite (7-FeOOH) of Fe(II)-dependent nitrite reduction. *Geochim. Cosmochim. Acta* **55**, 1289–1294 (1991).
- Yoshida, N. & Toyoda, S. Constraining the atmospheric N₂O budget from intramolecular site preference in N₂O isotopomers. *Nature* **405**, 330–334 (2000).
- Sutka, R. L. *et al.* Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundance. *Appl. Environ. Microbiol.* **72**, 638–644 (2006).
- Schmidt, H.-L., Werner, R. A., Yoshida, N. & Well, R. Is the isotopic composition of nitrous oxide an indicator for its origin from nitrification or denitrification? A theoretical approach from referred data and microbiological and enzyme kinetic aspects. *Rapid Commun. Mass Spectrom.* **18**, 2036–2040 (2004).
- Sutka, R. L., Ostrom, N. E., Ostrom, P. H., Gandhi, H. & Breznak, J. A. Nitrogen isotopomer site preference of N₂O produced by *Nitrosomonas europaea* and *Methylococcus capsulatus* Bath. *Rapid Commun. Mass Spectrom.* **18**, 1411–1412 (2004).
- Bol, R. *et al.* Dual isotope and isotopomer ratios of N₂O emitted from a temperate grassland soil after fertiliser application. *Rapid Commun. Mass Spectrom.* **17**, 2550–2556 (2003).
- Well, R., Kurganova, I., de Gerenyu, V. L. & Flessa, H. Isotopomer signatures of soil-emitted N₂O under different moisture conditions—a microcosm study with arable loess soil. *Soil Biol. Biochem.* **38**, 2923–2933 (2006).
- Toyoda, S. *et al.* Fractionation of N₂O isotopomers during production by denitrifier. *Soil Biol. Biochem.* **37**, 1535–1545 (2005).
- Ostrom, N. E. *et al.* Isotopologue effects during N₂O reduction in soils and in pure cultures of denitrifiers. *J. Geophys. Res. Biogeosci.* **112**, doi:10.1029/2006JG00 (2007).
- Toyoda, S. *et al.* Isotopomeric characterization of N₂O produced, consumed, and emitted by automobiles. *Rapid Commun. Mass Spectrom.* **22**, 603–612 (2008).
- Rakshit, S., Matocha, C. J. & Coyne, M. S. Nitrate reduction by siderite. *Soil Sci. Soc. Am. J.* **72**, 1070–1077 (2008).
- Schulte, M., Blake, D., Hoehler, T. & McCollom, T. Serpentinization and its implications for life in early Earth and Mars. *Astrobiology* **6**, 364–376 (2006).
- Mancinelli, R. L. & Banin, A. Where is the nitrogen on Mars? *Int. J. Astrobiol.* **2**, 217–225 (2003).
- Manning, C. V., McKay, C. P. & Zahnle, K. J. The nitrogen cycle on Mars: Impact decomposition of near-surface nitrates as a source for a nitrogen steady state. *Icarus* **197**, 60–64 (2008).
- Hutchinson, G. L. & Mosier, A. R. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.* **45**, 311–316 (1981).
- McIlvin, M. & Casciotti, K. L. Fully automated system for stable isotopic analyses of dissolved nitrous oxide at natural abundance levels. *Limnol. Oceanogr.* **8**, 54–66 (2010).

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Author contributions

V.A.S., M.T.M., M.W.B., and J.C.P. conducted the fieldwork; V.A.S. and S.B.J. designed experiments and V.A.S. and M.W.B. carried them out; K.L.C. led the natural abundance nitrogen isotopic analyses and interpretation; C.P.M. provided insight to the Mars nitrogen cycle; S.B.J. wrote the paper and all authors commented on it.

Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper on www.nature.com/naturegeoscience. Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions>. Correspondence and requests for materials should be addressed to S.B.J.