15. The sample in the transition aligns on the straight line that is defined by the cluster of samples above (disturbed glacier ice) and below (lake ice) but has a slope (4.88) significantly higher than 3.98. We interpret this alignment as a result of a diffusion process at a sharp transition between the two types of ice, and not as a freezing effect.
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23. This work is part of the joint project between Russia, France, and the United States to study the Vostok ice core. We are indebted to the Russian drill engineers from the St. Petersburg Mining Institute who conducted the field operations, and we thank all participants for field work and ice sampling. We acknowledge the Russian Antarctic Expeditions (RAE), the Institut Français de Recherches et Technologies Polaires (IFRTP), and the Division of Polar Programs (NSF) for logistic support. The project is supported in Russia by the Russian Ministry of Sciences; in France by the PNEDC (Programme National d'Etudes de la Dynamique du Climat) and by the Commission of European Communities (Environment Programme, ENV4-CT95-0130); and in the United States by NSF. R.S. and V.V. are grateful for the support of the Belgian Antarctic programme (Science Policy Office). We thank J. M. Barnola, J. Chappellaz, M. Delmotte, P. Duval, F. Ferron, G. Hoffmann, J. L. Jaffrezo, P. Jean-Baptiste, D. Paillard, L. Pépin, A. Salamatin, and D. Weis for helpful discussions and comments on the manuscript and T. Sowers and an anonymous reviewer for constructive criticism.

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Geomicrobiology of Subglacial Ice Above Lake Vostok, Antarctica

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Data from ice 3590 meters below Vostok Station indicate that the ice was accreted from liquid water associated with Lake Vostok. Microbes were observed at concentrations ranging from 2.8×10^3 to 3.6×10^4 cells per milliliter; no biological incorporation of selected organic substrates or bicarbonate was detected. Bacterial 16*S* ribosomal DNA genes revealed low diversity in the gene population. The phylotypes were closely related to extant members of the *alpha*- and *beta-Proteobacteria* and the Actinomycetes. Extrapolation of the data from accretion ice to Lake Vostok implies that Lake Vostok may support a microbial population, despite more than 10^6 years of isolation from the atmosphere.

Lake Vostok is the largest (~14,000 km^2) and deepest (maximum depth ~ 670 m) lake identified beneath Antarctic glacial ice (*1*, *2*). The residence time of the water in the lake has been estimated to be about 10,000 years, and the mean age of water, since deposition as surface ice, is about 1 million years (*2*). The ice above the lake has been cored to 3623 m, stopping ~120 m above the surface of the lake. The upper 3310 m is glacial ice that represents an environmental record covering four complete ice age climate cycles. Ice between 3310 and 3539 m is transitional between glacial and accretion ice; ice below 3539 m represents refrozen lake water accreted to the bottom of the glacial ice (*3*, *4*). Here we describe the geomicrobiological environment within the accretion ice and use the information to predict conditions in Lake Vostok.

We studied a core from a depth range of 3588.995 to 3589.435 m (core 3590) (*5*). Cross-polarized light observations of the optical section revealed two distinct ice crystals (Fig. 1). The crystal boundaries extended beyond the edge of the core, making it impossible to estimate the exact grain size of either crystal. The C axes of the two crystals made a three-dimensional angle of 24.3° with each other (*6*). The small and large crystals had declinations of 62° and 43° from the vertical direction, respectively. The horizontal and vertical crystal misalignment could have arisen from seed crystals that nucleated in the lake water or along the margins of the lake before attaching to the bottom of the overlying ice. Alternatively, sheer stresses may have reoriented or recrystallized the ice after accretion.

Unfiltered Cl^- and SO_4^{2-} concentrations in core meltwater fall between the Vostok modern and Vostok Last Glacial Maximum values, indicating that glacial and interglacial snow and ice have melted to produce Lake Vostok (Table 1). Elemental ratios for Al/Rb and Al/Ba in core 3590 were 714 and 192, which are similar to Earth crustal ratios of 704 and 116, respectively (*7*). NO_3^- in core 3590 was depleted relative to concentrations in ice from the Last Glacial Maximum and from the last interglacial period. It is not known whether the depletion of NO_3^- is related to its preferential retention in the lake or loss by biological incorporation or denitrification. Recent experiments (*8*) indicate little difference between liquid-solid water phase partitioning coefficients for Cl^- and NO_3^-, implying that NO_3^- was depleted biologically. Using liquid-solid chemical partitioning coefficients obtained from another Antarctic lake (*9*), we predict that the upper water column of Lake Vostok contains Na^+-SO_4^{2-} waters, similar to many lakes in North America (*10*).

The $\delta^{18}O$ (*11*) and δD values of core 3590 ice were −56.8‰ and −445‰, respectively, supporting the results of Jouzel *et al.* (*4*). If ice in this core was accreted from Lake Vostok water, as implied by our crystallography data, and the ice was in equilibrium with water at 0°C, the water in Lake Vostok should have isotopic values of −59‰ and −463‰ for $\delta^{18}O$ and δD, respectively (*12*). The δD value from core 3590 is within the range reported for Vostok glacier ice (−420 to −485‰) (*3*, *4*), again suggesting that the lake water is derived from a mix of melted ice from glacial and interglacial periods.

Mineral analysis showed that biotite (73%), quartz (13%), potassium feldspar (9%), plagioclase (2%), muscovite (2%), and iron oxide (1%) were the primary minerals (*13*). The distribution of mineral phases in these sediments does not reflect the expected proportions of minerals observed in common crustal granitoid rock types (biotite: <20%; quartz: 20 to 55%; potassium feldspar + plagioclase: 40 to 80%; and muscovite and iron oxide: trace amounts) (*14*). Whether through transport by air, glacier, or subglacial streams, a mechanical sorting process likely operated to concentrate biotite to relatively high levels in core 3590.

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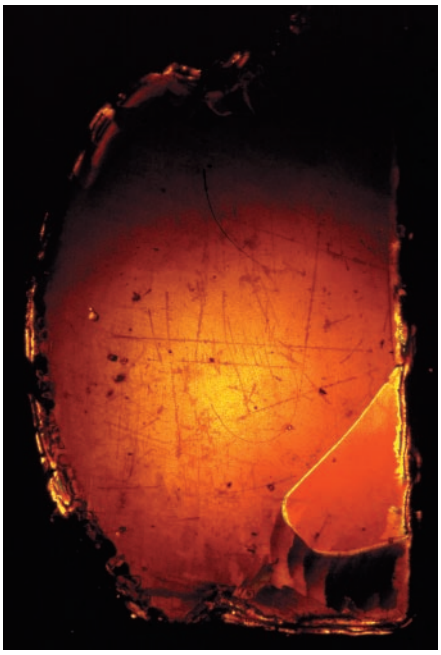

Fig. 1. Cross-polarized image of a 5-mm-thick core section taken from near the middle of core 3590. The horizontal core dimension is 9 cm.

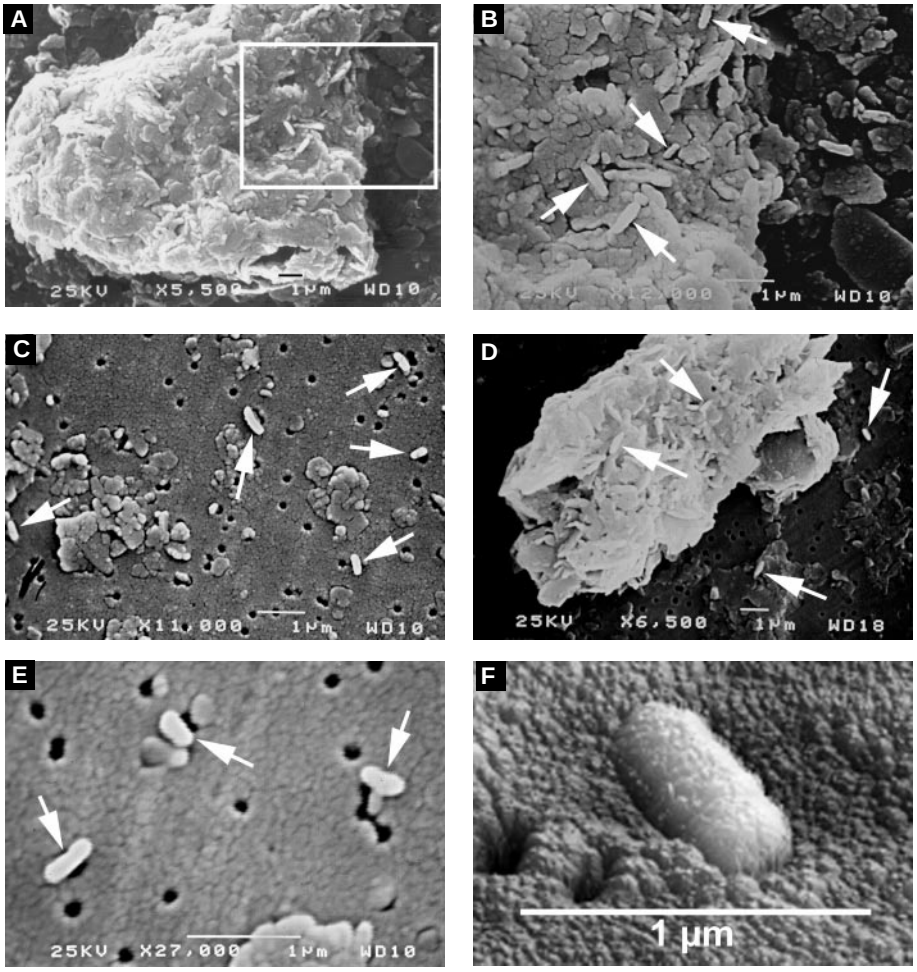

Fig. 2. Scanning electron (**A** to **E**) and atomic force (**F**) micrographs of particles within the core. (B) is a magnification of the area outlined in (A). Arrows indicate bacterial cells.

Microscopic analysis of melted ice revealed microbes in core 3590 (Fig. 2). Bacterial abundances determined by epifluorescence microscopy of DNA-stained samples and scanning electron microscopy (SEM) were 2.8×10^3 and 3.6×10^4 cell ml^{-1} of melted ice, respectively. These concentrations are similar to those from a Vostok core collected from 3603 m (*15*) and to those measured in the accretion ice of Lake Bonney, a permanently ice-covered lake in the McMurdo Dry Valleys, Antarctica (*16*). Assuming that the bacterial partitioning observed between the ice cover and the water column of Lake Bonney (*9*) is the same as the partitioning in the Vostok system, we estimate that the surface water of Lake Vostok had bacterial cell concentrations on the order of 10^5 to 10^6 ml^{-1} when the ice in core 3590 was accreted.

Genomic DNA was extracted (*17*) and amplified with archael and bacterial primers. No products were obtained from archael amplifications; bacterial 16*S* ribosomal DNA (rDNA) fragments were retrieved for terminal restriction fragment length polymorphism (T-RFLP) (*18*) and sequence analyses. About 12 peaks occurred on the DNA fingerprint of the bacterial 16*S* rDNA population (Fig. 3). Five peaks also occurred in the negative control and were considered artifacts. Most of the remaining peaks were assigned to major bacterial groups within either the *alpha-* or *beta-Proteobacteria* (*19*). Seven unique sequences were obtained from cloned polymerase chain reaction (PCR) fragments (*20*). Four clones were assigned to the *Acidovorax* and one clone to the *Comamonas*; extant members of both subgroups of the *beta-Proteobacteria* are known to have diverse habitats and physiologies. One clone was assigned to the *Afipia* subgroup of the *alpha-Proteobacteria* members, which are most commonly associated with root nodules, and one clone to the *Actinomyces* (typically commensal but often found in soil and water samples) (Table 2). *Actinomyces* have also been observed in Vostok glacial ice (*21*).

If microbes indeed grow within Lake Vostok, their metabolism may include heterotrophy. Dissolved organic carbon (DOC) concentration in core 3590 was 0.51 milligram of carbon per liter (mg C $liter^{-1}$) (*22*), indicat-

Table 1. Major ion and trace element concentrations in melted ice from core 3590. Predicted concentrations for water in Lake Vostok were derived from water-to-ice partitioning coefficients obtained from another Antarctic lake (*9*). Vostok modern and Vostok (LGM) represent concentrations in Vostok glacial ice from the present interglacial period and the Last Glacial Maximum, respectively (*32*). NA, not applicable. <, limits of detection.

Constituent	Major ions (µg $liter^{-1}$)							Trace elements (ng $liter^{-1}$)				
	Cl^-	NO_3^-	SO_4^{2-}	Na^+	K^+	Mg^{2+}	Ca^{2+}	Al	Rb	Ba	Al/Rb	Al/Ba
Unfiltered	29.6	<12	165	25	<2	28.7	21.3	5000	7	26	714	192
Filtered	37.6	<12	38	29	<2	7.6	9.7	NA	NA	NA	NA	NA
Vostok modern	10.3	18	125	23	<2	3.6	<5	NA	NA	NA	NA	NA
Vostok (LGM)	174	81	198	97	7.8	18.2	48.1	NA	NA	NA	NA	NA
Predicted Lake Vostok concentrations	1,924	NA	55,400	4,630	NA	3,270	2,261	NA	NA	NA	NA	NA

Table 2. Designation of taxonomic affiliation of PCR fragment clones from Lake Vostok core 3590. "No peak" indicates that the PCR fragment was not restricted by Sau3A. "Unknown" indicates that the T-RFLP analysis program did not identify a corresponding peak for *Comamonas*. "Artifact" indicates that it appeared in both the negative control and the sample. NA, not applicable.

Clones	Taxa	Similarity (%)	Fingerprint peak
4	*Acidovorax*	94	131 and 223
1	*Actinomyces*	NA	No peak
1	*Afipia*	98	204
1	*Comamonas*	97	Unknown
2	*Aquabacterium*	99	Artifact

ing an advected or internal biological source of organic carbon to the lake. The DOC concentration in core 3590 is similar to that measured in the bottom accretion ice of Lake Bonney (*16*). We know of no DOC data available for Antarctic glacial ice. Recent Greenland snow has a mean DOC concentration of 0.11 mg C liter^{-1} (*23*), which may actually be on the order of 0.01 mg C liter^{-1} if corrected for anthropogenic contamination (*24*). Applying observed partitioning coefficients of DOC obtained from accretion ice and the water column of Lake Bonney to core 3590 (*9*), we estimate that Lake Vostok had a DOC concentration of 1.2 mg C liter^{-1} when the ice in core 3590 was accreted. This concentration, if biologically labile, is adequate to support the growth of microbial heterotrophs.

Experiments to examine the incorporation of radiolabeled mannitol, thymidine, amino acid hydrozylate, and bicarbonate into cellular material revealed no heterotrophic or chemoautotrophic growth (*25*). Our incubation time (52 hours) may have been insufficient to measure cell growth in a slow growing population. Incubations were at 1 atm pressure (about 400 times as low as that in Lake Vostok) and in air, which may contain an unrealistically high oxygen concentration relative to Lake Vostok. The pressure and temperature in the lake should produce an environment low in oxygen owing to oxygen sequestration in gas hydrates (*26*). The pressure and oxygen conditions in our experiments could have suppressed or inhibited biosynthetic activity. Attempts to culture the cells at 1 atm and in air were negative (*27*). Microbes in extreme growth conditions might also be using substrates for maintenance activities rather than growth. Hence, metabolic results from core 3590 remain equivocal with respect to the actual viability of the microbes.

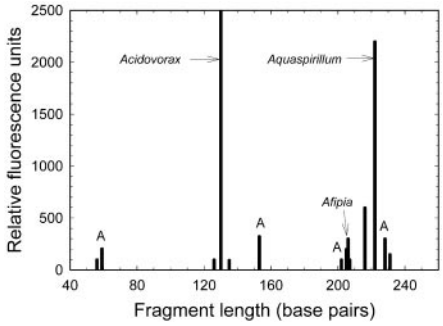

Fig. 3. DNA fingerprint derived from Sau3A digests of 46f(FAM-labeled)/519r amplified bacterial community DNA. Peaks have been designated as an artifact (A) or assigned a taxonomic identity.

Microbes within a liquid water habitat deep below a frozen surface provide an analog for possible life on Europa, one of the Galilean moons of Jupiter. Galileo spacecraft results imply that a subsurface ocean exists on Europa (*28*). Although the thickness of the overlying ice in Europa is unknown (*29*), ice would accrete to the bottom of the ice cover and would also form in cracks, possibly extending close to the surface (*30*). Similar to Lake Vostok accretion ice, this ice may retain evidence for life, if present, in the europan ocean.

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5. Core 3590 was a longitudinal cut from a 10-cm-diameter, 44-cm-long core obtained from Vostok hole 5G. The core had minor cracking propagating <3 cm into the sidewall and no exsolved clathrates. Nonaggregated sediments were dispersed throughout the core, and five sediment inclusions ranging from 0.5 to 1 mm in diameter were present. Core sections were cut for analyses with a saw sterilized with ethanol. Samples were processed under a sterile, positive-pressure laminar flow hood; sterile gloves, clean laboratory clothing, and hair covering were worn during handling. All core handling was conducted in a laboratory that had never been used to grow or process biological samples. Ion and trace chemical samples were rinsed thoroughly with 0.2-μm–filtered Barnstead-nanopure water until 4 to 10 mm of the outer surface had melted. The samples were then completely melted at room temperature in clean, sterile high-density polyethylene (HDPE) jars. Ions in filtered (0.2 μm) and unfiltered samples were analyzed by ion chromatography [K. A. Welch *et al.*, *J. Chromatogr.* **793**, 257 (1996)]; trace elements in unfiltered melt were determined by inductively coupled plasma mass spectroscopy (ICP-MS). Stable isotope samples were melted without rinsing and analyzed by mass spectrometry. SEM samples were rinsed and melted as for ion chemistry. Melted SEM samples were filtered onto sterile 0.2-μm filters with cleaned and sterilized equipment. A SEM control was prepared with 0.2-μm–filtered nanopure water frozen in a clean, sterile polycarbonate tube. The control core was melted, filtered, and analyzed by SEM with methods identical to those of the sample. Mineral and biological particles from the sample were unique with respect to that observed in the control core, indicating that the sample portions we analyzed were free from particulate matter contamination. A sample for epifluorescence microscopy of acridine orange–stained cells was melted in an acid-washed autoclaved bottle at 4°C. DNA staining and counting protocols are described in (*31*).
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and the δD notation is
$$\delta D = \left[\frac{(D/H)_{sample}}{(D/H)_{std}} - 1 \right] \times 1000$$
where std is the standard mean ocean water reference.
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17. Particulates from 250 ml of melted ice core were collected on a sterile 0.2-μm polycarbonate filter. Genomic DNA was extracted from the filter with Chelex 100 resin [P. S. Walsh, D. A. Metzger, R. Higuchi, *Biotechniques* **10**, 506 (1991)]. PCR primers corresponding to conserved sequences within the 5′ and 3′ regions of the 16*S* ribosomal DNAs of bacteria and archae were used. Primary amplification was performed with B1 (*E. coli* position 8 to 27) and B2 (*E. coli* position 1492 to 1510) [W. Liesack, H. Weyland, E. Stackenbrandt, *Microb. Ecol.* **21**, 191 (1991)] and Archael1 (*E. coli* position 3 to 20) and B2 [C. F. Brunk, E. Avaniss-Aghajani, C. A. Brunk, *Appl. Environ. Microbiol.* **62**, 872 (1996)]. A second amplification was performed with 1 μl from the first amplification and nested primers B3 (*E. coli* position 46 to 65) and B4 (*E. coli* position 536 to 519). PCR amplifications were performed following optimized protocols described by M. A. Voytek and B. B. Ward [*Appl. Environ. Microbiol.* **56**, 2430 (1995)].
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by nanopure water before melting at 4°C in a sterile HDPE container. Five-milliliter portions were placed in sterile test tubes and inoculated with isotopes to the following activities and concentrations: ^{14}C-bicarbonate (9.5×10^5 dpm ml^{-1}, trace addition), tritiated thymidine (2.1×10^8 dpm ml^{-1}; 36 nM), tritiated D-mannitol (2.2×10^8 dpm ml^{-1}; 7.5 nM), and tritiated L-amino acid mix (1.3×10^4 dpm ml^{-1}, 0.31 μg ml^{-1}). Three live replicates and one 5% formalin kill were included for each substrate addition. Samples were incubated in the dark at 4°C for 52 hours in air at 1 atm. Carbon 14–labeled bicarbonate and ^{3}H-labeled thymidine incubations were terminated by the addition of 0.5 ml of 6 M HCl and 5 ml of cold 10% trichloroacetic acid (TCA), respectively, before filtration onto 0.2-μm polycarbonate filters; ^{3}H-labeled mannitol incubations were terminated by filtration alone.

26. National Science Foundation Workshop–The Lake Vostok Study: A Curiosity or a Focus for Interdisciplinary Investigations, Washington, DC, 7 to 8 September 1998 (see www.ldeo.columbia.edu/vostok/).
27. Ice for cultures was rinsed with −20°C ethanol, followed by autoclaved, distilled, and organic free water. The ice was melted at 4°C for 12 hours in an autoclaved HDPE bottle that had been rinsed with organic free, deionized water. One milliliter of melted core water was inoculated into two liquid media: phosphate (1.44 g liter^{-1} Na_2HPO_4 and 0.24 g liter^{-1} KH_2PO_4)-buffered sterile deionized water (pH 7.4) and sterile water containing 0.05% (w/v) peptone (pH 7.4). Meltwater (0.5 ml) was also spread onto sterile agar (15 g liter^{-1}) plates prepared with the same media. The inoculated media were incubated for 4 months at 4°C in air at 1 atm in the dark (liquid media preparations were on a shaker during incubation).
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Microorganisms in the Accreted Ice of Lake Vostok, Antarctica

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Analysis of a portion of Vostok ice core number 5G, which is thought to contain frozen water derived from Lake Vostok, Antarctica (a body of liquid water located beneath about 4 kilometers of glacial ice), revealed between 2×10^2 and 3×10^2 bacterial cells per milliliter and low concentrations of potential growth nutrients. Lipopolysaccharide (a Gram-negative bacterial cell biomarker) was also detected at concentrations consistent with the cell enumeration data, which suggests a predominance of Gram-negative bacteria. At least a portion of the microbial assemblage was viable, as determined by the respiration of carbon-14–labeled acetate and glucose substrates during incubations at 3°C and 1 atmosphere. These accreted ice data suggest that Lake Vostok may contain viable microorganisms.

The existence of subglacial lakes in East Antarctica has been known for nearly three decades, but only recently have their large numbers and dimensions been revealed (*1*). Lake Vostok, one of nearly 80 subglacial lakes that have been discovered and mapped by means of airborne 60-MHz radio-echo sounding (*2*), is the largest (~14,000 km^2 surface area and ~1800 km^3 volume) and deepest (up to 670 m) of these unusual subglacial environments. The fresh water in Lake Vostok is kept liquid by the pressure of the ice overburden (equivalent to ~350 atm) and, perhaps, by geothermal heating. This lake and others like it may contain previously undescribed relic populations of microorganisms that are adapted for life in these presumably oligotrophic (low-nutrient, low-biomass, and low–energy flux) habitats.

In 1998, a team of Russian, U.S., and French scientists completed the drilling of Vostok hole number 5G (72°28′S, 106°48′E). At a termination depth of 3623 m, this is the deepest ice core ever obtained. The bottom of the core is ~120 m from the ice–Lake Vostok water interface. The upper 3300 m of Vostok ice core 5G provides a continuous record of Earth's paleoclimate over the past 400,000 years, including four complete glacial-interglacial periods (*3*). Ice samples extracted from core depths of 1500 to 2750 m (with corresponding ages ranging from 110,000 to 240,000 years) have shown (i) the presence of a diverse assemblage of prokaryotic and eukaryotic microorganisms (0.8×10^3 to 11×10^3 cells per milliliter of ice melt), (ii) a positive correlation between the presence of dust and the number of microorganisms, and (iii) the presence of viable mesophilic microorganisms as revealed by the consumption of ^{14}C-labeled organic substrates (*4*).

At greater depths in Vostok ice core 5G, between 3311 and 3538 m, the layers are disturbed by ice sheet dynamics; and beneath 3538 m, changes in the crystal structure, electrical conductivity, and stable isotope and gas composition of the ice suggest that the basal ice at this location (3538 to 3743 m) is refrozen Lake Vostok water (*3*, *5*). Because this lake is so remote and is largely inaccessible, the accreted ice provides the most reliable surrogate sample of the Lake Vostok ecosystem before the actual penetration of the ice-lake boundary and the collection of water samples.

A sample of the accreted Lake Vostok ice was analyzed for (i) microbial cell enumeration by epifluorescence microscopy, scanning electron microscopy (SEM), and dual laser flow cytometry (Figs. 1 and 2); (ii) microbial biomass estimation with two independent biomarker compounds (Table 1): adenosine-5′-triphosphate (ATP) and lipopolysaccharide (LPS); (iii) microbial cell viability and potential metabolic activity by analysis of rates of ^{14}C-CO_2 production and ^{14}C-incorporation into macromolecules after timed incubations with exogenous ^{14}C-labeled organic substrates (Tables 1 and 2); and (iv) the presence of potential carbon and nitrogen growth substrates (*6*–*9*). Our measurements from ice collected at 3603 m complement the independent ice core analyses of a sample from 3590 m (*10*). A major difference is that our core contained no sediment inclusions. Therefore, the results presented here may not be directly comparable to those of Priscu *et al.* (*10*), despite the fact that both samples were obtained from the accreted ice of Lake Vostok.

Epifluorescence microscopic examination of decontaminated, melted ice samples revealed numerous inorganic particles, many of which fluoresced under ultraviolet (UV) illumination (Fig. 1). The presence of these particles complicates precise enumeration of putative microbial cells; however, microbial cells (presumably bacteria) were readily and unequivocally detected (Fig. 1, A and B). There was a spectrum of cell sizes and morphologies, ranging from the abundant small (0.1 to 0.4 μm) coccoid cells that represented about half (43 ± 6%) of the community to a diverse mixture of thin rods and vibrios (0.5 to 3 μm) that made up the remainder (Fig. 1). Enumeration revealed a relatively low abundance of 2×10^2 to 3×10^2 cells per milliliter of melted ice, which extrapolates to ~3 ng of C per liter (Table 1). These biomass estimates are at least an order of magnitude lower than estimates of total prokaryotic cells present in low-nutrient, deep ocean environments (Table 3).

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