

# Temperature dependence of metabolic rates for microbial growth, maintenance, and survival

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Our work was motivated by discoveries of prokaryotic communities that survive with little nutrient in ice and permafrost, with implications for past or present microbial life in Martian permafrost and European ice. We compared the temperature dependence of metabolic rates of microbial communities in permafrost, ice, snow, clouds, oceans, lakes, marine and freshwater sediments, and subsurface aquifer sediments. Metabolic rates per cell fall into three groupings: (i) a rate,  $\mu_g(T)$ , for growth, measured in the laboratory at *in situ* temperatures with minimal disturbance of the medium; (ii) a rate,  $\mu_m(T)$ , sufficient for maintenance of functions but for a nutrient level too low for growth; and (iii) a rate,  $\mu_s(T)$ , for survival of communities imprisoned in deep glacial ice, subsurface sediment, or ocean sediment, in which they can repair macromolecular damage but are probably largely dormant. The three groups have metabolic rates consistent with a single activation energy of  $\approx 110$  kJ and that scale as  $\mu_g(T) : \mu_m(T) : \mu_s(T) \approx 10^6 : 10^3 : 1$ . There is no evidence of a minimum temperature for metabolism. The rate at  $-40^\circ\text{C}$  in ice corresponds to  $\approx 10$  turnovers of cellular carbon per billion years. Microbes in ice and permafrost have metabolic rates similar to those in water, soil, and sediment at the same temperature. This finding supports the view that, far below the freezing point, liquid water inside ice and permafrost is available for metabolism. The rate  $\mu_s(T)$  for repairing molecular damage by means of DNA-repair enzymes and protein-repair enzymes such as methyltransferase is found to be comparable to the rate of spontaneous molecular damage.

There is now both direct and indirect evidence that microorganisms live inside glacial and sea ice and permafrost at temperatures well below the freezing point of pure water (1–11). The thermodynamic stability of ion-rich liquid veins at triple grain-boundaries in polycrystalline ice (12) and of thin films of unfrozen water on microbial surfaces in permafrost (13, 14) enables transport of nutrients to and waste from them. Certain impurities such as mineral acids or salts can reduce the freezing point of water in narrow intergranular veins to as low as  $-90^\circ\text{C}$ . Acidophilic psychrophiles in a Greenland mine (15) survive the cold winters at  $-30^\circ\text{C}$ , probably by taking advantage of such a habitat. It has been conjectured that microbial life may have arisen under similar conditions in subsurface Martian permafrost or in warm diapirs in Europa's ice.

After some brief remarks about metabolism in a natural microbial community, we cite several examples of microbes in ice or permafrost at subfreezing temperatures that we cannot analyze quantitatively, either because information on both microbial concentration and metabolic rate in the same locations was not provided or because only isolates in pure cultures were studied. We then discuss methods and present quantitative data from the literature and from our unpublished work that enable us to calculate metabolic rates per cell. We display the results in Fig. 1 and, finally, discuss the implications.

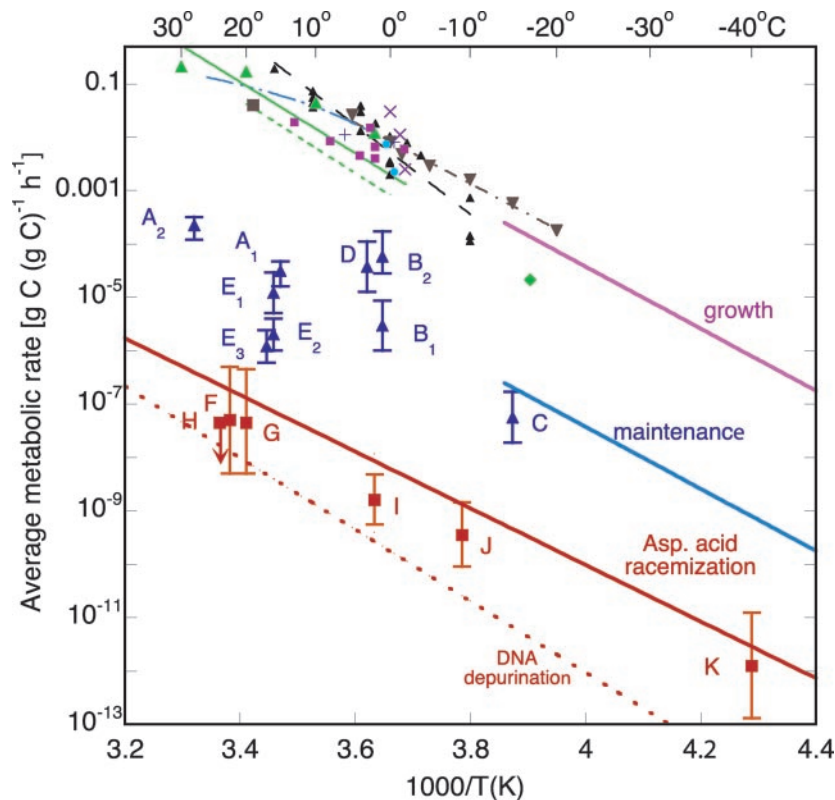
In a culture containing unlimited nutrients, energy sources, room for growth, and no predators, some microbial species can double in about an hour at room temperature. However, low growth rate, or intermittent periods of rapid growth rate interspersed with prolonged periods of nongrowth and starvation, is the norm for microorganisms in nature, for which the mean doubling time can be as long as millennia. Microbes grow until

they deplete the supply of nutrient or accumulate toxic byproducts of metabolism; then they adopt defensive measures. Some engage in cannibalism or develop ultramicrocells; some synthesize stress proteins or express starvation genes; some become dormant; some form spores. To survive at low temperature, microbes can reduce their cell size; reduce their capsular polysaccharide coat thickness; change their fatty acid and phospholipid composition; decrease the fractional volume of cellular water; increase the fraction of ordered cellular water; or extract energy by catalyzing redox reactions of ions in aqueous veins in ice or in thin aqueous films in permafrost.

- Abyzov *et al.* (1) filtered melt water from a section of an ice core from depths 1665 to 2750 m at Vostok Station (Antarctica), counted stained cells, incubated with  $^{14}\text{C}$ -labeled protein hydrolysate in the dark at  $12^\circ\text{C}$  and  $18^\circ\text{C}$  for 4–40 h, and measured the activities. Incubating for 4–7 h was sufficient for cell reactivation and consumption of labeled carbon whereas an 18- to 40-h incubation did not result in an increase in labeled carbon. They concluded that the cells were able to maintain vital functions but were not able to reproduce. We excluded these data because the incubations were not at *in situ* temperature and because they did not measure cell size.
- Priscu *et al.* (2) reported  $10^3$  to  $3 \times 10^4$  microbial cells per milliliter in samples of accretion ice (refrozen from subglacial Lake Vostok water below the overlying glacial ice) at a depth of 3,590 m in an ice core from Vostok Station, Antarctica; and Karl *et al.* (3) detected 200 to 300 per milliliter at 3,603 m. The high concentration was in a region containing rock fragments, and the low concentration was in ice without fragments.
- Willerslev *et al.* (4) identified 57 distinct taxa of eukaryotes from the Hans Tausen ice core, northern Greenland; Gruber and Jaenicke (5) estimated a concentration of  $\approx 3,000$  per milliliter in the same core; and Ma *et al.* (6) identified fungi from the GISP2 (Greenland Ice Sheet Project) ice core.
- Christner *et al.* (7) extracted bacteria from cores in glacial ice from Greenland, Antarctica, Tibet, and Bolivia. They were successful in cultivating only when it took place in a low-nutrient medium over a long time, from which they inferred that cells in low-nutrient media have sufficient time for cell repair before growth begins, whereas cells in rich-nutrient media attempt to grow before they have repaired all of the accumulated cell damage.
- Junge *et al.* (8) studied prokaryotes inside brine channels in Arctic sea ice with staining and epifluorescence microscopy on a cold stage at  $-2^\circ\text{C}$  to  $-20^\circ\text{C}$ . A large fraction of the microbes were attached to particles or to ice-crystal boundaries and were actively respiring even at  $-20^\circ\text{C}$ .
- Gilichinsky (16) reviewed research on viable microbes in permafrost at depths corresponding to ages of up to several million years. As indirect evidence of active metabolism, he cited the ability of immobilized enzymes in permafrost to become instantly activated in the laboratory; the simultaneous presence of nitrifying bacteria and nitrites and of methanogenic archaea and

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**Fig. 1.** Comparison of metabolic rates for prokaryotes as a function of temperature in various ecosystems (see text). In the top group, labeled "growth," lines join measurements by the same investigator at different temperatures: filled black triangles and dashed line, permafrost (27); filled brown inverted triangles and dot-dash line, permafrost (26); dashed triple-dot line, lake (31); solid green line, marine bacteria (33); short-dash green line, freshwater bacteria (33). Symbols without lines are labeled as follows: purple X, polar ocean and ice (35, 36, 38); +, permanently ice-covered Antarctic lake (39); ♦, South Pole ice and snow (28); ●, cloud (29); filled brown squares, ocean (32); filled green triangles, salt-marsh tidal creek (37); and filled purple squares, marine sediment (30, 34). To avoid clutter, error bars are not shown. Points labeled with letters in the middle and bottom groups are keyed to paragraphs in the text. Lines at the bottom are extrapolated from rates of racemization of aspartic acid (solid line, ref. 55) and rates of DNA depurination (dashed line, ref. 56), both measured at high temperature.

methane; and microcalorimetry experiments. As we will see later, this list can be expanded to include, for polar ice, the presence of  $N_2O$ ,  $CH_4$ , and  $CO_2$  in concentrations that correlate quantitatively with microbial concentration as a function of depth. Using a culture medium containing a cryoprotectant, Gilichinsky *et al.* (17, 18) observed cell growth at temperatures as low as  $-5^\circ C$  after 6 months and at  $-10^\circ C$  after 1.5 yr. Gilichinsky *et al.* (19) detected assimilation of  $[^{14}C]$ glucose at temperatures as low as  $-15^\circ C$  by bacteria in cryopegs (brine lenses) found in Siberian permafrost. They concluded that psychrophiles survive at  $-15^\circ C$  in cryopegs, probably repairing cell membranes and DNA damage resulting from background soil radiation and maintaining other vital functions, but without being able to reproduce.

- Using  $^{14}C$ -carbonate and  $^{14}C$ -acetate, Rivkina *et al.* (20) studied evolution of methane by a community of methanogenic archaea from permafrost at temperatures from  $+5^\circ C$  down to  $-16.5^\circ C$ .
- Bakermans *et al.* (21) studied reproduction and metabolism of a number of species of bacteria isolated from Siberian permafrost. One psychrophilic isolate grew at  $-10^\circ C$  at a specific rate of  $7 \times 10^{-4}$  per hour, as measured by culture turbidity and plate counts. The others showed the ability to reduce blue resazurin to pink resorufin but grew at a rate that was too small to measure with their techniques.
- Kappen *et al.* (22) detected  $CO_2$  exchange by polar lichens at  $-20^\circ C$  and found that photosynthetic carbon production and respiratory loss were measurable at  $-18^\circ C$  to  $-12^\circ C$  for certain species. The lichens recovered after having been stored 10 yr at  $-20^\circ C$ .

- Johnston and Vestal (23) estimated average turnover rates of  $\approx 4 \times 10^{-9}$  per hour for photosynthetic carbon incorporation in Antarctic cryptoendolithic microbial communities at ambient temperatures that were subzero for most of the year. The annual temperature cycle of these communities varies so much with sun angle that we are unable to compute metabolic rate as a function of temperature.

### Methods

To determine growth metabolism, we used data for communities, not isolates, whose growth was measured in time-course observations at *in situ* pressures and temperatures. Isolates in pure cultures are usually not representative of community behavior: they have bell-shaped growth curves with lowest, optimal, and highest temperature that depend on taxa, and they are not challenged by the factors that would be present in the natural environment. Methods for studying metabolism and growth include

- incorporation of  $[^3H]$ thymidine and  $[^3H]$ leucine ( $[^3H]$ Leu) into nucleic acids and proteins, respectively. When data with both methods were available, we used the thymidine data.
- $[2-^3H]$ adenine incorporation, which measures RNA and DNA synthesis.
- $[2-^{14}C]$ acetate or other labeled organic C for incorporation into glycolipids and for  $^{14}CO_2$  respiration.
- $^{35}SO_4^{2-}$  assimilation as a measure of total microbial community protein mass.
- fluorescently labeled oligonucleotide probes of quantity of rRNA and DNA.

- growth of turbidity of the medium or direct counts of dividing cells.

An exponential increase in the measured quantity with time is often used as evidence that growth is being measured.

To determine maintenance metabolism, i.e., metabolism for energy exclusive of growth, is a more difficult task. When activities of natural communities of bacteria are measured in terms of quantities such as oxygen consumption, sulfate respiration, or electron transport system activity, respiration independent of growth is measured (24). Radiolabeling can be used to detect respiration in which the labeled substrate is excreted to  $^{14}\text{CO}_2$ ,  $^{14}\text{CH}_4$ , or  $^{35}\text{SH}_2$ . A commonly used technique involves feeding a radiolabeled nutrient in an amount just sufficient to incorporate radioactivity at a detectable level but insufficient for growth. In some experiments, too rich a nutrient was used, which led to growth. We excluded such data as well as batch culture and chemostat data. We selected published experiments in which substrate had not been added and the observed quantity grew linearly, not exponentially.

The geochemical technique is a powerful method for measuring metabolism in an environment so hostile that rates are far too low to be detected on a laboratory timescale. This method requires that a microbial community has existed for a known time interval in a closed system and that the waste products of its metabolism, usually gaseous, are above a background level due to abiotic sources. This method is so sensitive that it can measure survival metabolism of a dormant community. The production rate  $\mu_s$  of excess gas of type  $j$  is given by

$$\mu_s = Y_j/f_j \cdot n \cdot m \cdot t, \quad [1]$$

where  $Y_j$  is the yield of biogenic gas of type  $j$ ;  $f_j$  is the fraction of reactions going to gas of type  $j$ ;  $n$  is the concentration of microbes;  $m$  is the mean mass per microbe; and  $t$  is the age of a closed system of ice or permafrost containing the microbes. In all geochemical cases that we analyzed, the products were the gases  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ , which were measured with standard gas chromatographic techniques. Note that the geochemical method measures average rate: it does not take into account changes in the nutrient supply with time or changes in physiological state of the microbial community. We use the symbol  $\mu_s$ , with the subscript "s" for survival, to suggest that metabolism measured in this way may be different from that measured in time-course experiments.

For growth metabolism, maintenance metabolism, and survival metabolism, when information on average carbon mass per cell was not provided, or when we could not calculate mass from electron micrographs, we used the values 10 fg for aquatic sediments, 20 fg for planktonic prokaryotes, 65 fg for marine sediments, 100 fg for soil, and 86 fg for a terrestrial aquifer, recommended in ref. 25.

Cell concentrations were determined by direct counts on stained cells rather than by cultivation. As a consequence, the metabolic rates represent averages over active, dormant, and even some dead cells.

## Data

Fig. 1 summarizes the results of our study. Points labeled with the symbols given in the legend show carbon-specific growth rates  $\mu_g$  in grams of carbon incorporated into cell material per gram of total biomass carbon per hour. The rates are for communities taken from permafrost, sea ice, snow, cloud, oceans, lakes, fresh and marine waters, and sediments, and measured in the laboratory under *in situ* conditions. Those data fall on a band near the top of the figure. To reduce clutter, several data sets with the greatest number of points were fitted with smooth curves without symbols, as indicated in the legend.

Points labeled A through E in blue show carbon-specific maintenance metabolic rates,  $\mu_m$ , for communities taken from glacial ice, Antarctic lake ice, soil, and marine sediment, and measured in the laboratory.

Points labeled F through K in red show rates,  $\mu_s$ , for *in situ* geochemical data for immobile communities in unperturbed closed systems: glacial ice, ocean sediments, and deep subsurface sandy and clayey aquifer sediments. The participating carbon is that converted into  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{CO}_3^-$ , or other waste.

A brief description of the growth data and nongrowth data follows.

- Rivkina *et al.* (26) monitored the incorporation of  $^{14}\text{C}$ -labeled acetate into glycolipids of a bacterial community from Siberian permafrost at seven temperatures from  $+5^\circ\text{C}$  to  $-20^\circ\text{C}$  for times up to 550 days. The brown dot-dashed curve near the top of Fig. 1 is a fit to their data for the exponential growth phase of those organisms that took up acetate (4). The nutrient uptake rate in the exponential phase reflects the growth potential under ideal conditions, which do not exist in nature, because of limited nutrient availability and limited rate of transport through the thin water film that exists between soil grains and ice in permafrost at low temperature.
- Jakosky *et al.* (27) monitored turbidity of the medium as a measure of growth rates of Siberian permafrost bacteria at temperatures from  $16^\circ\text{C}$  to  $-10^\circ\text{C}$ . The black long-dashed curve is an exponential fit to their data.
- Carpenter *et al.* (28) found concentrations of 200 to 5,000 bacterial cells per milliliter in surface snow and firm at the South Pole; 10–20% of them were members of the *Deinococcus-Thermus* group. From their scanning electron microscopy photos, we estimated a mass of 160 fg per cell. They measured uptake rates of  $^3\text{H}$ thymidine and  $^3\text{H}$ Leu by the community at  $-17^\circ\text{C}$ , the snow temperature at the time of their sample collection. Their result for  $^3\text{H}$ thymidine is plotted.
- Sattler *et al.* (29) used  $^3\text{H}$ thymidine and  $^{14}\text{C}$ Leu uptake rates at  $0^\circ\text{C}$  to measure cell multiplication rates and protein synthesis by bacteria in cloud droplets and snow. We plotted their  $^3\text{H}$ thymidine results. They suggested that airborne bacteria in clouds grow and divide at ambient air temperatures as low as  $-9^\circ\text{C}$  and that there is sufficient dissolved organic carbon in cloud droplets to support bacterial growth.
- Sulfate reducers active in polar sediments can metabolize at low temperatures. Using  $^{35}\text{SO}_4^{2-}$  tracer together with lactate or acetate in anoxic containers, Knoblauch *et al.* (30) calculated specific growth rates for a community of sulfate reducers from marine sediments off the coast of Svalbard. Assuming oxidation of 2 moles organic carbon per mole of reduced sulfate, we converted their rates to carbon-specific growth rates for psychrophiles at  $-1.8^\circ\text{C}$  and  $2.6^\circ\text{C}$  and included data for mesophiles from temperate sediments at  $4^\circ$ ,  $8^\circ\text{C}$ , and  $13^\circ\text{C}$ .
- Coveney and Wetzel (31) assayed specific growth rates of bacterioplankton as a function of temperature in oligotrophic Lawrence Lake, Michigan, using  $^3\text{H}$ thymidine. The blue triple-dot curve shows the rates they found for exponential growth with unlimited nutrient availability. They inferred lower rates for *in situ* growth under conditions when C, N, and P were limiting.
- Simon and Azam (32) used  $^3\text{H}$ thymidine and  $^3\text{H}$ Leu to measure specific growth rates in the exponential phase for marine bacterioplankton from the Southern California Bight at  $19^\circ\text{C}$ .
- White *et al.* (33) analyzed published pelagic bacterial growth rate data from 57 studies that had been conducted in fresh, marine, and estuarine/coastal waters mainly with  $^3\text{H}$ thymidine. They determined best-fit equations for specific growth rate as a function of temperature, algal chlorophyll-a concentration, and cell size. Our green short-dashed curve shows the dependence on *in situ* temperature from  $2$ – $20^\circ\text{C}$  for freshwater bacteria, with cell size =  $0.083 \mu\text{m}^3$ . Our green solid curve



shows the temperature dependence for marine bacteria, with chlorophyll-*a* = 62  $\mu\text{g}\cdot\text{liter}^{-1}$  (nutrient-unlimited).

- Novitsky (34) added [ $^2\text{-}^3\text{H}$ ]adenine to sediment slurries taken at 1-cm intervals at six depths from the water-sediment interface to a depth of 5 cm from a coastal marine sediment in Halifax Harbor and measured specific growth rates of the aerobic microbial communities at the *in situ* temperature 2°C. The average growth rates for the interface and for the depth interval 0–5 cm are plotted.
- Bird and Karl (35) used [ $^3\text{H}$ ]Leu to measure the exponential growth rate of bacteria from shallow water near the Antarctic peninsula, at a temperature of  $\approx 0^\circ\text{C}$ . They filtered out grazers and phytoplankton and obtained rates with and without amino acid amendments.
- Smith and Clement (36) used [ $^3\text{H}$ ]thymidine augmented with glucose to study exponential growth rate of bacteria from the bottom few centimeters of sea ice in the Canadian Arctic, at the *in situ* temperature of  $-1.5^\circ\text{C}$ .
- Shiah and Ducklow (37), using [ $^3\text{H}$ ]thymidine, found that the specific growth rate of heterotrophic bacterioplankton in a salt-marsh tidal creek near Chesapeake Bay depended strongly on water temperature. Results at four *in situ* temperatures from 2–30°C are plotted.
- Kottmeier *et al.* (38), using [ $^3\text{H}$ ]thymidine, measured specific growth rate of bacteria in sea ice in McMurdo Sound, Antarctica, and of bacterioplankton in the Southern Ocean at a temperature of  $\approx -1.9^\circ\text{C}$ .
- Ward and Priscu (39) used turbidity measurements to study growth of denitrifying bacteria from various depths in the permanently ice-covered Lake Bonney, Antarctica, at *in situ* temperatures and salinities of  $-0.5^\circ\text{C}$  to  $6^\circ\text{C}$  and 60–99 g/liter.

**A.** Anderson and Domsch (40) determined maintenance carbon requirements of the microbial communities in “starving” agricultural and forest soils. They measured the amount of carbon required to keep a community at a constant maintenance level, with no net microbial carbon gain or loss. They analyzed the  $\text{CO}_2$  output of 100 g of soil in a closed container to which glucose had been added. Point **A**<sub>1</sub> was an average metabolic rate for three soil types after incubation for 2 weeks at 15°C, and point **A**<sub>2</sub> was for 1 week at 28°C. The rates were lower by a factor  $\approx 10^3$  than the glucose consumption rate in pure culture where growth was allowed.

**B.** In sealed anoxic containers at 1°C, Franzmann *et al.* (41) measured  $^{14}\text{CH}_4$  yields resulting from uptake of  $\text{NaH}[^{14}\text{C}]\text{O}_3$ , [ $^{14}\text{C}$ ]formate, and [ $^{14}\text{C}$ ]acetate by microbes that were living at 1°C in Ace Lake, Antarctica. We calculated the microbial concentrations from data of Mancuso *et al.* (42), who measured the composition of phospholipids as a function of depth in water-column and sediment samples. Franzmann *et al.* concluded that methanogenesis occurs both in the sediments and in the water column. Point **B**<sub>1</sub> is our estimate of the maintenance metabolic rate in the sediment for methanogenesis from acetate. Point **B**<sub>2</sub> is the maximum maintenance rate they found, corresponding to methanogenesis from  $\text{CO}_2$  at a 20-m water depth.

**C.** Christner (43) studied the metabolism of psychrotrophs from polar ice cores. He exposed them to [ $^3\text{H}$ ]thymidine and [ $^3\text{H}$ ]Leu for 100 days at  $-15^\circ\text{C}$ . Taking into account the high concentration ( $\approx 10^7$  per milliliter), the genome size (3 Mbp), and average protein size (36 kDa), he inferred that  $<1\%$  of the genome had been replicated and only  $\approx 100$  protein molecules had been synthesized per cell. He concluded that bacteria can synthesize macromolecules in ice at  $-15^\circ\text{C}$  at a rate that is very low but sufficient to repair macromolecular damage. From his data, we infer that the rate of cell reproduction was too slow for the cells to have gone beyond the lag phase into an exponential growth phase. He conjectured that cells remain metabolically active in

solute-enriched water films on the surfaces of mineral grains in ice or in liquid veins at triple junctions (12) in ice.

**D.** Karl *et al.* (3) studied metabolism in accretion ice from subglacial Lake Vostok. Incubating the ice melts with  $^{14}\text{C}$ -labeled acetate and glucose for up to 18 days at 3°C (slightly above the *in situ* temperature) in the dark led to production of  $^{14}\text{CO}_2$ , which indicated respiration by metabolizing cells. The rate for acetate respiration (point **D**) was  $\approx 800$  times higher than for glucose respiration. Rates of  $^{14}\text{C}$  incorporation into nucleic acids and protein were not above background rates.

**E.** Parkes *et al.* (44) determined anaerobic respiration rates of bacteria in marine sediment from the Peru Margin at 16°C, using [ $^{35}\text{S}$ ]sulfate and [ $^{14}\text{C}$ ]bicarbonate to measure rates of sulfate reduction and methanogenesis. Points **E**<sub>1</sub>, **E**<sub>2</sub>, and **E**<sub>3</sub> show the rates for depths of 0.12–3.5 m, 8–40 m, and 90 m, respectively. The metabolic rate decreases with depth due to nutrient deprivation.

**F.** Chapelle and Lovley (45) measured metabolism in deep aquifers in the South Carolina coastal plain where aerobic and anaerobic reactions, mostly leading to  $\text{CO}_2$  production, take place in different regions. From measurements of concentrations of dissolved organic carbon as a function of distance along the flowpaths of each aquifer, they estimated a production rate of  $\approx 10^{-2}$   $\mu\text{mol}$  of  $\text{CO}_2$ /liter per year by a community of  $3 \times 10^6$  bacteria per gram of sediment during 80 million years, with an uncertainty of a factor 10.

**G.** Phelps *et al.* (46) studied microbial respiration in the same aquifers. They extracted groundwater from boreholes at five points along the flowpath of the Middendorf aquifer at depths 250–400 m, at temperatures of 23–25°C, in complete isolation from atmospheric  $\text{CO}_2$ . Dissolved oxygen concentration along the flow path decreased from near saturation in the recharge zone to anaerobic 80 km downstream at the last borehole. The rate of microbial respiration was calculated from groundwater chemistry, cell counts, and groundwater age determined from the  $^{14}\text{C}$  activity of the dissolved inorganic carbon. Comparing metabolic rates inferred from geochemical methods and from radioisotope time-course experiments, they concluded that radiotracer results overestimated *in situ* rates by factors  $10^3$  to  $10^6$ . They concluded that radioisotope methods are unsuitable for estimating *in situ* metabolic rates in the deep subsurface, mainly because of the much lower specific activity, but perhaps also because of failure to reproduce the *in situ* distribution of nutrients and various microorganisms in the radiolabeling experiments.

**H.** Both Phelps *et al.* (46) and Chapelle and Lovley (45) found microbial concentrations to be a factor  $\approx 10^3$  lower and specific metabolic rates to be much lower in clayey sediments than in sandy sediments. In clays, pore size is so small that microbes are immobile and must depend on diffusion of gaseous or liquid nutrients toward them.

**I.** D’Hondt *et al.* (47) used depth profiles of methane and sulfate to make an *in situ* assessment of the metabolic activity of sulfate reducers and methanogens in marine sediments down to several hundred meters below sea floor at various ocean-margin sites and open-ocean sites. From these profiles and from cell counts by R. J. Parkes and B. A. Cragg, they determined subsurface respiration rates of  $\text{CO}_2$  per cell and mean  $\text{SO}_4^{2-}$  reduction rates per cell as a function of depth. The specific metabolic rates at  $\approx 2^\circ\text{C}$  in sediment at sulfate-rich open-ocean sites were typically three orders of magnitude lower than at methane-rich ocean-margin sites, and both rates were found by the geochemical method to be orders of magnitude lower than had been found with radiotracers. They concluded that sub-seafloor metabolic activity is greatly concentrated in relatively narrow zones of sulfate-reducing methane

oxidation along ocean margins, and that microorganisms in sub-seafloor sediments are adapted for extraordinarily low metabolic activity. Point **J**<sub>1</sub> gives the average metabolic rate for three open-ocean sites: equatorial Pacific, Lau Basin, and Izu-Bonin Trench.

**J.** Tison *et al.* (9) found huge increases in CO<sub>2</sub> (up to 130,000 ppm by volume) and CH<sub>4</sub> (up to 6,000 ppm by volume) in the bottom 6 m of the European borehole [Greenland Ice Core Project (GRIP)] at Summit, Greenland, within a few meters of subglacial till. We suspected that those strong gradients were due to *in situ* microbial metabolism. For the present article, we analyzed CH<sub>4</sub> in the basal ice from the U.S. borehole (GISP2, near GRIP) and found even higher concentrations: 1.2% CH<sub>4</sub> in air extracted from the ice at a depth of 3,044 m, at which the *in situ* temperature was  $-9^{\circ}\text{C}$ . In collaboration with one of us (T.S.), V. Miteva examined GISP2 basal ice. She found  $6\text{--}9 \times 10^7$  cells per milliliter of melt water at the same depth (11) and estimated a live:dead ratio of  $\approx 5:1$ . Point **J** shows the average metabolic rate, which we calculated for an average carbon mass of 15 fg for the dwarf cells observed by Miteva. Based on her successful cultivation of anaerobes at  $-2^{\circ}\text{C}$ , it is likely that methanogens were mainly responsible for the excess methane.

**K.** Sowers (10) reported peaks in the concentrations of N<sub>2</sub>O and  $\delta^{15}\text{N}$  of N<sub>2</sub>O and dips in the values of  $\delta^{18}\text{O}$  of N<sub>2</sub>O occurring at the same depths in Vostok ice where Abyzov *et al.* (1) found peaks in bacterial concentration and where Petit *et al.* (48) found peaks in dust concentration. The ice temperature at that depth is  $-40^{\circ}\text{C}$ . The remarkable correlation strongly suggests that, during the penultimate glacial maximum  $\approx 140,000$  years ago, dust and microorganisms were windborne with greater than normal efficiency and deposited onto Antarctic ice. The observed high  $\delta^{15}\text{N}$  and low  $\delta^{18}\text{O}$  of N<sub>2</sub>O indicated nitrification, in contrast to denitrification, which would have resulted in a high  $\delta^{15}\text{N}$  and high  $\delta^{18}\text{O}$  of N<sub>2</sub>O. Sowers inferred *in situ* N<sub>2</sub>O production by nitrifying bacteria that oxidize ammonia present in the ice. At low but finite oxygen concentration, nitrifiers such as *Nitrosomonas* can convert  $\approx 10\%$  of the total fixed ammonia to N<sub>2</sub>O. The N<sub>2</sub>O is produced both by the denitrification of the NO<sub>2</sub><sup>-</sup> product of NH<sub>4</sub><sup>+</sup> oxidation and by the oxidation of NH<sub>2</sub>OH intermediate. This interpretation is consistent with studies of N<sub>2</sub>O production in Antarctic lake ice (Lake Bonney) at depths where there was a strong excess of nitrifiers (49). Nitrifiers require an aqueous, dark medium, at low O<sub>2</sub> pressure: conditions that exist in liquid veins deep in the Vostok ice. Carbon for growth is obtained from fixation of CO<sub>2</sub> by means of the Calvin cycle. From the measurements in Lake Bonney (49), together with calculations (50), we used a ratio (C fixed)/(N<sub>2</sub>O produced)  $\approx 0.04$  to convert from the rate of nitrification to the rate of carbon metabolism.

We add to this list the fascinating work of Campen *et al.* (51), who reported very large excesses of CO<sub>2</sub> ( $\approx 32\times$  tropospheric value), CH<sub>4</sub> ( $8\times$ ), and N<sub>2</sub>O (up to  $240\times$ ) at depths corresponding to ages of 14,000 yr (for CO<sub>2</sub> and N<sub>2</sub>O) and 15,400 yr (for CH<sub>4</sub>) in air extracted from an ice core from the Sajama (Bolivia) glacier. They concluded that the excesses of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O were products of metabolism due to microbes probably situated in liquid veins along triple junctions of ice grains (12). Unfortunately, not knowing the microbial concentrations at those depths, we cannot estimate metabolic rates.

## Discussion

Morita (52) proposed two levels of metabolism for starving, nongrowing microbes. Maintenance energy is the energy required for osmotic regulation, maintenance of intracellular pH, futile cycles, turnover of macromolecules, motility, and energy dissipation by proton leak and ATP hydrolysis, exclusive of biomass production; and survival energy is the energy required just for repair of

macromolecular damage. Remarkably, in Fig. 1 the metabolic data for microbial communities do fall into three groupings: the rate for exponential growth (uppermost band) is orders of magnitude greater than most of the rates for maintenance without growth (blue letters), and the rates for communities trapped in glacial ice or deep subsurface sediments (red letters) are lower by another three orders of magnitude. The clear separation justifies using Morita's term, survival metabolism, to distinguish the extremely weak metabolism of immobile, probably dormant communities from maintenance metabolism of communities more accessible to nutrient and free to move but still at too low a level for growth. Maintenance metabolism depends, of course, on nutrient availability as well as on temperature. The data of Parkes *et al.* (3) provide an example of the dependence on nutrient availability: maintenance metabolic rate decreases with sample depth in marine sediment depth as shown by points **E**<sub>1</sub>, **E**<sub>2</sub>, and **E**<sub>3</sub>.

We added lines in Fig. 1 to denote the three groupings into which the data fall. The upper solid line, in purple, for exponential growth of a community of permafrost bacteria (fed in the laboratory) has a slope corresponding to an "effective" activation energy  $U_g \approx 110$  kJ/mole. The solid line in blue through the points labeled **A**–**E** is drawn parallel to the purple line; the errors on the middle grouping of points are too large to enable us to determine the effective activation energy accurately, but it is not inconsistent with the value for growth:  $U_m \approx U_g$ , with  $\mu_g(T)/\mu_m(T) \approx 10^3$ .  $U_g$  and  $U_m$  are not activation energies in the chemical sense, but are measures of the temperature response of microbial communities. They are higher than activation energies of  $\approx 40\text{--}75$  kJ/mole for growth of individual species (53, 54) measured on the low-temperature side of the maximum in the growth curve.

Instead of drawing a line through points **F**–**K** that would be parallel to the purple and blue lines, we draw attention to the remarkable similarity of the rates in the bottom grouping to empirical rates of spontaneous macromolecular damage. Brinton *et al.* (55) recently determined the rate and activation energy for spontaneous aspartic acid racemization of microbes in wet samples of Siberian permafrost. The solid red line is an extrapolation of their data into the temperature range of our study. Rates for the other amino acids are much lower. The short-dashed red line is an extrapolation of data on the rate of depurination of DNA (56). The errors in the extrapolated rates for racemization and depurination are roughly a factor two.

We know that DNA-repair enzymes in a living organism efficiently reverse the effects of spontaneous degradation because fewer than  $10^{-3}$  of the accidental base changes in DNA cause a mutation (57). Some damaged proteins can also be repaired and replaced. Almost all cells contain a methyltransferase that can recognize and repair aging proteins such as the L-isopartyl residue and the racemized D-aspartyl residue (58) by means of a methyl esterification reaction. Other protein-repair enzymes may also exist. Brinton *et al.* (55) have presented evidence that permafrost organisms can maintain active recycling of D-amino acids for at least 30,000 yr.

The approximate agreement of the data on rates of aspartic acid racemization (55) and DNA depurination (56) with points **F**–**K** for metabolic rates for survival of immobilized, starved, possibly dormant microbes suggests that rates of repair of DNA and protein damage in living microbes are similar to rates of damage. We suggest a concrete definition of dormancy as the state of a microorganism in which it expends energy at the rate  $\mu_s(T)$  solely to repair molecular damage.

Nutrients can be transported to immobile and mostly dormant microbes as H<sub>2</sub> gas (59, 60) or as water-soluble organic compounds such as humic and fulvic acids (61, 62). For glacial ice (12), the access paths for gaseous and liquid nutrients and waste would be micrometer-diameter veins; for shale and clay (61, 62), they would be sub-micrometer-size pores.

The extremely low expenditures of survival energy enable microbial communities in extreme environments to survive indefinitely. Nitrifying bacteria with metabolic rate  $\approx 10^{-12}$  per hour at  $-40^{\circ}\text{C}$  (point L) have been encased in liquid veins in Vostok ice for  $>140,000$  yr. At a rate of  $10^{-12}$  per hour, it takes  $\approx 10^8$  yr to turn over the carbon in their cells without growth. Even more impressive is the ability of microbes to survive while deeply buried and immobilized in confining clays and shale for  $\approx 10^8$  yr (45, 46).

Our results disprove the view (63) that the lowest temperature at which life is possible is  $\approx -17^{\circ}\text{C}$  in an aqueous environment, as well as the remark (27) that “the lowest temperature at which terrestrial and presumably martian life can function is probably near  $-20^{\circ}\text{C}$ .” Our data show no evidence of a threshold or cutoff in metabolic rate

at temperatures down to  $-40^{\circ}\text{C}$ . A cell resists freezing, due to the “structured” water in its cytoplasm (64). Ionic impurities prevent freezing of veins in ice and thin films in permafrost and permit transport of nutrient to and products from microbes. The absence of a threshold temperature for metabolism should encourage those interested in searches for life on cold extraterrestrial bodies such as Mars and Europa.

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