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Iron cycling in the anoxic cryo-ecosystem of Antarctic Lake Vida

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Abstract Iron redox cycling in metal-rich, hypersaline, anoxic brines plays a central role in the biogeochemical evolution of life on Earth, and similar brines with the potential to harbor life are thought to exist elsewhere in the solar system. To investigate iron biogeochemical cycling in a terrestrial analog we determined the iron redox chemistry and isotopic signatures in the cryoencapsulated liquid brines found in frozen Lake Vida, East Antarctica. We used both in situ voltammetry and the spectrophotometric ferrozine method to determine iron speciation in Lake Vida brine (LVBr). Our results show that iron speciation in the anoxic LVBr was, unexpectedly, not free Fe(II). Iron isotope analysis revealed highly depleted values of $-2.5‰$ for the ferric iron of LVBr that are similar to iron isotopic signatures of Fe(II) produced by dissimilatory iron reduction. The presence of Fe(III) in LVBr therefore indicates dynamic iron redox cycling beyond iron reduction. Furthermore, extremely low $\delta^{18}O–SO_4^{2-}$ values ($-9.7‰$) support microbial iron-sulfur cycling reactions. In combination with evidence for chemodenitrification resulting in iron oxidation, we conclude that coupled

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difficult since they can co-exist and compete (Weber et al. 2006; Ionescu et al. 2015). Iron isotope ratios provide a useful proxy for gaining insight into Fe sources and biogeochemical processes—including mineral precipitation and dissolution, sorption, as well as bacterially-mediated processes such as dissimilatory iron reduction (DIR) (Beard et al. 1999; Bullen et al. 2001; Johnson et al. 2008). In this paper, we have investigated the Fe chemistry—including Fe speciation experiments and isotope analysis of LVBr—to understand the interplay of biotic and abiotic Fe biogeochemistry in this temperature-limited but active microbial ecosystem.

Methods

Study area, sample collection and storage

Lake Vida is located in Victoria Valley—a closed, evaporative basin in the northernmost McMurdo dry valleys (MDV; Supplementary Fig. 1), a dry polar desert that receives generally less than 35 mm per year snowfall (Fountain et al. 2010). The mean annual temperature at the surface of Lake Vida approaches $-30\ °C$ in some years (Doran et al. 2002). The geochemistry and microbiology of Lake Vida brine (LVBr) have recently been described by Murray et al. (2012), indicating a distinct microbial assemblage compared to other lakes in the MDV. The Basement Sill and Penneplaine Sill of the Ferrar Dolerite, which contain Mg–Fe rich pyroxenes, dominate the geology around Lake Vida. The dominating Fe compound in the dolerite is iron oxide (FeO), where Fe occurs as Fe(II) (Zavala et al. 2011). The floor of Victoria Valley also is covered by glacial till dominated by dolerite clasts.

Brine samples were collected during an expedition in 2010 (Murray et al. 2012). Two boreholes were drilled for the extraction of ice cores (20 and 27 m); the 20 m borehole was used for brine collection. LVBr entered the 20 m borehole at a depth of ~ 18 m and was collected using sterilized submersible pumps (Waterra Monsoon pumps) and sterile polytetrafluoroethylene tubing (Doran et al. 2008). Brine samples were directly pumped into bottles under N_2 atmosphere, over-pressurized, and stored at $-10\ °C$ under dark, anoxic conditions. For bulk iron [Fe(II) + Fe(III)] isotope analyses, triplicate samples were filtered using a 0.2 µm Acrodysic polypropylene PES membrane filter ("filtered brine"); three were filtered for speciation analysis ("filtered speciation brine"); three remained unfiltered ("whole brine"). Sulfate analyses were performed on brine samples preserved in a gas-tight glass syringes that were flash frozen and kept at $-78\ °C$ wrapped in foil until analysis.

In addition, five rock and sand samples were collected in the study area (Supplementary Fig. 1b): (1) a sample from a porous, frozen sand layer in contact with LVBr collected between 25.28 and 25.48 m depth in the ice core recovered during the 2010 expedition (Hole #1), "LVS"; (2) a sample collected in the center of the Basement Sill of the Ferrar Dolerite on the South shore of Lake Vida, displaying millimeter-size pyroxenes "LVDCC"; (3) a sample collected on the southwest shore of Lake Vida in the Bull Drift, a moraine dominated by dolerite clasts, "LVS3". This sample was representative of the sand in the moraine that constitutes the Southwest and Northwest portion of the lakeshore; (4) A sand sample was collected on the north shore of Lake Vida, downwind from a major dune, "LVS4". This sand is representative of aeolian material that accumulates on the northeast shore of the lake. (5) "LVS9" is a sample collected on the Lake Vida ice surface and was representative of windblown material. Orthopyroxene grains, derived from orthopyroxene crystals of the Ferrar dolerite, were present in all sand samples (between 13.3% of the grain in LVS and 7.8% in LVS-3). Dolerite clasts also were present in all sand samples (between 17.8% for LVS and 10.8% for LVS4).

Fe speciation by voltammetric electrochemistry

Voltammetry is capable of collecting real-time, simultaneous measurements of dissolved O_2, H_2S, Mn(II), Fe(II), $S_2O_3^{2-}$, $S_4O_6^{2-}$, S_x^{2-}, S(0), as well as aqueous species of Fe(III) and FeS (Luther et al. 2008). A solid-state, gold-amalgam (Au/Hg) working electrode (deployed without membrane) enables simultaneous voltammetric measurements of multiple redox species in situ and recently has been shown to also differentiate between varieties of reduced sulfur species (Luther et al. 2008).

In the field, we used a ~ 33 m cable attached to a DLK-70 three-electrode potentiostat (Analytical

Instrument Systems, Inc.) to apply a voltage ramp to the working electrode (i.e., Au/Hg solid-state electrode) versus a reference electrode (i.e., Ag/AgCl solid-state electrode). The current at the working electrode surface was simultaneously measured through a counter electrode (i.e., Pt electrode). For any given analyte, the current is proportional to concentration, so that the analyte concentration can be calculated from current peak heights obtained in voltammograms using calibration coefficients established during laboratory standardizations made prior to field deployment [analogous to measuring absorption peaks at given wavelengths in spectroscopy (Brendel and Luther 1995)]. Each redox species, if present at detectable levels, produces a current peak that can be discriminated from others in one potential scan from -0.1 to -2.0 V. Because the voltammetric electrode scans a range of potentials, rather than constantly resting at a single potential, consumption of the chemical species measured is undetectable.

Fe speciation by ferrozine spectrophotometry

Due to the high Fe concentration in the sample (256–308 μmol L^{-1}) (Murray et al. 2012), LVBr was diluted (1:300) to work within comparable concentration ranges established in previous studies and to remove matrix issues. For laboratory Fe speciation experiments, ~ 100 μL Lake Vida brine (0.2 μm-filtered) were subsampled from a gas-tight vial under an Argon atmosphere and diluted into Argon-purged high purity water (Millipore-element >18 MΩ cm), hereafter called MQ.

The ferrozine reagent prepared daily at a concentration of 10 mmol L^{-1} in MQ containing 0.1-mol L^{-1} ammonium acetate (CH_3COONH_4) was added to the sample at a final concentration of 1 mmol L^{-1} ferrozine (Viollier et al. 2000). After waiting 2 min. to allow the magenta Fe(II)-ferrozine complex to form, the sample was transferred to an acid-cleaned 10 cm Quartz cuvette and the absorbance recorded at 562 nm with an Ocean Optics spectrometer (USB2000) and light source (DT-MINI-2-GS). This initial absorbance measurement represents an estimate of the labile Fe(II) concentration. In a second step, the reductant sodium sulfite (Na_2SO_3), prepared daily in MQ at a concentration of 0.01 mol L^{-1} (O'Sullivan et al. 1995), was added to the sample to a final concentration of 0.6 mmol L^{-1}, and the absorbance was measured again. The second measurement yields

an estimate of the total dissolved Fe concentration in the sample and is suitable for calibration with an Fe(III) standard.

Consistency of calibrations was confirmed with both Fe(II) and Fe(III) standards in Argon-purged MQ, and complete reduction of Fe(III) in the standard was achieved in less than 10 min. For calibrations with Fe(II), a 10 mmol L^{-1} standard of ammonium iron(II) sulfate hexahydrate was prepared in 0.1 mol L^{-1} Seastar® Baseline hydrochloric acid (HCl) and considered stable in the refrigerator for up to a month. Calibrations with Fe(III) were performed using a Fluka certified iron standard for AAS (TraceCERT) of 1000 mg L^{-1} diluted with 0.1 mol L^{-1} Seastar® HCl.

In order to test whether organic complexation of Fe plays a role in LVBr, a subsample of the brine (1:600 dilution) was subjected to UV oxidation as follows. In a Teflon® cup with a quartz window, ~ 50 μL brine were added to 30 mL Seastar® HCl (0.01 mol L^{-1}). Acidification was deemed necessary to prevent precipitation of Fe(III) after UV oxidation. The solution was UV oxidized for 2 h in a UVO-Cleaner (Jelight, model no. 144AX) and buffered to pH ~ 4.3 with 40 μL concentrated ammonium hydroxide (NH_4OH) before analysis by ferrozine spectrophotometry as described above. Weighing of the solution before and after UV oxidation confirmed that there was no loss due to evaporation.

Fe isotope analysis

Sample preparation for stable isotope analyses of Fe was carried out in a metal-free clean room facility supplied by HEPA-filtered air. All lab ware was pre-cleaned in dilute acid baths using ultra pure acids (Seastar® Baseline) and rinsed with 18 MΩ MQ prior to use. For analysis of the mineral samples, appropriate amounts of sample material were weighed into cone-shaped Savillex® Teflon beakers. A combination of concentrated ultra high purity acids (Seastar®) ($HF + HNO_3$, $HCl + HNO_3$ and HCl) and HCl-only leaching were used for acid digestion of the mineral samples (Table S1). Brine samples (both whole and 0.2 μm filtered) were directly loaded onto the ion exchange columns. Analysis of the same samples digested with concentrated HCl only showed δ^{56}Fe values about 0.1‰ higher than the HF-digested samples (Table S1).

Samples were purified by anion exchange chromatography using EiChrom AG 1-X 100–200 mesh

resin. The resin was cleaned with 5 mL 5 M HNO_3 and 2 mL of MQ and preconditioned with 4 mL 6 M HCl before loading the sample. Matrix elements were then removed from the column with 6 mL 6 M HCl before Fe was eluted from the column with 2 mL of MQ and 5 mL 5 M HNO_3. The sample was then dried on a hot plate overnight and re-dissolved in 100 μL of concentrated nitric acid to remove organic and chloride complexes. The nitric acid was then heated to 100 °C and dried once again before re-dissolved in 3% nitric acid for measurement by multi collector inductively coupled plasma mass spectrometer (MC-ICP-MS, Thermo Scientific, Neptune). Sample recovery from this procedure is greater than 99%, as determined by isotope dilution, which ensures no fractionation occurs during sample preparation. Ion exchange blanks were typically 15–20 ng representing a 0.7% contribution to the total amount of Fe in the sample. This would result in at most a 0.02‰ uncertainty of reported $\delta^{56}Fe$ values. Iron isotope ratios are reported in the standard δ notation in per mil (‰): $\delta^{56}Fe = [(^{56}Fe/^{54}Fe_{sample})/(^{56}Fe/^{54}Fe_{standard}) - 1] \times 1000$. Precision of $\delta^{56}Fe$ values was 0.09‰.

$\delta^{18}O$–SO_4^{2-} Isotope analysis

The pretreatment procedure for oxygen isotope analysis of barites has been previously described in detail (Bao et al. 2008). In brief, the initial acid-treated sulfate precipitates were further processed with a DDARP method (Bao 2006) and a 2 M NaOH solution (Bao et al. 2007) to precipitate pure barite ($BaSO_4$). The $\delta^{18}O$ of barite was measured using CO gas converted from $BaSO_4$ using a thermal conversion elemental analyzer (TCEA) at 1450 °C coupled with the MAT253 isotope ratio mass spectrometer in a continuous-flow mode at Louisiana State University OASIC Lab. The standard deviation (σ) associated with $\delta^{18}O_{SO_4}$ is ±0.5‰. Here the $\delta = (R_{sample}/R_{standard} - 1) \times 1000$, in which R is the ratio of $^{18}O/^{16}O$, $^{17}O/^{16}O$, or $^{34}S/^{32}S$, in which the $\delta' \equiv \ln(R_{sample}/R_{VSMOW})$ (Assonov and Brenninkmeijer 2005).

Results and discussion

In situ voltammetric measurements in the Lake Vida borehole showed neither vertical chemical stratification of the brine in the borehole nor any change in brine fluid chemistry during discrete sample collection (Fig. 1a). The voltammetry verified that the brine was anoxic (oxygen MDL <1 μM, Fig. 1a), but interestingly, no detectable reduced sulfur species (HS^-/H_2S, FeS(aq), $S_{(0)}$, S_X^{-2}, $S_2O_3^{2-}$, $S_4O_6^{2-}$). A significant and reproducible current peak at -0.15 V indicated the presence of aqueous Fe(III) species (Fig. 1a), while no free Fe(II) was observed. This technique has a Fe(II) detection limit of 10 μmol L^{-1}. For comparison, in situ profiles were also collected at Lake Hoare, two valleys ∼35 km to the south of Lake Vida, using the same voltammetry method. This lake represents a weakly stratified freshwater system with strong vertical changes in redox conditions near the bottom (Green and Lyons 2009). At Lake Hoare, O_2 was detected between lake surface and 26 m depth. At 29 m depth, no oxygen, but trace sulfide was detected. At 31 m depth, Fe(II), Mn(II), FeS_{aq}, and free sulfide were all detected (Fig. 1b). The comparison between the two lakes demonstrates the contrasting redox profile for Lake Vida, which lacks the presence of freely-available reduced species despite the absence of O_2.

Voltammetry measures electro-active aqueous chemical species (i.e., not particulates). Dissolved aqueous organic Fe(III) species are qualitatively detectable using this method, but the variety of potential organic complexes in natural fluids and variability in their electro-activity makes quantification of Fe(III) difficult because of a lack of calibration standards, i.e., the quantification of in situ uncharacterized Fe(III)-organically complexed species is impossible (Jones et al. 2011). However, the in situ voltammetric data reveal that the dominant species of Fe present in the brine is not free Fe(II), and suggest that aqueous Fe(III) is present.

Independently, laboratory ferrozine analysis on discrete LVBr samples also showed that Fe(III) was the dominating Fe species. Two experiments were conducted with diluted (1:300) LVBr: In the first experiment, the reductant was added at t = 0 min and in the second experiment at t = 60 min. Initial Fe(II) readings were low (∼0.034 μmol L^{-1}) and consistent between both experiments (Fig. 1c). This translates to a total of about 10 μmol L^{-1} Fe(II) for LVBr, representing <4% Fe(II) of the total iron. This is at the detection limit of the voltammetry method and therefore consistent with the
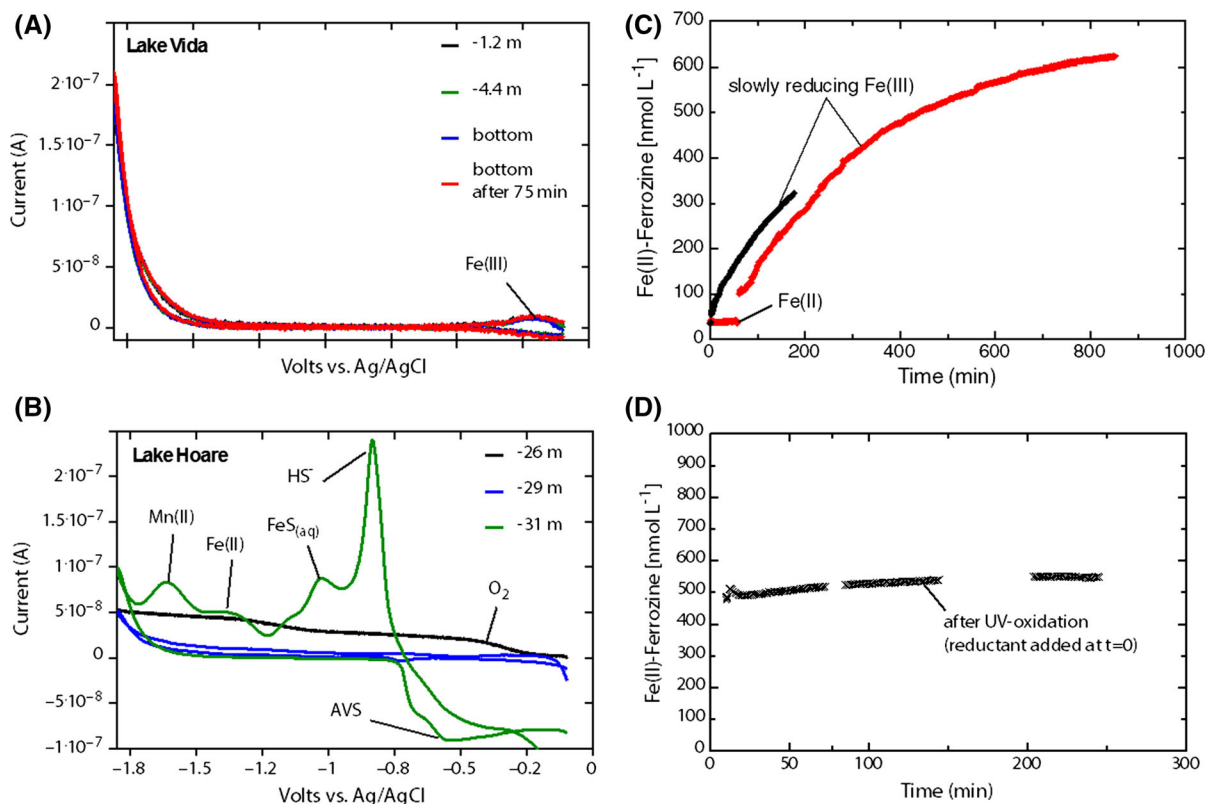
Fig. 1 Iron speciation in Lake Vida brine. Representative in situ cyclic voltammogram scans at 500 Mv s^{-1} in Lake Vida brine (LVBr *panel A*) and Lake Hoare (*panel B* for comparison). Voltage scanning was from -0.1 to -1.8 V, back to -0.1 V.

Panel C evolution of two Fe redox speciation experiments in diluted (1:300) LVBr with ferrozine, with reductant added at time $= 0$ min (*black*) and 60 min (*red*). *Panel D* UV-oxidized LVBr (1:600 dilution), with reductant added at time $= 0$ min
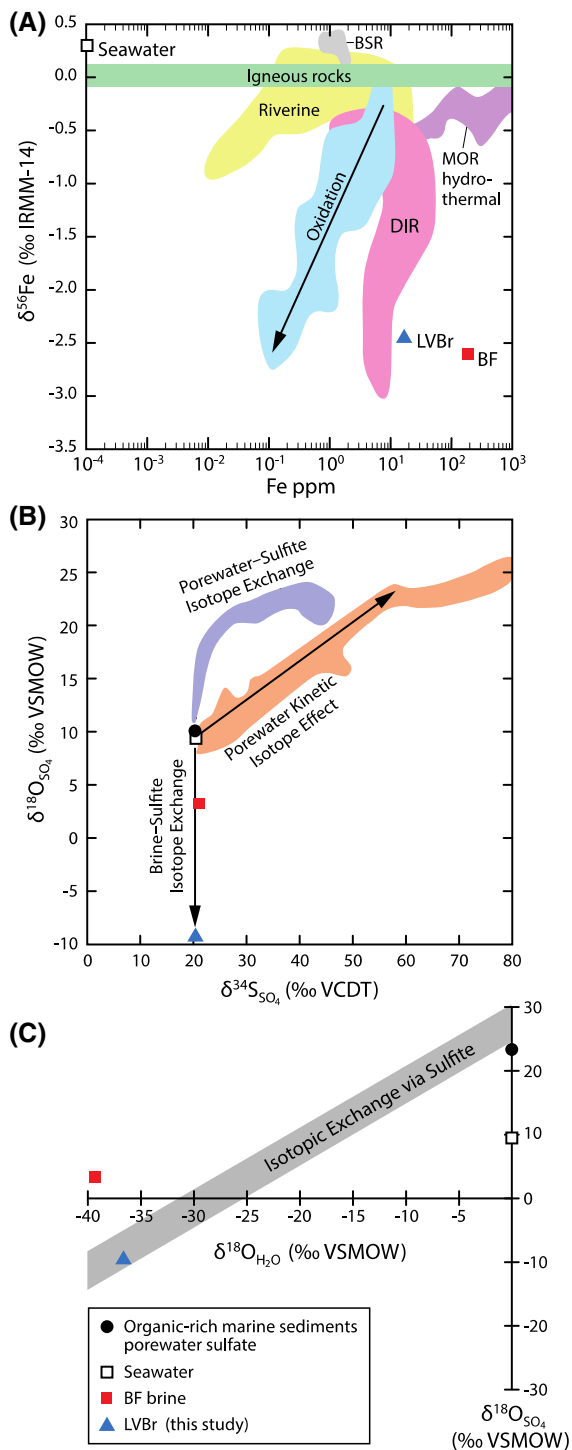
in situ data. After the addition of the reductant, the ferrozine method reveals slowly reducible Fe(III) approaching ~ 0.650 µmol L^{-1}, which accounts for the remainder of iron in LVBr (Fig. 1c).

In a separate laboratory experiment, LVBr was diluted (1:600) and UV-oxidized at low pH, a procedure known to destroy organic material (Gledhill and van den Berg 1994). Approximately 90% of the Fe present in the sample was reduced within 10 min of addition of the reductant (Fig. 1d), with the remainder being reduced extremely slowly (Fig. 1d). We therefore conclude that organic ligands likely complex the majority of the Fe(III) present in the Lake Vida brine. This explains the possibility of Fe(III) presence well above the solubility limit for Fe(III) in a system with high ionic strength such as LVBr (Liu and Millero 2002) and is consistent with high levels of high molecular weight dissolved organic matter in the LVBr (Cawley et al. 2016). For example, organic complexation by uncharacterized extracellular

polymeric substances (EPS), has been invoked as an explanation for this excess Fe(III) to remain in solution, much as is the case in seawater (Gledhill and van den Berg 1994; Liu and Millero 2002). Likewise, EPS can serve as a cryoprotectant in subzero brines (Marx et al. 2009). EPS were observed in scanning electron micrographs and high levels of carbohydrate were determined in LVBr (Murray et al. 2012). Also, the slow reaction (>500 min.) with the reductant [i.e., orders of magnitude slower than what would be expected based on theoretical calculations for uncomplexed Fe(III) (Millero et al. 1995)] can be explained by the presence of strong organic Fe(III) ligands, inhibiting the reaction. For comparison, the same experiment was carried out with inorganic Fe(III) standards with full Fe(III) reduction completed within 2 min (data not shown).

Iron isotope analysis revealed that all brine samples were extremely depleted in ^{56}Fe compared to δ^{56}Fe values typically reported in nature (Fig. 2a). The Fe

(−2.6 ± 0.5‰) (Mikucki et al. 2009). DIR, the microbial reduction of Fe(III) oxides and hydroxides, is associated with Fe isotope fractionation, that was hypothesized to be occurring in Blood Falls (Mikucki et al. 2009). However, this would result in Fe(II) as the dominant species, which is the case for Blood Falls, but not for Lake Vida.

The $\delta^{56}Fe$ values of Lake Vida sediment obtained from the ice core and Victoria Valley mineral samples (for locations, see Fig. S1) were up to ∼2.8‰ different than those of LVBr. The $\delta^{56}Fe$ values of mineral samples were nearly identical (averaging 0.20 ± 0.02‰; n = 5) (Table S1). These values are typical for the isotopic composition of dolerite (Craddock and Dauphas 2010). Like the majority of igneous and metamorphic rocks, the Ferrar Dolerite of the MDV contains most of its Fe in the form of Fe(II) (as FeO) (Zavala et al. 2011; Ojha et al. 2015). The low $\delta^{56}Fe$ values in LVBr compared to the parent material demonstrate that the brine has undergone significant isotope fractionation, that could be the result of several processes: (i) DIR is associated with Fe isotope fractionation, and $\delta^{56}Fe$ values of dissolved reduced Fe(II) have been reported to be up to 3‰ lower than the parent substrate (Beard et al. 1999; Crosby et al. 2005). This process implies that the source of Fe in LVBr was Fe(III), potentially a weathered product of

isotopic signature of LVBr is −2.45 ± −0.16‰ for the filtered (n = 3) and −2.47 ± 0.19‰ for the total brine (n = 3). These values are very similar to $\delta^{56}Fe$ values of the subglacial brine of Blood Falls

the Ferrar Dolerite rather than the rock itself (Fe(II)). The resulting Fe(II) from DIR is consequently re-oxidized via chemodenitrification, leaving Fe(III) depleted in ^{56}Fe. This step assumes that chemodenitrification would not be associated with significant iron isotope fractionation, which is currently unknown. (ii) Abiotic oxidation of Fe(II) to Fe(III) may cause formation of isotopically-enriched ferrihydrite (Fe_2O_3), leaving the Fe(II) in solution up to 3‰ depleted in ^{56}Fe (Bullen et al. 2001). Although abiotic oxidation has the potential to lead to the low δ^{56}Fe values observed in LVBr, brine anoxia, lack of mineral precipitate such as iron oxides and high concentrations of Fe(III) in solution suggest that this process is unlikely responsible for the Fe isotopic signature in LVBr. (iii) In laboratory soil extraction experiments, ligand-mediated dissolution of Fe(II)-bearing minerals has been shown to result in ^{56}Fe depletion in the dissolved phase (Brantley et al. 2004), suggesting that organic molecules remove isotopically light iron from incongruently dissolving silicates in Earth's surface environments. This process of Fe isotope fractionation has yet to be demonstrated in natural systems, including sub-zero environments, but may explain the low δ^{56}Fe values associated with organically-complexed Fe(III) as observed here. To date, it appears that δ^{56}Fe values as observed in LVBr have only been observed in nature for Fe(II) resulting from DIR. We therefore conclude that LVBr δ^{56}Fe values are a new outlier for Fe(III) found in nature.

In order to investigate the possibility of microbial sulfur reactions in LVBr and possible interactions with the iron cycling, we also determined the oxygen isotopic composition of sulfate (δ^{18}O–SO_4^{2-}), -9.7 ± 0.5‰. Although the LVBr δ^{34}S–SO_4^{2-} values ($+20.3 \pm 0.1$‰) (Murray et al. 2012) alone did not provide evidence for microbial processing, δ^{18}O–SO_4^{2-} values in LVBr were $\sim$20‰ depleted relative to modern marine barites (Stevens et al. 2015) suggesting microbial sulfur cycling (Fig. 2b) that does not result in a sulfidic system. This compares to a δ^{18}O–SO_4^{2-} value of $+3.3$‰ for the subglacial brine of Blood Falls (Mikucki et al. 2009), indicating that there was even a greater degree of oxygen isotope exchange between SO_3^{2-} and H_2O [δ^{18}O–$H_2O = -36.7 \pm 2.3$‰ (Murray et al. 2012)] in LVBr compared to Blood Falls brine (Fig. 2c). The sulfur cycling in Lake Vida between $SO_4^{2-} \Leftrightarrow SO_3^{2-}$ could be microbially-mediated as proposed for Blood Falls, where DIR was proposed to be coupled to the sulfur oxidation.

This is further supported by the presence of epsilonproteobacteria related to sulfur-oxidizers (*Sulfurovum* sp.) and sulfate-reducing bacteria detected by 16S rRNA gene sequencing in LVBr (Murray et al. 2012). Although no reduced sulfur intermediates were detected by in situ voltammetric measurements, abiotic oxygen isotope exchange with water itself is not a very likely explanation for the δ^{18}O–SO_4^{2-} values observed here. Under LVBr conditions (temperature and pH), sulfate-water equilibration would take on the order of 10's of millions of years to generate observed δ^{18}O–SO_4^{2-} values, which does not agree with current LVBr age estimates of 2800 ^{14}C years (Doran et al. 2003; Murray et al. 2012; Cawley et al. 2016).

High concentrations of NO_2^- (23.7 ± 1.0 μmol L^{-1}) and NO_3^- (904.4 ± 30.0 μmol L^{-1}) in LVBr strongly support abiotic Fe oxidation (chemodenitrification), which results in N_2O production (58.8 μmol L^{-1}) (Murray et al. 2012; Ostrom et al. 2016). This process also has been suggested to drive high N_2O concentrations measured in other MDV locations, such as in the soil pore space near Don Juan Pond (Wright Valley, Southern Victoria Land, Antarctica) and constitutes a newly recognized abiotic mechanism for the production of the greenhouse gas N_2O (Samarkin et al. 2010; Peters et al. 2014; Jones et al. 2015). The dominance of Fe(III) over Fe(II) found in LVBr is consistent with chemodenitrification. Iron oxidation also can be biologically mediated, possibly via reactions with Lake Vida microbes. Organisms capable of NO_3^- reduction in LVBr (Murray et al. 2012) could be a site for biological or abiotic Fe oxidation as shown for *Paracoccus denitrificans* (Klueglein et al. 2014), resulting in cell surface encrustation. Indeed, Fe capsular structures have been observed in LVBr microbes (Kuhn et al. 2014).

A parsimonious interpretation of our data leads us to postulate that this system is the result of coupled N–Fe–S reactions (Fig. 3). In such a scheme, Fe could be reduced in a coupled, catalytic reaction with sulfur oxidation and oxidized via chemodenitrification. The catalytic sulfur cycle proposed for Blood Falls (Mikucki et al. 2009) suggests that DOC pools are oxidized and Fe(II) accumulates via SO_4^{2-} cycling to sulfur intermediates (SO_3^{2-}, $S_2O_3^{2-}$, S^0), which are then re-oxidized back to SO_4^{2-}. This catalytic sulfur pathway could provide a source of reduced Fe to LVBr, a source needed for chemodenitrification proposed above. Lake Vida differs from Blood Falls in the abundance of oxidized N-species, in addition to
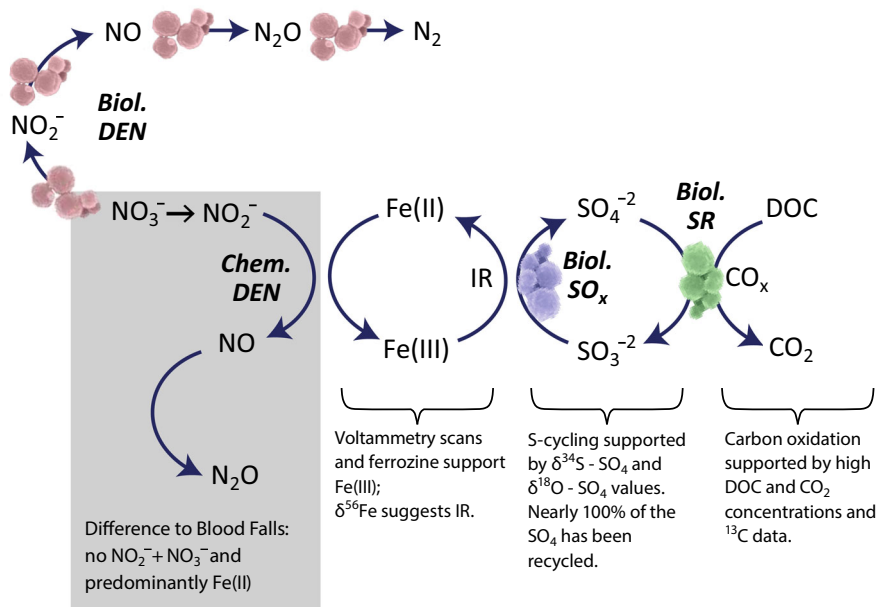
Fig. 3 Proposed mechanism for coupled model of chemodenitrification (*Chem. DEN*) as well as Fe and S redox pathways in Lake Vida brine. Abiotic processes may drive coupled Fe oxidation (chemodenitrification) and Fe reduction (IR) cycling in Lake Vida. Biological (*Biol.*) pathways including denitrification (DEN), dissimilatory IR, SO_4^{2-} reduction (SR), and S oxidation (SO_x) potentially coupled to Fe reduction (as proposed for Blood Falls) also may occur at very low rates and interact with the abiotic pathways. Heterotrophic respiration may be coupled to the Biol. SR and Biol. DEN pathways while S oxidation may be carried out chemo-autotrophically

much higher levels of organic carbon. Furthermore, DIR, as implicated by $\delta^{56}Fe$ values, may also be responsible for providing Fe(II) for chemodenitrification. For example, LVBr *Marinobacter* spp., which have been cultivated from LVBR and are well represented in LVBR 16S rRNA gene clone libraries (Murray et al. 2012), harbor a putative Fe reductase (e.g. *Marinobacter* sp. LVR2A5A20, locus Ga0070251_2837).

Although the reaction kinetics at −13 °C and drivers of the Fe speciation are not yet fully understood, the high abundance of oxidized species in the anoxic Lake Vida brine is provocative, providing a rare opportunity to study the geochemical interactions of biology and abiotic processes in a cryo-ecosystem. Our results suggest that this tightly coupled N–Fe–S cycle is sustainable in hypersaline, sub-zero environments—offering a habitable ice-covered refuge for life during the Cryogenian Period (Snowball Earth glacial episodes) and that may exist on other frozen planets in the present solar system.

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