Research Paper

A Viable Microbial Community in a Subglacial Volcanic Crater Lake, Iceland

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ABSTRACT

We describe a viable microbial community in a subglacial lake within the Grímsvötn volcanic caldera, Iceland. We used a hot water drill to penetrate the 300-m ice shelf and retrieved lake water and volcanic tephra sediments. We also acquired samples of borehole water before and after penetration to the lake, overlying glacial ice and snow, and water from a nearby subaerial geothermal lake for comparative analyses. Lake water is at the freezing point and fresh (total dissolved solids = 260 mg L⁻¹). Detectable numbers of cells were found in samples of the lake water column and tephra sediments: 2×10^4 ml⁻¹ and 4×10^7 g⁻¹, respectively. Plate counts document abundant cold-adapted cultivable organisms in the lake water, but not in the borehole (before penetration) or glacial ice. Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments amplified from genomic DNA extracted from Grímsvötn samples indicates that the lake community is distinct from the assemblages of organisms in borehole water (before penetration) and the overlying ice and snow. Sequencing of selected DGGE bands revealed that many sequences are highly similar to known psychrophilic organisms or cloned DNA from other cold environments. Significant uptake of ¹⁴C-labeled bicarbonate occurred in dark, low-temperature incubations of lake water samples, indicating the presence of autotrophs. Acetylene reduction assays under similar incubation conditions showed no significant nitrogen fixation potential by lake water samples. This may be a consequence of the inhibition of diazotrophy by nitrogen in the lake. **Key Words:** Extreme environments—Microbial ecology—Subglacial lakes. *Astrobiology* 4, 327–344.

INTRODUCTION

RECENT INVESTIGATIONS have reported microorganisms beneath temperate glaciers (Sharp *et al.*, 1999; Skidmore *et al.*, 2000) and in ice that has

accreted to the base of the ice sheet covering Lake Vostok in Antarctica (Karl *et al.*, 1999; Priscu *et al.*, 1999). There is evidence that microbes mediate the subglacial geochemistry of carbon and sulfur (Tranter *et al.*, 1994; Bottrell and Tranter, 2002;

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Wadham *et al.*, 2004). Biologically useful light levels are not found at depths exceeding 100 m in glacial ice (Warren *et al.*, 2002), and therefore life in these environments must rely on chemical sources of energy. Nitrate reduction, sulfate reduction, and methanogenesis have been observed in samples of basal ice from a high Arctic glacier incubated at near-freezing temperature in the dark (Skidmore *et al.*, 2000).

Active subglacial volcanoes and their concomitant hydrothermal systems may be especially propitious sites for life. Besides their intrinsic scientific interest as “extreme” environments, subglacial volcanoes are potential analogs to past or present habitats on an early “Snowball” Earth, in possible martian glaciers, and in the subsurface ocean of the satellite Europa (Gaidos *et al.*, 1999; Gaidos and Marion, 2003). Iceland hosts several active volcanoes under glacial ice as a consequence of its location on both the Mid-Atlantic Ridge and at the Arctic Circle (Fig. 1). Volcanic heat maintains subglacial ice-dammed lakes that drain episodically and catastrophically, causing jökulhlaups, the largest floods on present-day Earth (Björnsson, 1992). One such lake lies within the caldera of the active Grímsvötn volcano beneath the Vatnajökull ice cap. Over the period 1922–1991, 78% of the lake water was supplied by basal melting of the ice shelf (Björnsson and Gudmundsson, 1993). The remaining water probably originated as summer snow melt that drains into the lake at the rim of the shelf. Based on basal melting rates, the heat flow through the caldera averaged 50 W m^{-2} over the past 70 years (Björnsson and Gudmundsson, 1993). Such a high heat flow is indicative of hydrothermal activity (Turcotte and Schubert, 1982), probably arising from the interaction of glacial melt water with a shallow magma body beneath the caldera (Gudmundsson and Milsom, 1997) and the circulation of the hot water along faults in the subsiding caldera floor (Björnsson *et al.*, 1982).

Ágústsdóttir and Brantley (1994) used hot water drilling to penetrate the glacial ice shelf above the lake at two locations in 1991, retrieved water and sediment samples, and reported the chemical analyses of those samples. At their borehole #1 the ice shelf was 250 m thick, and the lake was 140 m deep (Fig. 1). Ágústsdóttir and Brantley (1994) made four observations that are relevant to any subglacial lake biology. First,

lake waters are fresh and slightly acidic (pH 5.7–7.0), with total dissolved solids (TDS) of only a few hundred milligrams per liter. Lake chemistry appears to be dominated by glacial melt and acidification by volcanic CO_2 , with only a minor contribution by hydrothermal fluids (Ágústsdóttir and Brantley, 1994). It is distinct from that of subaerial acid-brine crater lakes in which evaporative concentration occurs (Varekamp *et al.*, 2000). Second, the average temperature of the water column at both locations was near the pressure-depressed freezing point (-0.2°C), a consequence of the melting ice shelf. Third, sulfur was present as sulfate (SO_4^{2-}) throughout most of the water column, and sulfide was detected in only two samples from borehole (#1) closest to the lake center (Fig. 1). This indicates that the lake was at least partially oxic, perhaps because of oxygen released from melting glacial ice. Finally, the lake sediment consisted of volcanic glasses as well as feldspar, olivine, and other basaltic minerals (Ágústsdóttir and Brantley, 1994). This tephra was subglacially erupted within the Grímsvötn volcanic complex, *e.g.*, the Gjálp fissure in 1996 and the southeast edge of the Grímsvötn caldera in 1998, and reached the lake by subglacial slumping or by being deposited on the ice shelf and released when the ice eventually melted at its base (M. Gudmundsson, personal communication). Such sediments are an additional potential habitat for microorganisms. The lake water was sampled again in 1997 and 1998 (M. Gudmundsson, personal communication). The lake’s biology has never been investigated.

We drilled through the Vatnajökull ice cap in June 2002 and sampled the subglacial lake within the caldera of the active Grímsvötn volcano. Our objectives were (1) to determine if there is a viable microbial community in the lake; (2) to evaluate whether the community is distinct from the assemblage of organisms in the overlying snow and ice; and (3) to assay the ability of the community to fix inorganic carbon and nitrogen. We also carried out supporting geochemical analyses of the lake environment. The results of our investigation, presented here, demonstrate the existence of a microbial community in the lake distinct from that in the surrounding environment and capable of autotrophy. To our knowledge, this is the first report of a viable microbial community in a subglacial lake.

FIG. 1. Top: Location of zones of volcanic activity (dotted lines), volcanoes (dots), and major glaciers (including Vatnajökull) on Iceland. **Middle:** Aerial view of the Grímsvötn caldera and ice shelf from the northwest. The approximate locations of the sampling sites of Ágústsdóttir and Brantley (1994) (open circles AB1 and AB2) and this project (solid circle) are shown. (Photo: Óddur Sigurdsson.) **Bottom:** Drilling operation on the Grímsvötn ice shelf.

MATERIALS AND METHODS

Hot water drilling

Hot water drilling equipment was provided by the Science Institute of the University of Iceland (Fig. 1). Twin diesel burners heated melt water to 80–90°C in the drilling system. Some of this hot water was recirculated into a container of snow to produce additional melt water, and the remainder was pumped through a hose to the steel drill head where it was ejected under pressure from four outlets. The use of hot water mitigates the potential for contamination of the lake by exogenous organisms through the borehole water, particularly by psychrophiles. The location of our borehole (64°25.01' N, 17°18.84' W) was 300 m to the east of Ágútsdóttir and Brantley's (1994) borehole #1 (Fig. 1). *In situ* borehole temperatures were measured with a Seagron temperature logger (Hugrún ehf., Reykjavík, Iceland).

Sampling

Lake and borehole water samples were retrieved with a 0.7-L Model 429 stainless steel point-source bailer (Solinst Canada Ltd., Georgetown, ON, Canada) equipped with flow-activated Teflon ball stops. Samples were collected from a depth of 80 m in the borehole (before and after penetration to the lake), from the glacier–lake interface (300 m), from the mid-point of the lake water column (310 m), and from the bottom of the lake (320 m). The bailer was cleaned with 95% ethanol before retrieving each sample to minimize cross-contamination by cells or organic debris. Lake sediment (tephra) samples were recovered using a 95% ethanol-cleaned steel gravity corer. Ice samples were extracted from an ice core recovered from a depth of 85–90 m in the ice shelf adjacent to the hot water drilling site (Thorsteinsson *et al.*, unpublished data). The exterior surface of the ice core was washed with 95% ethanol and melted in a sterile polypropylene container at 0°C before sampling. Clean snow samples were obtained from a snow pit dug 200 m upwind from the drill site, and melted as for the ice core. We also collected water from a subaerial geothermal-heated lake (hereafter referred to as the Jacuzzi) at the base of the southern caldera scarp. All of the samples were divided into separate aliquots for water chemistry, cell counts, plate counts, nitrogen and carbon fixation assays, carbon isotope analysis, and DNA extraction and analysis. (The

three lake samples were pooled to increase the yield of cells and DNA.)

Water chemistry

Aliquots (25 ml) of water samples were added to serum vials, and their pH (WTW Model 330i, WTW, Ft. Myers, FL) and conductivity/TDS (Corning 320, Corning Instruments, Corning, NY) were immediately measured. Samples were observed for degassing and any sulfidic odor indicative of anoxic conditions. Major ion analysis was performed at the University of Hawaii with a DX-300 Ion Chromatograph (Dionex Corp., Sunnyvale, CA). F^- , Cl^- , SO_4^{2-} , oxalate, Br^- , and NO_3^- were analyzed with an AS11-HC column (Dionex), ASRS-ULTRA self-regenerating suppressor (Dionex), and 25 mmol L⁻¹ NaOH eluent, while Na^+ , NH_4^+ , K^+ , Mg^{2+} , and Ca^{2+} were analyzed with a CS12 column (Dionex), CSRS-ULTRA self-regenerating suppressor (Dionex), and 20 mmol L⁻¹ MSA eluent (Dionex). Detection limits ($\mu g\ L^{-1}$) were as follows: F^- , 2; Cl^- , 3; Br^- , 15; Na^+ , 4; K^+ , 10; Mg^{2+} , 10; Ca^{2+} , 15 ppb; SO_4^{2-} , 6; NO_3^- , 10–15; NH_4^+ , 6; and oxalate, 10.

Particulate organic matter (POM) analysis

Aliquots (0.5 L) of sample were filtered, and particulate matter was collected on precombusted (550°C, 16–24 h) 47-mm GFF filters (Whatman, Maidstone, Kent, UK). Analyses of bulk particulate organic carbon (POC) and its isotopic distribution were performed on untreated and acid-fumed fractions of the filters (Hedges and Stern, 1984) using an on-line CHN analyzer (Verardo *et al.*, 1990) coupled with an isotope ratio mass spectrometer (ConFlo II/MAT Delta-Plus, Thermo Electron Finnigan, Waltham, MA). Carbon isotope ratios are reported as $\delta^{13}C$ in ‰ notation relative to the Pee Dee belemnite standard. Multiple analyses of reference samples and well-characterized laboratory standard materials indicated precision and accuracy using this technique was better than $\pm 0.2\%$. For single lake and ice samples, the total abundance of particulate organic nitrogen (PON) was also measured via the CHN analyzer.

Cell counts

Approximately 40-ml aliquots of water were added to combusted (550°C) EPA vials containing 2 ml of 37% stock formaldehyde, mixed, and

stored for transport at 4°C. Cells were incubated with the DNA staining dye 4',6-diamidino-2-phenylindole dihydrochloride (DAPI), filtered, and counted as previously described (Porter and Feig, 1980; Kirchman *et al.*, 1982), with the exception that the volume of water examined was varied from 1 to 25 ml to obtain sufficient cells for statistically accurate counts (20–200 per field, >500 total). A procedural blank consisting of 300 ml of sterile, filtered, Nanopure water was examined in parallel; no cells were observed in the procedural blank (data not shown). For the tephra sample, cells were extracted for counting by a modified version of a previously described method (Bottomley, 1994). Briefly, triplicate samples of 1 g of sediment, sterile borosilicate glass beads, and 9 ml of sterile, filtered, 0.15 mol L⁻¹ NaCl were vigorously shaken, followed by 3 × 30 s sonication on ice in a HEPA-filtered laminar flow hood to extract cells. Nanopure water (30 ml) was added, and the samples were mixed. After settling for 1 h at 4°C, 25 ml of clear supernatant was removed and fixed with formaldehyde (1%). The supernatant was stained with DAPI and counted as described for water samples. A procedural blank lacking sediment was examined in parallel; no cells were observed in the procedural blank (data not shown).

Viable plate counts

Samples were shipped on ice and delivered to the laboratory within 1 week after collection. One milliliter of each sample plus 1:10 and 1:100 dilutions in 0.2-μm-pore-size-filtered lake water were plated on 15-mm DIFCO R2A agar (Becton-Dickinson, Franklin Lakes, NJ) plates at room temperature. Procedural controls (plates without inoculum or plates with sterile water inoculum) were also made. No colonies were seen in any of the procedural controls. Four replicate plates were incubated under room temperature (25°C) or at 5°C and either under aerobic conditions or in BBL Gaspack System anaerobic jars (Becton-Dickinson). Colonies were counted after 8 days; if colonies on replicates with different titers were in the countable range (20–200 cells per plate), the weighted average of the titers was used to calculate the colony-forming unit concentration.

Bicarbonate uptake assays

The protocol described here is based upon a method described by Joint *et al.* (1993). Samples

(snow, ice, lake, and borehole before penetration) were transported to the laboratory at 4°C in sterile Nalgene bottles. Five 100-ml aliquots were placed in acid-washed, sterile borosilicate glass vials (160 ml), capped with butyl rubber stoppers, and sealed with aluminum crimp seals. For each sample, three bottles were used as experimental replicates, a fourth had 1% (final) formaldehyde added as a killed control (the concentration chosen to minimize chemical effects), a fifth had no radiolabel added as a no-¹⁴C control, and a sixth bottle was prepared with deionized H₂O as a no-cell control. Where appropriate, 5 μCi of ¹⁴C-labeled sodium bicarbonate (American Radiolabeled Chemicals, St. Louis, MO) was added to the samples on ice. Immediately following addition of radiolabel and at the indicated periods thereafter, 10 ml was removed from each bottle using a sterile needle. The volume removed was replaced by room air filtered through 0.2-μm-pore-size syringe filter. Particulate material was collected from each sample on a 0.2-μm-pore-size Nucleopore filter, which was placed in a dry scintillation vial and frozen at -80°C for later processing. The full volume of the remaining liquid (~40 ml) was filtered for the final time point (day 60). The order of sample processing was randomized at each time point to prevent artifacts from cross contamination of samples. A procedural blank that consisted of 10 ml of deionized H₂O filtered onto a 0.2-μm-pore-size Nucleopore filter was prepared at the beginning and end of each time point. After collection of all time points, 5 ml of Betacount scintillation cocktail (Fisher Scientific, Hampton, NH) was added to each scintillation vial, and the radioactivity of the particulate material was determined in a Beckman LS-6500 scintillation counter (Beckman-Coulter, Fullerton, CA). To convert counts per minute to molar concentration, a standard curve consisting of serial dilutions of ¹⁴C-labeled sodium bicarbonate was determined (data not shown). Samples were incubated for 60 days at 4°C in the dark. No radioactivity above background was detected in the procedural blanks (data not shown).

Acetylene reduction assays

Nitrogenase activity was assayed by the reduction of acetylene to ethylene (Hardy *et al.*, 1968). Approximately 10-ml aliquots of sample (actual measured average, 11.2 ml) were added to 25-ml serum vials, and the headspace was re-

placed with an 85%:15% mixture of N₂ and acetylene (C₂H₂) at a temperature of 5°C. Aliquots were killed at regular intervals by addition of 1% by volume formalin. Three negative controls (sample without acetylene, deionized H₂O with acetylene, killed sample with acetylene) were run. Ethylene concentration was measured by gas chromatography using an SRI 310 GC (SRI Instruments, Torrance, CA) with a 5-ft Hayesep T 80/100 column maintained at 50°C (Alltech Associates, Deerfield, IL) and a flame ionization detector. Calibrations were performed immediately before and after each set of assays using a 100 ppm ethylene standard (Alltech Associates).

DNA extraction

The liquid samples (lake, snow, Jacuzzi, borehole before and after penetration to the lake) were transferred to acid-cleaned, autoclaved polypropylene bottles. Frozen samples (snow, ice) were allowed to melt at near-freezing temperatures. Samples were filtered in the field through 0.2- μ m-pore-size Supor filters (Pall, Ann Arbor, MI). The filters were frozen in the presence of lysis buffer (20 mmol L⁻¹ Na-EDTA, 400 mmol L⁻¹ NaCl, 0.75 mol L⁻¹ sucrose, 50 mmol L⁻¹ Tris-HCl, pH 9.0), shipped on ice (~1 week), and stored at -80°C. Total nucleic acids were extracted from the filters and purified as described elsewhere (Giovannoni *et al.*, 1996). Sediment samples were stored at 4°C for shipping (~1 week). A subsample for DNA extraction was frozen at -80°C upon arrival in the laboratory for later processing. Total DNA was extracted from tephra samples (sediment) with the FastDNA Spin Kit for Soil (QBioGene, Carlsbad, CA) following the manufacturer's recommendation. DNA extracted from 10 0.5-g samples was combined for further study.

Polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE)

A nested-PCR approach was used to amplify the variable region 3 (V3) of the Eubacterial 16S rRNA gene. The full-length Eubacterial 16S rDNA was amplified from the genomic DNA using the primers 27f and 1512r (Lane, 1991). The PCR products were purified and used as a template to amplify the V3 region with the GC-clamp primer 341F-GC and primer 518R (Muyzer *et al.*, 1993). The final concentrations of the different

components were as follows: 0.2 μ mol L⁻¹ each primer, 200 μ mol L⁻¹ each deoxynucleoside triphosphate, 1.5 mmol L⁻¹ MgCl₂, 10 $\times$ Reaction Buffer (MgCl₂-free), 2 U 50 μ l⁻¹ Taq DNA polymerase (Promega, Madison, WI), 400 ng μ l⁻¹ bovine serum albumin, and DNase- and RNase-free filter-sterilized H₂O. PCR conditions including the hot-start and touchdown for primer annealing were similar to those used by Muyzer *et al.* (1993). DGGE was performed using the Bio-Rad Decode System (Bio-Rad, Hercules, CA). The PCR product generated by the 341F-GC and 518R primers was loaded onto 160 $\times$ 160 $\times$ 1 mm 6% (wt/vol) polyacrylamide gels in 1 $\times$ TAE (20 mmol L⁻¹ Tris, 10 mmol L⁻¹ acetate, 0.5 mmol L⁻¹ EDTA, pH 7.4). The polyacrylamide gels were made with a denaturing gradient ranging from 40% to 60% (where 100% denaturant contains 7 M urea and 40% formamide) by a Model 475 Gradient Former (Bio-Rad). The electrophoresis was run for 8 h at 100 V or 4 h at 200 V in 1 $\times$ TAE buffer at a constant temperature of 60°C. The gel plate was cooled in ice water for 5–10 min and then stained for 10–20 min in 200 ml of 1 $\times$ TAE buffer containing 100 μ g ml⁻¹ ethidium bromide. The stained gel was photographed on a UV trans-illuminator with a CCD system (Bio-Rad).

Analysis of DGGE patterns

The DGGE gel images were analyzed with the GelCompar II software version 2.5 (Applied Maths, Kortrijk, Belgium). Calculation of similarity between gel band patterns is based on the Pearson (product-moment) correlation coefficient (Pearson, 1926) and results in a distance matrix. The Pearson correlation is an objective coefficient that does not suffer from the typical peak/shoulder mismatches that are often found when band-matching coefficients are used.

Sequencing and sequence analysis of DGGE bands

To determine the source of individual bands, a piece of gel from the middle of each of the 21 target bands was excised with an ethanol-cleaned knife. The crushed gel was placed into a 1.5-ml sterile tube, and 0.1 ml of TE buffer was added. After three freeze-thaw cycles (-80°C to 65°C), 1 μ l of the resulting supernatant was PCR-amplified with the primers 341F and 518R using the

PCR conditions described above and, for all samples except for borehole before penetration, cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad). Five to six clones were chosen for each band, and prepared plasmid was sequenced with an ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA). DNA sequences were compared for similarity with those available in public databases by BLAST analysis of the GenBank database (Altschul *et al.*, 1990). Sequences from the borehole sample prior to penetration were to be used only as a check for lake contamination, and so DNA in bands was not cloned but directly re-amplified and sequenced.

RESULTS

Continuous drainage since the 1998 Grímsvötn eruption resulted in a low lake water level in the summer of 2002, and we chose our drill site based on where water would most likely be present (H. Björnsson and M. Gudmundsson, personal communication) (Fig. 1). The ice–water interface was reached after 6 days of drilling through 300 m of ice; the water depth at the drill site was approximately 20 m. *In situ* temperatures were at the pressure-depressed freezing point (-0.2°C). We found, as did Ágústsdóttir and Brantley (1994), that the lake bottom consisted of coarse volcanic tephra.

Lake chemistry

Borehole water samples from a depth of 80 m below the ice surface, taken prior to penetration into the lake, actively degassed, and the pH ranged from 5.4 to 7.5, probably because of de-

gassing of CO_2 . Unlike several samples obtained by Ágústsdóttir and Brantley (1994), neither our post-penetration borehole water samples nor lake samples exhibited any degassing. The pH of three lake samples was more consistent, ranging from 4.87 to 5.13. The average conductivity of the three lake samples was $388\ \mu\text{S cm}^{-1}$, and that of the ice sample $20.4\ \mu\text{S cm}^{-1}$. These correspond to estimated TDS values of approximately 260 and 14 mg L^{-1} , respectively. Also in contrast to Ágústsdóttir and Brantley (1994), no sulfide odor was detected from any of the samples. The major ion abundances in samples from the lake and other locales are compared in Table 1. The sample of the borehole water after penetration of the ice shelf is chemically indistinguishable from the lake water column and significantly different from the borehole water before penetration. This indicates that, as expected, lake water entered the borehole after penetration.

Total particulate carbon and the corresponding $\delta^{13}\text{C}$ values are given in Table 2. Note that multiple measurements (both non-acidified and acidified) from one site are from different subsections of the same membrane. The mean particulate carbon on the three non-acidified filter subsamples is $0.57 \pm 0.15\ \text{mg L}^{-1}$, and that of the acidified subsamples $0.33 \pm 0.02\ \text{mg L}^{-1}$. (We excluded one acidified subsample measurement that was anomalously high compared with the other three.) This difference indicates that a fraction of the particulate carbon was acid-soluble, *e.g.*, carbonate. We use the acidified subsample results as a measure of the POC. Total dissolved organic carbon was not measured; however, the concentration of oxalate measured by ion chromatography ($0.2\ \mu\text{M}$, or $0.005\ \text{mg of C L}^{-1}$) suggests that dissolved organic carbon is low. Particulate ni-

TABLE 1. MAJOR ION ABUNDANCES

Source	Abundance (mg L^{-1})										
	TDS	F	Cl	Br	Na	K	Mg	Ca	S [SO_4^{2-}]	NO_3^-	NH_4^+
Lake	260	0.075	9.95	0.035	5.47	0.40	0.81	0.65	0.80	0.54	N/D
Ice	14	0.023	0.23	N/D	0.40	0.18	0.020	0.085	0.010	0.012	0.049
Snow	—	0.021	0.92	N/D	0.82	0.24	0.073	0.30	0.064	0.071	0.091
Borehole before	—	0.082	2.29	N/D	4.27	0.32	0.16	0.57	0.44	0.89	N/D
Borehole after	—	0.076	10.07	0.039	5.88	0.43	0.80	0.80	0.82	0.56	N/D
Jacuzzi	—	0.614	46.6	0.078	160	15.5	23.0	70.0	48.7	N/D	N/D
AB1 (average)	237	0.70	6.89	—	25	3.05	5.75	25.0	25.2	—	—
AB2 (average)	205	0.04	1.77	—	5.57	1.27	2.66	6.22	1.13	—	—

N/D, not detected.

TABLE 2. PARTICULATE CARBON ABUNDANCE AND ISOTOPIC FRACTIONATION

Source	Number of subsamples	Mean C (mg L^{-1})	Standard deviation (mg L^{-1})	Mean $\delta^{13}\text{C}$ (‰)
Lake	3	0.57	0.15	-23.2
Lake (acidified)	3	0.33	0.02	-22.8
Ice	3	0.25	0.10	-21.1
Ice (acidified)	4	0.22	0.09	-25.3
Snow	1	0.52	—	-25.6
Snow (acidified)	1	0.58	—	-26.5
Borehole before	1	0.13	—	-21.2
Borehole before (acidified)	4	0.31	0.29	-28.1
Borehole after	1	0.22	—	-25.4
Borehole after (acidified)	1	0.28	—	-28.1
Jacuzzi	2	0.18	0.04	-24.1
Jacuzzi (acidified)	1	0.31	—	-26.3

trogen was measured as 0.034 mg L^{-1} for the lake and 0.018 mg L^{-1} for ice (both samples acidified) (Fig. 2). Dissolved inorganic nitrogen (as nitrate; ammonium was not detected) is $8.7 \mu\text{mol L}^{-1}$. If we neglect any contribution by dissolved organic nitrogen in forms other than ammonia, we estimate the total nitrogen concentration in the lake to be $11 \mu\text{mol L}^{-1}$. All acidified and non-acidified samples were found to be enriched with the light carbon isotope, with $\delta^{13}\text{C}$ values between -28.2 and -21.1 ‰. The weight-averaged $\delta^{13}\text{C}$ of acid-insoluble particulate carbon in the lake is -22.8 ‰,

that in ice -25.3 ‰ (0.22 mg L^{-1}), and that in snow -26.5 ‰ (0.58 mg L^{-1}).

Lake microbial abundance

DAPI cell counts are given in Table 3. The “error” is the standard deviation calculated from replicate counts of the same sample, and the “variation” is the standard deviation among multiple samples. Significant numbers of cells were counted in all samples, including the lake water column and tephra sediments. The viable plate

FIG. 2. POC and PON concentrations in the Grímsvötn lake (filled square) and glacial ice (filled triangle) compared with subaerial high arctic lakes (open circles) (Michelutti *et al.*, 2002).

TABLE 3. DAPI AND VIABLE CELL COUNTS

Source	DAPI (10^3 ml^{-1})	Error (10^3 ml^{-1})	Variation (10^3 ml^{-1})	Plate counts (10^3 CFU ml^{-1})
Lake	21	5.7	3.6	7.1
Ice	4.1	1.8	—	0
Snow	32	10	—	14.0
Borehole before	38	13	16	0
Borehole after	18	8.6	—	5.0
Jacuzzi	340	95	—	0
Tephra (g^{-1})	38,000	7,600	5,200	—

CFU, colony-forming units.

counts should be considered as qualitative rather than quantitative because of the possibility of cell growth or death during delays associated with shipping.

Plates of two of the three lake samples contained abundant colonies, which indicates that psychrophilic, or at least psychrotolerant, species were present. In contrast, no colonies were seen on 5°C -incubated plates of two different borehole samples retrieved *before* penetration through the ice shelf, although colonies were present on plates incubated at room temperature (data not shown). Counts of colonies on plates inoculated by the borehole sample obtained after penetration to the lake were similar to those of the lake samples. These organisms were not initially present in the borehole but were introduced when the borehole was flooded with lake water after penetration to the lake. This scenario is consistent with the chemical analyses. No growth was observed for the ice sample at either 5°C or room temperature. In contrast, plates of the snow sample incubated at 5°C contained abundant colonies. No colonies were seen on any plates incubated under anaerobic conditions, perhaps because of insufficient concentrations of a suitable alternative electron acceptor in the R2A medium.

Carbon fixation

Carbon fixation rates were determined in select samples (lake water, ice, snow, and water from the borehole before penetration) by measuring incorporation of H^{14}CO_3 into particulate material over time in active samples relative to various negative controls (including a no-cell, a killed cell, and a no-radiolabel control) at near *in situ* conditions (4°C , dark) (Fig. 3). All active samples showed significant ^{14}C incorporation; no activity above background was detected for any of

the controls. The lake, snow, and borehole samples showed similar-shaped incorporation curves, with minimal lag time and rapid incorporation of label into particulate material. For these samples, the incorporation signal reached an asymptote by day 27. The initial slopes of the incorporation curves (days 0–15) are $3.2 \pm 0.5 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the lake, $2.35 \pm 0.05 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the snow, and $3.1 \pm 0.4 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the borehole water. The ice sample showed a different pattern of incorporation. No significant incorporation was observed prior to day 27, at which point activity increased rapidly to $5.0 \pm 0.3 \text{ nmol L}^{-1} \text{ day}^{-1}$ (days 27–57). No approach to an asymptote was observed in this sample.

Nitrogen fixation

Reduction of acetylene (C_2H_2) to ethylene (C_2H_4) was measured as a proxy for nitrogen fixation activity. The acetylene used in the assay was contaminated with a small amount of ethylene, resulting in a background level of approximately 3.2 ppm. Formaldehyde-poisoned and deionized water negative controls contained the background level after 50 days of incubation. Significant ethylene production was observed in the incubations of the borehole, snow, and ice samples, but not in the incubation of the lake sample (Fig. 4). Furthermore, acetylene reduction in the borehole, snow, and ice incubations occurred only after 33–39 days, at which time incubation conditions, *i.e.*, the “bottle effect,” had probably altered the microbiota. Headspace C_2H_4 concentrations were converted into total moles in the 25-ml incubation vials by assuming a sample volume of 11.2 ml (measured average) and a headspace volume of 13.8 ml. The Henry’s constant of C_2H_4 is $8.4 \times 10^{-6} \text{ atm}$ at 5°C (the incubation temperature), allowing us to calculate

FIG. 3. Incorporation of $H^{14}CO_3$ into particulate material in Grímsvötn samples. Snow, borehole, and lake error bars represent 1 standard deviation about the mean ($n \geq 3$); ice error bars represent the coefficient of variation ($n = 2$).

FIG. 4. Production of ethylene by lake, borehole before penetration into the lake, ice, and snow samples in acetylene reduction assays for nitrogen fixation potential. The baseline level of ~ 3.2 ppm is due to contamination of the acetylene gas by ethylene. One part per million ethylene is approximately equivalent to 2.2 pmol ml^{-1} of acetylene reduction activity.

FIG. 5. Cluster analysis of the DGGE patterns based on a Pearson correlation. Densitometric histograms are shown to the right of the appropriate DGGE pattern. Clones were obtained from the bands related to the numbered electropherogram peaks (see Table 4 for sequences).

that 70% of C_2H_4 was in the headspace and 30% was dissolved in the sample. One part per million of C_2H_4 (at 1 atm) in the headspace is equivalent to a specific acetylene reduction activity of 2.2 pmol ml^{-1} .

Molecular analysis

A DGGE gel showing 16S rRNA gene fragments amplified by PCR on various Grímsvötn samples is shown in Fig. 5. Each pattern of bands represents a "fingerprint" of the community, modulated by the biases imposed by the relative abundance in the microbial population, extractability of DNA, and biases of PCR amplification (Suzuki and Giovannoni, 1995; Polz and Cavanaugh, 1998). Similarities or differences between patterns indicate similarities or differences between communities at the molecular level. The samples are organized by similarity according to nearest-neighbor clustering of the Pearson correlation coefficients. Visual inspection and the clustering analysis showed that the samples from the lake and the borehole after penetration were essentially identical, and distinct from all other samples, including from the Jacuzzi. Table 4 lists the closest matches (most with >95% identity) found in public databases to each of five sequenced clones obtained from six bands from the lake sample, two bands from the snow sample, two from the ice sample, five bands from the tephra sample, and four bands from the Jacuzzi sample. Thirteen bands from the borehole sam-

ple obtained before lake penetration were also directly sequenced. Currently, 16S rRNA gene sequences with >97% identity are considered to come from organisms of the same "species," while those with >95% identity are of the same "genus" (Ludwig *et al.*, 1998).

DISCUSSION AND CONCLUSIONS

Lake hydrology and chemistry

We found the lake to be significantly shallower in 2002 (20 m) than in 1991 (140 m). Volcanic eruptions and variation in caldera heat flow are accompanied by changes in the frequency and volume of Grímsvötn jökulhlaups (Björnsson and Kristmannsdóttir, 1984). The jökulhlaups associated with the 1996 Gjálp and 1998 Grímsvötn eruptions shortened the hydraulic residence time (the average interval between jökulhlaups) from an average of 5 years over the 4 decades prior to 1993 (Gudmundsson *et al.*, 1995) to a few months and led to a low stand in the lake. Chemical analyses of our water samples show, as did those of Ágústsdóttir and Brantley (1994), that the lake is dominated by glacial melt (Table 1). Our average TDS value agrees with that of Ágústsdóttir and Brantley (1994), although the total abundance of the individual species that we measured with ion chromatography was much lower (18.7 mg L^{-1}). Aqueous CO_2 species may account for this discrepancy, but the necessary concentration is in-

TABLE 4. SEQUENCES FROM GRÍMSVÖTN ENVIRONMENTS

Source	Band	Clone ^a	Closest sequence (affiliation)	Accession number	Identity (%)	Comment
Lake	1	1	<i>Acinetobacter</i> sp. (γ proteobacteria)	AF417863	100	
		1	Clone ZZ15C3 (β proteobacteria)	AY214204	98	Benzene-contaminated groundwater
		1	Clone BM89PA1BbC7 (CFB)	AF365784	96	Coral-associated
		1	Clone N2.71PG (β proteobacteria)	AF431305	95	Rhizosphere soil
		1	<i>Pedobacter</i> sp. MSB3023 (sphingobacteria)	AY275498	94	Maple sap
	2	1	Clone LTUE03014 (ϵ proteobacteria)	AY154390	99	Petroleum-contaminated sandy soil
		2	Clone ZZ15C3 (β proteobacteria)	AY214204	97–98	Benzene-contaminated groundwater
		1	Clone c230 (CFB)	AY154587	97	Earthworm intestinal cast
		1	Clone NMS8.130WL (TM6)	AY043961	93	Forest mineral soil
	3	1	Clone B9 (β proteobacteria)	AJ557835	98	Antarctic soil
		2	<i>Aquaspirillum arcticum</i> (β proteobacteria)	AB074523	97	Psychrophilic snow/ice bacterium
		1	<i>Flavobacterium</i> sp. MTN11 (CFB)	AY162137	96	
		1	<i>Herbaspirillum</i> sp. Hg 1 (β proteobacteria)	AY154366	96	Naphthalene-degrading soil bacterium
	4	3	<i>A. arcticum</i> (β proteobacteria)	AB074523	9798	Psychrophilic snow/ice bacterium
		2	<i>H. lusitanum</i> (β proteobacteria)	AF543312	95–96	Nitrogen-fixing endophyte
	5	5	<i>H. lusitanum</i> (β proteobacteria)	AF543312	97–98	Nitrogen-fixing endophyte
	6	2	<i>H. lusitanum</i> (β proteobacteria)	AF543312	97–98	Nitrogen-fixing endophyte
		1	<i>A. arcticum</i> (β proteobacteria)	AB074523	97	Psychrophilic snow/ice bacterium
		1	Clone ZZ15C3 (β proteobacteria)	AY214204	97	Benzene-contaminated groundwater
		1	<i>Flavobacterium</i> sp. MTN11 (CFB)	AY162137	92	
Ice	1	3	<i>Acinetobacter</i> sp. (γ proteobacteria)	AF417863	97–99	
		1	<i>Streptococcus</i> sp. (low G+C firmicutes)	AY278632	99	
		1	Clone Wuba70 (β proteobacteria)	AF336360	97	Karstic aquifer
	2	1	WCHB1-87 (α proteobacteria)	AF050533	99	Contaminated aquifer
		1	Clone KD8-62 (γ proteobacteria)	AY218689	98	Antarctic penguin droppings
		3	Clone WCB186 (γ proteobacteria)	AY217472	92–97	Cave sediment
Snow	1	1	Clone Wuba70 (β proteobacteria)	AF336360	98	Karstic aquifer
		4	Clone P4-20 (β proteobacteria)	AF523331	93–97	Uranium mine sediment
	2	1	Clone SMW4.133WL (acidobacteria)	AY043828	99	Mineral soil
		1	Clone LO13.5 (β proteobacteria)	AF358019	97	Peat soil microcosm
		2	Clone c230 (CFB)	AY154587	96–97	Earthworm intestinal cast
		1	Clone ZZ15C3 (β proteobacteria)	AY214204	97–98	Benzene-contaminated groundwater

TABLE 4. SEQUENCES FROM GRÍMSVÖTN ENVIRONMENTS (CONTINUED)

Source	Band	Clone ^a	Closest sequence (affiliation)	Accession number	Identity (%)	Comment
Tephra	1	1	<i>Pseudomonas</i> sp. (γ proteobacteria)	AY162141	100	Arctic sea-ice melt ponds
		1	Antarctic bacterium sp. (β proteobacteria)	AJ440982	100	Antarctic lake, soils, nutrient-limited cave
		2	<i>Xanthomonas</i> sp. (γ proteobacteria)	AY169434	99–100	Greenland ice core, polar soil, saline lakes
		1	Clone ARKMP-92 (β proteobacteria)	AY198108	99	Arctic sea-ice melt ponds
	2	3	<i>Pseudomonas</i> sp. (γ proteobacteria)	AY158037	98–100	Subantarctic marine system
		1	Clone KD8-62 (γ proteobacteria)	AY218689	98	Antarctic penguin droppings, forest soil
		1	Clone Elb270 (β proteobacteria)	AJ421940	94	Polluted river biofilm
	3	3	Isolate ARK10172 (β proteobacteria)	AF468348	97–98	Arctic sea-ice melt pond
		1	<i>Delftia acidovorans</i> (β proteobacteria)	AF538930	96	Rhizosphere, herbicide- degrading bacterium
		1	Clone ARKMP-92 (β proteobacteria)	AY198108	94	Arctic sea-ice melt pond
	4	1	<i>D. acidovorans</i> (β proteobacteria)	AF538930	100	Rhizosphere, herbicide- degrading bacterium
		1	Clone ARKMP-27 (β proteobacteria)	AF468325	100	Arctic sea-ice melt pond
		1	Isolate BrG2 (β proteobacteria)	U51102	99	Fe-oxidizing denitrifier (freshwater mud)
	5	2	Clone GOBB3-CL121 (β proteobacteria)	AF388908	99	Estuarine water
		1	<i>Pseudomonas</i> sp. (γ proteobacteria)	AY162141	99	Arctic sea-ice melt ponds
		1	<i>Pseudomonas</i> sp. (γ proteobacteria)	AF321048	99	
		1	<i>D. acidovorans</i> (β proteobacteria)	AF538930	98	Rhizosphere, herbicide- degrading
		1	Clone WCB190 (β proteobacteria)	AY217460	98	Cave sediment
		1	<i>Stenotrophomas</i> sp. (γ proteobacteria)	AF368754	97	
Jacuzzi	1	2	Bacterial clone MTU-7 (firmicutes)	AY099232	98	Thermophilic bioleaching consortium
		2	<i>Desulfurella</i> sp. clone (δ proteobacteria)	AF402982	97–98	New Zealand hot spring
	2	1	<i>Aquificales</i> strain 1B-6	AF311357	97	Icelandic hot spring
		1	Sphingomonadacea (α proteobacteria)	AY177713	99	Marine environment
		1	Bacterial clone MTU-7 (firmicutes)	AY099232	98	Thermophilic bioleaching consortium
		1	<i>H. lusitanum</i> (β proteobacteria)	AF543312	98	Nitrogen-fixing endophyte
		1	<i>Desulfurella</i> sp. clone (δ proteobacteria)	AF402982	97	New Zealand hot spring
		1	Clone N42.50PG (β proteobacteria)	AF431301	95	Rhizosphere soil
	3	2	<i>Cytophagales</i> clone OPB73 (CFB)	AF027008	98–99	Yellowstone hot spring
		1	Bacterial clone MTU-7 (firmicutes)	AY099232	98	Thermophilic bioleaching consortium
		1	<i>Aquificales</i> strain 1B-6	AF311357	97	Icelandic hot spring
		1	<i>Desulfotomaculum</i> sp. (firmicutes)	AF295656	91	Thermophilic aquifer community
	4	2	Clone FL13B01 (β proteobacteria)	AY293516	98–99	Yellowstone travertine

(continued)

TABLE 4. (CONTINUED)

Source	Band	Clone ^a	Closest sequence (affiliation)	Accession number	Identity (%)	Comment
Borehole after penetration to lake		1	<i>Aquificales</i> strain 1B-6	AF311357	98	Icelandic hot spring
		1	Clone 1068 (ϵ proteobacteria)	AB030591	98	Petroleum-contaminated groundwater
		1	<i>Meiothermus</i> sp. (<i>Deinococcus/Thermus</i>)	AF418002	95	Moderate thermophile
	1	3	Clone Wuba70 (β proteobacteria)	AF336360	95–98	Karstic aquifer
		2	Clone ZZ15C3 (β proteobacteria)	AY214204	97–98	Groundwater
	2	2	<i>H. lusitanum</i> (β proteobacteria)	AF543312	97–99	Nitrogen-fixing endophyte
	2	2	<i>H. lusitanum</i> (β proteobacteria)	AF543312	97–99	Nitrogen-fixing endophyte
		1	Clone Spb111 (β proteobacteria)	UBE422161	97	
		1	<i>Pseudomonas veronii</i>	AY179328	98	Cold-adapted soil bacterium
		1	Clone ARK1072 (β proteobacteria)	AF468348	98	Arctic sea-ice melt pond
Borehole before penetration to lake	2	*	<i>Nitrospira</i> clone rt16S-5 (β proteobacteria)	AJ271099	96	Arable soil
	3	*	Clone 100 AGG X42 (β proteobacteria)	AF063635	95	Mediterranean sea
	4	*	<i>H. lusitanum</i> (β proteobacteria)	AF543312	93	Nitrogen-fixing endophyte
	5	*	Clone S52.50PG (β proteobacteria)	AF431329	90	Rhizosphere soil
	6	*	Clone JG36-GS-54 (β proteobacteria)	AJ582038	98	Uranium mining waste pile
	7	*	<i>Burkholderia</i> sp. (β proteobacteria)	AB011287	80	
	8	*	Clone GOBB3-B07-4-2 (γ proteobacteria)	AF494505	84	Estuary
	9	*	Clone BOGG3-B07-4-2 (γ proteobacteria)	AF494505	91	Estuary
	10	*	Clone WCHB1-87 (α proteobacteria)	AF050533	89	Contaminated aquifer
	11	*	Clone SW9 (actinobacteria)	AJ575553	86	German freshwater lake
	12	*	<i>Burkholderia tropicalis</i> (β proteobacteria)	AY128105	91	
	13	*	Clone LiUU-9-273 (actinobacteria)	AY497001	90	Freshwater
	14	*	Clone Wd13 (<i>Nitrospira</i>)	AY147385	89	Epilithic river community

^aAn asterisk indicates sequences were obtained directly from DGGE bands.

consistent with the lack of degassing from lake samples. The lack of sulfide odor indicates that lake waters were oxygenated at the location and time of sampling.

The level of POM is comparable to the lowest levels observed in high latitude oligotrophic Arctic lakes and ponds (Fig. 2) (Michelutti *et al.*, 2002). Concentrations of POC in the ice and snow samples are 0.22 mg L⁻¹ and 0.58 mg L⁻¹, respectively, and comparable to that of the lake (0.33 mg L⁻¹). Allocthonous sources may be important

contributors to lake carbon. Although the precise amount of isotopic fractionation in the POC relative to the inorganic source was not determined, our absolute fractionation values are in the range of biologically fixed organic carbon (Freeman, 2001; Schidlowski, 2001). The average $\delta^{13}\text{C}$ of lake POC was -22.8‰ , 14‰ lighter than atmospheric CO₂ and 18‰ lighter than volcanic CO₂. Note that the light isotopic signal appears in the snow and ice as well as lake samples and thus is not direct evidence of autotrophy in the lake.

Lake biota

Our DAPI counts, viable plate counts, and sequence data are compelling evidence for a viable microbial community in the water column and tephra sediments of the lake. Lake cell counts are low ($2 \times 10^4 \text{ ml}^{-1}$) compared with other freshwater aquatic habitats and similar to that in the deep ocean (Whitman *et al.*, 1998). Counts in tephra are much higher ($4 \times 10^7 \text{ cells g}^{-1}$), a situation paralleling other marine and lacustrine systems. Lake samples formed abundant colonies on agar plates at 5°C, but colonies did not grow from samples of ice and borehole water before penetration to the lake. This indicates that the lake contains microorganisms adapted to growth at low temperature. These organisms were not present in the borehole (or were killed) prior to penetration to the lake.

Small-subunit rRNA gene DGGE band patterns and cloned fragment sequences (Fig. 5 and Table 4) demonstrate that the lake and tephra communities are distinct from the overlying ice and snow. With the exception of a sequence matching *Herbaspirillum lusitanum* (a possible contaminant, see below), the sequences obtained from borehole samples before penetration to the lake do not match those obtained from the lake or the borehole water after penetration. Although we did not attempt a thorough characterization of the total microbial community, and the lack of overlap of samples may be an artifact of limited library size, the combined distinctiveness of the DGGE patterns and sequence data suggest that our lake samples were not significantly contaminated by borehole water. This conclusion is also consistent with our viable count data.

The different DGGE gel patterns also indicate that the lake community is distinct from that in the subaerial hot spring waters of the Jacuzzi. Sequences from lake and tephra samples are not similar to known thermophiles or gene sequences obtained from high-temperature environments. Instead, many clones have high sequence similarity to previously identified psychrophilic organisms or DNA cloned from cold environments. This is in contrast to the match of sequences from the Jacuzzi (where the input of hot spring water was directly observed) to known thermophiles or those obtained from other high-temperature environments. The non-detection of such organisms in the subglacial lake and the appearance of psychrotolerant-like sequences indicate that the com-

munity is adapted to a cold glacial melt, not hydrothermal environment. Six of the 13 clones from the lake match sequences classified as belonging to the β proteobacteria, the dominant taxa in many oxygenated, oligotrophic lakes (Urbach *et al.*, 2001).

One-half centimeter of sediment at our borehole site contained biomass roughly equal to that in the entire 20-m water column. The low biomass in the water column raises the possibility that migration or detachment of cells from the benthos could be a significant contributor to the pelagic community. However, the dissimilarity of the lake and tephra DGGE band patterns and the lack of overlap between the sequences from the two respective clone libraries suggest that the two communities are distinct. The proportion of matches to known psychrotolerant organisms or to 16S RNA sequences obtained in cold environments is higher for clones from the tephra sediment sample than for those from the lake water. The predominance of cold-adapted microbes in the tephra sediment could be a result of a more stable, higher biomass community in the sediments, which is more fully adapted to near-freezing temperatures than the lake community. It may also be a sampling artifact, as only a few of the most dominant DGGE bands were chosen for cloning and sequence analysis in this initial study.

A few of the clone sequences in the lake are highly similar to those from samples of snow and ice, which suggests that at least some lake microbes derive from overlying snow and glacial ice melt. The appearance of sequences related to soil and sea-ice microorganisms in our clone libraries may also provide insight into the source of the lake biota. Sandy deserts are widespread in the Icelandic highland (Arnalds *et al.*, 2001), and numerous layers of dust found in a 115-m ice core drilled on the Grímsvötn ice shelf concurrently with our sampling program are believed to result from enhanced summertime deposition of wind-borne dust that originated in these deserts (Thorsteinsson *et al.*, unpublished data). Soil microorganisms associated with dust grains may be transported to Grímsvötn in this way. The major ion content of Iceland's glaciers reflects a marine aerosol source (Gíslason, 1990), and psychrotolerant microbes attached to marine aerosol particles may be another potential source of organisms for the lake. These organisms are deposited onto the glacier surface, buried during glacial ice formation, and released into the lake when that ice even-

tually melts at the base of the shelf. Alternatively, microbes could be carried by summer snowmelt draining into the lake at the rim of the ice shelf.

The interpretation of the similarity between sequences from this study with other database sequences is less clear. Gene databases represent an agglomeration of sequences from environments of human concern rather than an unbiased molecular view of the microbial world, and matches to sequences cloned from contaminated environments are not by themselves evidence of contamination. Of greatest concern is the match of several lake, Jacuzzi, and borehole water clones to *Herbaspirillum* spp. or *Acinetobacter* spp. With the exception of clinical isolates, *Herbaspirillum* spp. are nitrogen-fixing endophytes (Valverde *et al.*, 2003). The genus *Acinetobacter* includes many soil and pathogenic organisms. Sequences closely related to the genera *Acinetobacter*, *Stenotrophomonas*, and *Herbaspirillum* have been identified in contaminant libraries generated from the amplification of cell-free control extractions and low-biomass habitats (Tanner *et al.*, 1998). Although our deionized water negative control samples produced no amplified product, and *Herbaspirillum* spp.- or *Acinetobacter* spp.-like sequences were not identified in any of the clones from the low-biomass ice sample, we cannot rule out the possibility that this sequence is a contaminant.

Microbial carbon and nitrogen fixation

Our lake water incubations with ^{14}C -labeled bicarbonate demonstrate that the lake community contains autotrophs. The initial fixation rates observed in the lake water samples are higher than those in the snow or the borehole water samples, which, again, indicates that the lake community is not simply a dilution of snow melt or contamination from the borehole. The high specific activity for carbon fixation observed in the ice sample was unexpected. However, activity in the ice incubation lagged the other samples by about 27 days and may have been due to an adaptation of the ice community to the incubation conditions or growth of rare chemoautotrophic cells in the sample. After 15 days, the amount of ^{14}C incorporated into particulate material in incubations of lake, borehole water, and snow samples remained unchanged or decreased slightly through the end of the experiment. It is unclear why continuing carbon fixation was inhibited, but it is intriguing that the incorporation stabilized at ap-

proximately the same level in all three cases. The lack of significant acetylene reduction in our incubations of water column samples may be a consequence of inhibition by the high ($\sim 9 \mu\text{mol L}^{-1}$) levels of nitrate in the lake.

In summary, we have demonstrated the existence of viable microbial communities in the water column and tephra sediments of the Grímsvötn subglacial lake. These communities contain psychrotolerant and autotrophic microbes, which reflects the low-temperature and nutrient-poor conditions in the lake, and they are distinct (based on 16S rDNA sequence fragment analysis) from the assemblage of microorganisms in the overlying ice and snow, and borehole water before penetration into the lake. We speculate that the lake is colonized by organisms transported on desert soil or marine aerosol particles and deposited on the Vatnajökull ice cap. These organisms are either entombed in the glacier and released upon melting of the base of the ice shelf above the lake, or are transported with surface meltwater to the rim of the ice shelf and directly into the lake.

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ABBREVIATIONS

DAPI, 4',6'-diaminidino-2-phenylindole hydrochloride; DGGE, denaturing gradient gel electrophoresis; PCR, polymerase chain reaction; POC, particulate organic carbon; POM, particulate organic matter; PON, particulate organic nitrogen; TDS, total dissolved solids.

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