Microbial life at −13 °C in the brine of an ice-sealed Antarctic lake

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The permanent ice cover of Lake Vida (Antarctica) encapsulates an extreme cryogenic brine ecosystem (−13 °C; salinity, 200). This aphotic ecosystem is anoxic and consists of a slightly acidic (pH 6.2) sodium chloride-dominated brine. Expeditions in 2005 and 2010 were conducted to investigate the biogeochemistry of Lake Vida's brine system. A phylogenetically diverse and metabolically active *Bacteria* dominated microbial assemblage was observed in the brine. These bacteria live under very high levels of reduced metals, ammonia, molecular hydrogen (H_2), and dissolved organic carbon, as well as high concentrations of oxidized species of nitrogen (i.e., supersaturated nitrous oxide and ∼1 mmol·L^{-1} nitrate) and sulfur (as sulfate). The existence of this system, with active biota, and a suite of reduced as well as oxidized compounds, is unusual given the millennial scale of its isolation from external sources of energy. The geochemistry of the brine suggests that abiotic brine-rock reactions may occur in this system and that the rich sources of dissolved electron acceptors prevent sulfate reduction and methanogenesis from being energetically favorable. The discovery of this ecosystem and the in situ biotic and abiotic processes occurring at low temperature provides a tractable system to study habitability of isolated terrestrial cryoenvironments (e.g., permafrost cryopegs and subglacial ecosystems), and is a potential analog for habitats on other icy worlds where water-rock reactions may cooccur with saline deposits and subsurface oceans.

astrobiology | geomicrobiology | microbial ecology | extreme environment

The observation of microbes surviving and growing in a variety of icy systems on Earth has expanded our understanding of how life pervades, functions, and persists under challenging conditions (e.g., refs. 1–3). Studies of the physical characteristics, the geochemical properties, and microbes in ice (triple point junctions, brine channels, gas bubbles) have also changed our perceptions of the environments that may contain traces of, or even sustain, life beyond Earth [e.g., Mars (4), Europa (5), and Enceladus (6)].

Solute depression of ice crystal formation or solar radiation melting of water ice are key processes that provide liquid water—the key solvent that makes life possible—within icy systems. Microbial communities in these conditions are often sustained by a supply of energy that ultimately derives from photosynthesis (present or past). The understanding of ecosystems based on energy sources other than the Sun comes mainly from realms where hydrothermal processes have provided reduced compounds necessary to fuel chemosynthetically driven ecosystems. Methane derived from thermogenic or biogenic sources can also support microbial communities in deep sea (7) and high arctic cold saline seeps (8). More recently, discoveries of life and associated processes in deep terrestrial subsurface ecosystems (9) provide compelling evidence of subsurface life that in some cases is fueled by nonphotosynthetic processes. Our knowledge of geochemical and microbial processes in aphotic icy environments remains mostly unknown, however, especially at subzero temperatures.

Lake Vida is located in Victoria Valley, the northern most of the McMurdo Dry Valleys of East Antarctica (Fig. S1). Initial studies of Lake Vida's thick ice cover described a −11.6 °C, wet, saline (estimated 245, practical salinity scale) ice at 15.8 m (10). This brine has been isolated by the thick lake ice cover and underlying 800–970 m of permafrost (11, 12), prohibiting input of ground water or of annual glacial melt and associated nutrients. ^{14}C-dating of organic matter sampled at 12 m in the lake ice cover suggests that the brine has been isolated for more than 2,800 y (10). The Lake Vida brine represents a cryoecosystem that is a suitable, accessible analog for glacial and subglacial systems, including soils, sediments, wetlands, and lakes underlying the Antarctic ice sheet, some of which may harbor saline waters at depth (13), and for the icy worlds of our solar system. The goal of the present study is to examine the inorganic and organic geochemistry as well as the biology of the brine and determine the capacity of this sealed, aphotic cryoecosystem for harboring and sustaining microbial life.

Results and Discussion

During coring of the lake ice in 2005, brine infiltrated the borehole 16.0 m below the surface of the ice. The brine then rose in the borehole to 10.5 m below the ice surface (14), indicating that the thick ice cover of the lake is at least partially grounded. The brine consistently returned to the same level in the borehole following sample collections with submersible pumping, indicating connection with an extensive brine network in the lake ice. In 2010, the brine hydrologic system behaved similarly. During this second expedition, we retrieved a 27-m ice-core that was interlaced with layers of sediments and briny ice below 21 m. The bottom depth of the lake is currently unknown, although ice was present at the bottom of the core, suggesting that the lake bottom may be > 27-m deep. A second ice-core to 20 m was retrieved at the same location and brine was collected as in 2005 for geochemical and microbiological analysis (14).

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Brine Geochemistry. Lake Vida brine (LVBr) is anoxic, slightly acidic (pH 6.2), and turns from light yellow to dark orange upon exposure to the atmosphere as a result of ferric iron precipitation. NaCl is the dominant salt (salinity of 176–200 based on refractive index; ion concentrations) (Table 1 and Table S1) with a water activity of 0.87, which limits life in LVBr to moderately halophilic organisms (15). LVBr geochemistry was very similar in 2005 and 2010, revealing that the distinctive composition of the brine is quite stable with respect to time (Table 1 and Table S1). LVBr has high levels of reduced and oxidized forms of inorganic nitrogen (ammonia, 3,600–3,885 μmol·L^{-1}; nitrate, 904–1,120 μmol·L^{-1}; nitrite, 23.7–27.8 μmol·L^{-1}; ranges provided cover data for both 2005 and 2010 samples). High levels of inorganic nitrogen ions in the system are presumably derived from initial atmospheric precipitation on dry valley soils (16). Subsequent glacial processes and weathering cycles likely resulted in the introduction of these N salts into Lake Vida during earlier ice-free stages of the lake. Dissolved inorganic carbon (DIC) levels are also high (61.2–72.3 mmol·L^{-1}) relative to lakes and oceans but similar to levels reported from the Taylor Valley, where the Blood Falls surface outflow of deep subsurface brine presents under Taylor Glacier as it enters the west end of Lake Bonney (17) (Fig. S1 and Table S1).

LVBr also contains high levels of dissolved gasses. Nitrous oxide is supersaturated (58.8–86.6 μmol·L^{-1}, 2005 and 2010, respectively) and stands above all values reported in other lakes of the dry valleys (Table S1) (18). A substantial amount of H_2 (10.5 μmol·L^{-1}) was detected in 2010 LVBr, the source of which is discussed below. Additionally, the carbon dioxide levels were also high (8.9 mmol·L^{-1}), in line with the high DIC levels.

Stable isotope data provide insight into the origins of dissolved species and may reflect the extent of microbial activity. The δ^{13}C-DIC values ranged between 1.44‰ and 2.68‰, suggesting a predominantly inorganic origin, although lack of pre-encapsulation data and unknown rates of internal DIC to dissolved organic carbon (DOC) cycling limits interpretation of these values. Although sulfate levels are higher than those of other dry valley lakes (58–66 mmol·L^{-1}) (Table S1), no other sulfur intermediates (e.g., S_2O_3) or sulfides (mono- or polysulfide in particulate or dissolved forms) were detected. The sulfate δ^{34}S value, indistinguishable from that of modern seawater (Table 1) (19), reveals that sulfate did not undergo any significant microbial processing (e.g., no significant bacterial sulfate reduction or sulfur disproportionation that would have imparted kinetic isotope effects). The isotopic compositions of NO_3^-, NH_4^+, and N_2 (δ^{15}N = 0.3‰) are all consistent with an atmospheric origin (Table 1) (20). The bulk δ^{15}N and site preference values for N_2O (Table 1) are consistent with an origin from microbial denitrification or an inorganic origin. An inorganic origin by chemodenitrification is likely, because soils surrounding nearby Don Juan Pond have been shown to produce N_2O by this process with similar but variable δ^{15}N and site preference values of −45.4‰ to −34.5‰ and −45.2‰ to 4.1‰, respectively (21). The δ^2H of H_2 in the brine is similar to expected values for production from radiolysis and microbial hydrogenase activity (−692‰ and −793‰, respectively) based on the isotopic composition of the water (Table 1) and fractionation factors for these processes (22, 23). Microbial H_2 consumption has been shown to catalyze nonproductive H_2-water exchange and drive the δ^2H of H_2 toward isotopic equilibrium with water on a monthly time scale (24). The expected δ^2H value for H_2 in equilibrium with Lake Vida water at −13.4 °C is −850‰ (25), which indicates isotopic disequilibrium. Isotopic equilibrium between H_2 and water is expected, however, to take between 1,000 and 10,000 y (22), perhaps longer at the temperature of Lake Vida. Consequently, we conclude that H_2 in Lake Vida likely has an inorganic origin, radiolysis or serpentinization, and that microbial H_2 consumption and the passage of time have not been sufficient for the H_2 in Lake Vida to reach isotopic equilibrium. Thus, the isotopic composition of DIC, sulfate, N_2O, and H_2 provide little to no indication of alteration by microbial processes and are largely consistent with inorganic origins.

Table 1. Geochemical characteristics of Lake Vida brine collected in 2005

Parameter	Value
Physical and major ions	
Temp (°C)	−13.4
pH	6.2
Salinity (psu)	188.0
Ca^{2+} (mmol·L^{-1})	30.1 ± 1.2
Cl^- (mmol·L^{-1})	3,318 ± 112
F^- (mmol·L^{-1})	1.5 ± 0.1
K^+ (mmol·L^{-1})	82.8 ± 2.8
Mg^{2+} (mmol·L^{-1})	664.9 ± 22.5
Na^+ (mmol·L^{-1})	1,914 ± 60
SO_4^{2-} (mmol·L^{-1})	58.4 ± 2.3
Gasses (μmol·L^{-1})	
N_2O	58.8
CO_2	8,860 ± 190
H_2	1,0.47 ± 0.02*
MeSH	0.2
DMS	0.1
DMSO	25.0
H_2S	ND
CH_4	<1.0
Oxygen	Anoxic
Carbon and nutrients	
DIC (mmol·L^{-1})	61.2 ± 0.6
DOC (mmol·L^{-1})	48.2 ± 9.7
NH_4^+-N (μmol·L^{-1})	3,885.2 ± 43.0
NO_2^--N (μmol·L^{-1})	23.7 ± 1.0
NO_3^--N (μmol·L^{-1})	904.4 ± 30.0
PO_4^--P (μmol·L^{-1})	5.0 ± 0.2
Metals (μmol·L^{-1})	
Al	10.2 ± 10.8
As	0.9 ± 0.1
Ba	0.5 ± 0.03
Cd	ND
Cr	0.8 ± 0.4
Cu	0.5 ± 0.04
Fe	307.9 ± 22.6
Mn	81.9 ± 3.7
Mo	0.4 ± 0.12
Ni	1.3 ± 0.3
Pb	0.9 ± 0.4
Sr	447.5 ± 17.8
U	0.6 ± 0.04
Zn	10.4 ± 8.0
Stable isotopes	
DIC δ^{13}C ‰	2.7 ± 0.1
DOC δ^{13}C ‰	−14.6 - -19.5
SO_4^{2-} δ^{34}S ‰	20.3 ± 0.1*
NO_3^- δ^{15}N ‰	−7.9 ± 0.2*
NO_3^- δ^{18}O ‰	31.7 ± 0.3*
NH_4^+ δ^{15}N ‰	−4.8 ± 0.1*
N_2 δ^{15}N ‰	−0.3 ± 0.3*
N_2O δ^{15}N ‰	−22.2 ± 0.1*
N_2O δ^{18}O ‰	2.97 ± 0.1*
N_2O SP	−3.64 ± 0.3*
H_2 δ^2H ‰	−704 ± 12*
H_2O δ^2H ‰	−240 ± 20*
H_2O δ^{18}O ‰	−36.7 ± 2.3*

The accompanying data collected in 2010 in addition to results of additional analyses can be found in Tables S1 and S2. Site preference (SP) is the difference in the δ^{15}N values between the central and outer N atoms in N_2O reported in per mil units.
*Data collected in 2010.

LVBr contains very high concentrations of iron (256–308 μmol·L^{-1}) that are similar for the total and dissolved fractions (passing < 0.2-μm filter), suggesting that the majority of elements are in the dissolved state (Table S1). The metals, iron in particular, most likely originate from weathering of the pyroxene-rich dolerites dominating the landscape around Lake Vida (discussed below) (Figs. S2 and S3).

The DOC (48.3–64.7 mmol·L^{-1} C) levels were very high in LVBr, in which carbohydrates comprised a significant fraction (~7%) of the 2010 LVBr DOC (9.56 ± 0.81 mmol·L^{-1} C; $n = 6$). Small amounts of low molecular weight organic compounds (0–50 nmol·L^{-1} CH_4, and 2.7 nmol·L^{-1} and 5.2 nmol·L^{-1} ethane and ethene, respectively) support potential for biological carbon processing in the system. Fluorescence spectroscopy of dissolved organic material (DOM) fractions reveal that the DOM is microbially derived: the level of the fluorescence index values of 1.73–1.79 are at the upper end of the full range in potential fluorescence index values from 1.2 to 1.8, corresponding to predominantly microbial sources compared with sources that include plant degradation products. These values are similar to those for DOM in other microbe-dominated Antarctic lakes (Table S2) (e.g., ref. 26). The $\delta^{13}C$ of the high molecular weight DOC fractions (~50% of the DOC) averaged −15.0‰ (>1 kDa), −18.6‰ (fulvic acid, LV-FA), and −17.4‰ (transphilic acid, LV-TPIA), and are consistent with autotrophic production (Table S2). The >1-kDa fraction has radiocarbon ages 2,955 ± 15 and 3,585 ± 20 y BP, somewhat older than the algal mat in the ice 12.8 m below the surface of 2,800 y BP (10), confirming the millennial scale of the age of encapsulation of the brine. The radiocarbon ages of the potentially more microbially recalcitrant DOM fractions are older, with LV-FA and LV-TPIA fractions (10% and ~8% of the DOM, respectively) having similar radiocarbon ages at ~4,000 ^{14}C y BP, suggesting that the > 1-kDa fraction may include a greater proportion of younger DOM produced in the brine (Table S2).

Microbial Abundance, Activity, and Diversity. Microscopic observation using fluorescent nucleic acid targeted stains (DAPI and SYBRGold) revealed stained particles in the size class of typical aquatic bacteria (>0.2 to 1.0-μm diameter) at a cell density of 0.1–0.6 × 10^6 mL^{-1} and a second class of particles (≤ 0.2-μm diameter) that are almost two orders-of-magnitude more abundant (~49–60 × 10^6 mL^{-1}). Scanning electron micrographs showed doublets of particles in both size classes (potentially dividing cells) and many cell clusters (two or more) connected with strands of an uncharacterized extrapolymeric substance (EPS), particularly for the small-size class (Fig. 1). EPS production, a cellular response to low water activity and temperature (27), could be a significant contributor to the accumulated DOC (the carbohydrate fraction in particular) in LVBr, where DOC concentrations are 20-fold greater than in the bottom waters of other lakes of the dry valleys.

Assays for ^3H-leucine incorporation indicate that the bacterial assemblage synthesizes proteins under anoxic conditions at −12 to −13.4 °C (for the 2005 and 2010 experiments, respectively) (Table 2) at very low rates. Protein synthesis is enhanced three- to fivefold at 0 °C, and slightly depressed—yet still significantly above killed controls—in the presence of an aerobic headspace (at 0 and −12 or −13.4 °C).

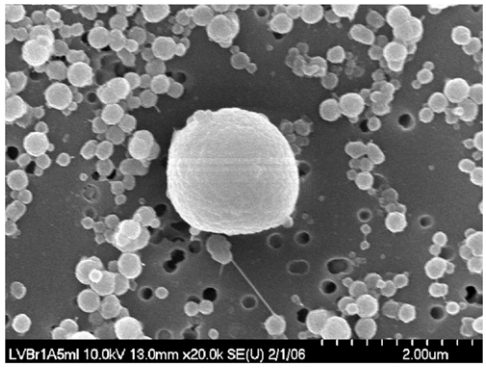

Fig. 1. Lake Vida brine micrograph. Scanning electron micrograph with ~1.0-mm diameter cell, and small particles (~0.2 μm in diameter). Note that the pore size of the 25-mm diameter membrane is 0.2 μm; and that 5 mL of brine was filtered onto the membrane.

Small subunit (SSU) rRNA gene surveys conducted with the 2005 LVBr revealed the presence of 32 unique sequences (of 154 total; 0.01 distance) distributed across eight bacterial phyla (*Proteobacteria*, *Lentisphaera*, *Firmicutes*, *Spirochaeta*, *Bacteroidetes*, *Verrucomicrobia*, TM7, and *Actinobacteria*) (Fig. 2). Several phyla were not previously observed in high salinity systems (e.g., the *Epsilonproteobacteria*, *Verrucomicrobia*, TM7, and the deep-branching *Lentisphaera* group) (28). In addition, surveys of complementary SSU rRNA (crRNA) led to the detection of rRNA, from which biological activity can be inferred for 8 of these 32 LVBr sequences (Figs. S4 and S5). *Psychrobacter* sp. and *Marinobacter* sp. affiliated *Gammaproteobacterial* SSU rRNA gene sequences dominate the environmental gDNA-derived SSU rRNA gene clone library (39%) and crRNA results (Fig. 2, and Figs. S5 and S6). Several strains of these two *Gammaproteobacteria* species were cultivated under aerobic isolation and anaerobic enrichment (N_2 atm) in our laboratory as well as under aerobic isolation in a separate laboratory (Fig. S5) (29). Similarly, *Epsilonproteobacteria*, which form the second-most abundant subgroup detected in the *Proteobacteria* phylum (16%), also have strong crRNA signals in the denaturing gradient gel-electrophoresis profile (Fig. S4). Environmental representatives of this class, best known from deep sea habitats, grow heterotrophically, chemolithoheterotrophically, and chemolithoautotrophically using both Calvin-Benson cycle and reductive tricarboxylic acid (TCA) cycle-based CO_2 fixation coupled to sulfur oxidation (30). The closest cultivated relative (i.e., *Sulfurovum* sp.; 95% sequence

Table 2. ^3H-leucine incorporation (into protein as DPM; averages ± SD) was determined for LVBr sampled in 2005 (LVBR; 0 and −12°C) and 2010 (LVH2b; 0 and −13.5°C)

Year	Experiment	N_2, 0 °C	Air, 0 °C	N_2, −12 and −13.5 °C	Air, −12 °C
2005	LVBR1 30 d	2,262 ± 227**	1,513 ± 209**	502 ± 23**	338 ± 111
	LVBR2 10 d	987 ± 102*	664 ± 33	NA	NA
	LVBR3 10 d	577 ± 23**	533 ± 55**	132 ± 25**	147 ± 24**
2010	LVH2b 6 d	105 ± 41*	NA	13 ± 15	NA
	LVH2b 11 d	358 ± 73*	NA	111 ± 57*	NA
	LVH2b 16 d	429 ± 127*	NA	187 ± 62*	NA

Experimental duration (d) is listed following experiment designation, and experimental conditions including incubation atmosphere and temperature are listed in the columns.

**$P < 0.01$, *$P < 0.05$ Mann–Whitney U test.

"NA" indicates these experiments were not conducted. Killed control values (overall average of 266 ± 94 dpm for 2005 LVBR and 190 ± 67 for the 2010 LVH2b) were subtracted from the values reported (n = 4–6).

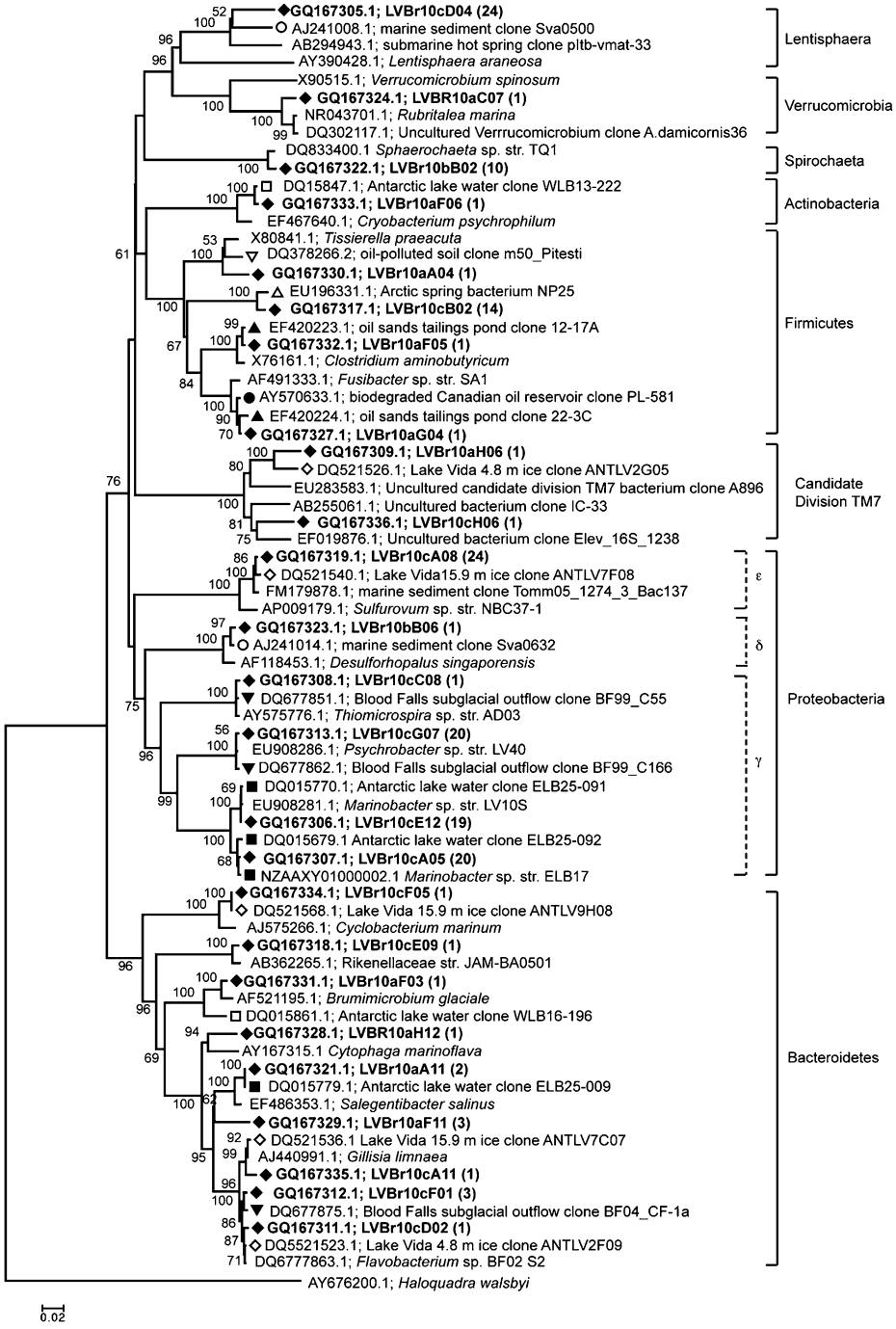

Fig. 2. The evolutionary relationships of LVBr (♦) bacterial SSU rRNA gene sequences, related environmental clones, and cultured isolates were inferred using minimum evolution, and distances computed using maximum composite likelihood. The number of LVBr sequences with distance ≤0.01 are shown following the clone identifier in parentheses. Symbols designate libraries used in comparative analyses (Lake Vida Ice, LVI ♢; East Lobe Lake Bonney, ELB, ▪; West Lobe Lake Bonney, WLB, □; Oil sands tailings pond, OSTP, ▲; Gypsum Hill and Color Peak, GH/CP, △, oil-contaminated soil, PIT, ▽; Canadian biodegraded oil reservoir, PL, ●; Svalbard sediments SVA, ○; Blood Falls, BF, ▼) (see Fig. S6 for principle components analysis). There were a total of 1,020 positions in the final dataset; 1,000 bootstrap trees were calculated and the consensus tree is shown.

identity) of the LVBr *Epsilonproteobacteria* also uses H_2 as an energy source (31). We also detected a unique, abundant group (15%) of SSU rRNA gene sequences with no close cultivated relatives and branching deeply within the *Lentisphaera* phylum (Fig. 2). The presence of diverse members of the *Firmicutes* (11% of the SSU rRNA gene library), which can grow by fermentation, was supported by detection of low numbers of germinable endospores (800 spores/L^{-1} out of a total of 200,000 spores/L^{-1}). Another fermentation-capable group, free-living pleiomorphic spirochaetes isolated from anaerobic environments and enrichment cultures (32), are the closest relatives (97% sequence identity) of LVBr *Sphaerochaeta* sp. SSU rRNA gene sequences (7% of the SSU rRNA gene library). The *Bacteroidetes*-associated SSU rRNA gene sequences (9% of the SSU rRNA gene library) harbored the greatest diversity and were related in several cases to sequences detected in other Antarctic studies (33–35). PCR surveys to detect eukaryal and archaeal SSU rRNA genes from genomic DNA were negative. The lack of detection of an archaeal signal is noteworthy because many comparable cold briny habitats contain significant, diverse archaeal populations (e.g., refs. 36 and 37). LVBr contains such high levels of resources (inorganic and organic) that methanogens, nearly always found in nutrient depleted systems, are not expected to be significant constituents of the assemblage. Consistent with this finding, methane was not detected in 2005 LVBr, and trace levels (50 nM) were detected in 2010 (Table 1 and Table S1).

The LVBr microbial assemblage is distinct from those of other saline lakes of the McMurdo Dry Valleys, such as Lake Bonney (33) and other Antarctic lakes of the Vestfold Hills (36), as indicated by

principle components analysis (Fig. S6*A* and Table S3). The LVBr microbial assemblage is most similar to that inhabiting the subglacial brine of Blood Falls and, as expected, the deeper portion of the Lake Vida ice cover (35) (Fig. S6), where representatives of the *Marinobacter* genus, known to have denitrifying capabilities, are dominant. Phylogenetically, the LVBr assemblage can be distinguished from those of the Lake Vida ice samples and Blood Falls brine because it contains several exclusive phylotypes, including a sizeable *Epsilonproteobacteria* group ($P = 6.90\ 10^{-9}$) and a cluster of *Lentisphaera*-related sequences ($P = 6.05\ 10^{-7}$). Additionally, the LVBr assemblage harbors Spirochaetes and many *Clostridium*-related sequences unlike those of the Blood Falls brine and Lake Vida ice cover. These unique organisms and their associated metabolisms (e.g., H_2 utilization, EPS production, and fermentation) may hold clues to the functional ecology of the brine ecosystem.

Brine Geobiology and Ecosystem Function. The abundance and diverse redox states of compounds found in LVBr raise the question of resource supply and sustenance if the ecosystem is truly encapsulated. Could the life we observe be dependent on the organic inventory accumulated in the lake water before encapsulation? By scaling reaction rates for different types of biodegradable organic matter (e.g., ref. 38) to low temperatures in the brine, the timescales for exponential decay of an initial organic inventory in Lake Vida are on the order of several hundred to a few thousand years. Thus, given the timing of biodegradation and the millennial scale of the age of the sealed system, ~3,000 ^{14}C y at a maximum based on DOC ^{14}C-dating presented here, we would expect that LVBr should be in the final stage of decomposition, and the microbial processes would likely be dominated by methanogenesis, or a mixture of sulfate reduction, H_2-generating fermentation, and methanogenesis. In contrast, we observed in LVBr a complex geochemistry with enormous amounts of dissolved metals, inorganic and organic carbon, N_2O, and H_2, as well as large amounts of both reduced and oxidized forms of dissolved nitrogen, together with a microbial assemblage that is potentially capable of metabolisms ranging from denitrification to chemolithoautotrophy and fermentation, and if present, only very low levels of methanogenesis, as inferred from detection of SSU rRNA genes and SSU rRNA. Thus, the geochemistry and microbial complement of LVBr are more indicative of (i) an ecosystem that receives an influx of energy, other than sunlight, but without a significant influx of mass, or (ii) a system that is severely limited by temperature, some limiting (micro)nutrients, or a combination of the two.

Next, we considered the LVBr system itself. The carbon inventories are high but do not appear to be significantly modified by the microbial processes. Support for this inference is provided by DIC and biomass production, which when converted to a rate of respiration, indicates that the δ^{13}C-DIC value would have only shifted by 0.002‰ in response to 2,800 y of respiration (assuming that respiration is equal to production, the leucine incorporation rate was converted to biomass production; e.g., $3.1\ 10^{-8}$ gC·L^{-1}·d^{-1} using vales of 1.5 kg C/mol and 10 fg C/cell) (39). This production rate results in a generation time averaging 120 y, which slightly exceeds the predicted value for maintenance metabolism at the LVBr temperature (40). Therefore, it is plausible that low heterotrophic metabolic rates could be supported by autochthonous resources.

Alternatives to the decomposition and metabolic scenarios may exist but are currently poorly understood. For example, a recent study of Blood Falls subglacial brines (17) proposed a coupled sulfate-iron (III) reduction cycle that occurs catalytically to oxidize organic matter and regenerate sulfate because of low DOC concentrations (> 100 times lower than in LVBr). This possible path may explain the absence of sulfides in LVBr, but is clearly discordant with the high concentration of DOC, and the lack of sulfur intermediates observed. Another study conducted with soils near the hypersaline (>600), ice-free Don Juan Pond of a neighboring dry valley, demonstrated that abiotic rock-brine reactions, akin to serpentinization, produce copious amounts of H_2 and N_2O via coupling of oxidation of Fe^{2+}-rich minerals of dolerite, with reduction of nitrite and nitrate to nitrous oxide (21). Although Don Juan Pond does not support life because of low water activity, there are several parallels between the abiotic reaction proposed and LVBr geochemistry. There are two major sills of the Ferrar dolerite in Victoria Valley (41) that could contribute iron-rich minerals to the LVBr cryoecosystem (Figs. S2 and S3) as a result of weathering and erosion during successive glacial periods, leaving dolerite clasts over most of the Victoria Valley floor (Fig. S3) (41). The high levels of dissolved nitrogen species, N_2O and H_2 are also consistent with abiotic production.

One implication of abiotic H_2 production is that it could provide energy to this closed system to facilitate metabolism, either autotrophically or as a supplement to heterotrophic metabolism. The lack of isotopic equilibrium between the H_2 and the water argues for little biological contribution to H_2, but cannot discount biological H_2 production completely. Similarly, H_2 can be used by many organisms as an energy source (42), several groups of which were detected by 16S rRNA gene-sequence analysis (i.e., members of the *Firmicutes* and *Bacteroidetes* phyla). Additionally, active and distinctive components of the LVBr microbial assemblage, such as Thiovulgaceae-associated *Epsilonproteobacteria*, are able to use the H_2 lithoautotrophically or lithoheterotrophically (30). Both DNA and crRNA data indicate that these organisms are well represented, active, and unique to Lake Vida in comparison with other Antarctic lakes, although whether they are using H_2 is currently not known.

Conclusions. Lake Vida is an ice-bound system that was presumably isolated with solar-derived organic carbon and coincident microbial life and that has survived for millennia since isolation. The high levels of electron acceptors (i.e., NO_3^-) and a slow rate of metabolism, near maintenance level, prevent biogeochemical processes within the encapsulated system from depleting energetic reserves and from going down the redox couple ladder toward sulfate reduction and methanogenesis. The high levels of dissolved and gaseous nitrogen compounds, Fe and H_2, suggest that serpentinization-like reactions may also occur in LVBr. With the LVBr H_2 levels as high as they are, regardless of their source (abiotic or biogenic production), this H_2 could at least supply readily utilizable energy to combat depurination and racemization processes in this harsh ecosystem (42). LVBr microbial life is reasonably diverse, maintains cellular rRNA, and retains the ability to synthesize proteins at very low levels. We contend that metabolism in this encapsulated brine ecosystem may last for a prolonged period, well in excess of its ~2,800 y of existence.

Materials and Methods

Field Sampling Campaign. Expeditions to Lake Vida resulted in successful extraction of ice cores (16.5 m in 2005, 20 and 27 m in 2010) and collections of brine that entered the borehole at a depth of ~16 m (see Table S1 for station coordinates). We followed a carefully designed sampling approach in both field campaigns to ensure clean access (14). We collected LVBr from within the borehole using sterilized submersible pumps (SS Monsoon, Waterra) and sterile polytetrafluoroethylene tubing (14) at a depth of 16 m in 2005 and 18.5 m in 2010 (from the 20-m borehole).

Geochemical Analyses. LVBr was pumped directly into bottles in a glove bag (N_2 atmosphere) and stored at −10 °C under anoxic conditions for geochemical analyses in both 2005 and 2010. Oxygen measurements were conducted using a Winkler titration in 2005 and by gas chromatography in 2010, neither approach detected oxygen in the brine. Ion constituents were determined using a Dionex DX-120 chromatograph (43); in addition for the 2010 brine, sulfate and nitrate were samples were passed through a 0.2-μm filter and Ag-filter to remove chloride from the brine. Details of DIC and DOC determinations are presented in *SI Materials and Methods*. Inorganic nutrients were determined on a Latchet Autoanalyzer at the Desert Research Institute following standard practices. Analysis for sulfur intermediates (S^o, $S_2O_3^{2-}$, and SO_3^{2-}) and sulfides are described in *SI Materials and Methods*.

Elemental determinations of LVBr were by high-resolution inductively coupled plasma mass spectrometry (Thermo-Finnigan Element 2). The 2005 elemental analysis were operationally defined as "total-recoverable" as per Environmental Protection Agency Method 200.8 (Rev 5.4). The 2010 determinations included the analyses of "total" (acid digestion) and "dissolved" (≤ 0.2-μm diameter) fractions (*SI Materials and Methods*).

N_2O concentrations were measured (headspace technique) for 2005 LVBr with a portable Photo-Acoustic Infrared Trace Gas Analyzer, (Europa Scientific). Details of the N_2O and N_2 determination method and mass spectrometry for 2010 LVBr are presented in *SI Materials and Methods*. Methylthiol (MeSH) and dimethylsulfide (DMS) in the 2005 LVBr were measured (headspace technique) using an SRI 310 gas chromatograph with a sulfur specific FPD detector. Attempts to quantify CH_4 (headspace technique) using this approach were negative for the 2005 LVBr. The approach for CH_4 quantification, in addition to other low molecular-weight organic compounds, in 2010 LVBr is described in *SI Materials and Methods*. The concentration and isotopic fractionation of H_2 dissolved in the 2010 LVBr samples was determined using headspace equilibration and mass spectrometry (*SI Materials and Methods*).

Microbial Analyses. Bacterial cells were observed in glutaraldehyde-preserved (0.1% vol/vol) brine that was stained with either DAPI or SYBR GOLD (Invitrogen) and then filtered onto both 0.2- and 0.02-μm pore-size polycarbonate and aluminum oxide membrane filters (Whatman Anodisc), respectively, followed by epifluorescent microscopic detection and enumeration. Endospore viability assays based on dipicolinic acid triggered terbium ion (Tb^{3+}) luminescence was used to enumerate the germinable and total concentrations of endospores in LVBr (44). Cell preparations on 0.2-μm pore-size polycarbonate filters also were also visualized using scanning electron microscopy using a cold field emission Hitachi S-4700-II Scanning Electron Microscope.

Leucine incorporation was evaluated by incubating 5 mL of brine (five biological replicates with two technical replicates for each sample) with ^{3}H-labled leucine (20-nM final concentration) followed by cold TCA extraction [5% (vol/vol) final concentration] and microcentrifugation (45). TCA and formalin [5% (vol/vol) final concentration] or TCA and autoclaved brine were used to provide killed controls for the 2005 and 2010 experiments, respectively. The 2005 samples were incubated at 0 °C and −12 °C for 10–30 d with nitrogen or ambient air as the headspace in three separate experiments. The 2010 samples were incubated at 0 °C and −13.5 °C for 6, 11, and 16 d with nitrogen headspace. Attempts to measure levels of ^{3}H-thymidine, ^{3}H-acetate and ^{14}C-bicarbonate into cellular macromolecules were not significantly different from killed controls (*SI Materials and Methods*). Cultivation details and molecular analysis details are presented in *SI Materials and Methods*.

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