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Bacteria beneath the West Antarctic Ice Sheet

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Summary

Subglacial environments, particularly those that lie beneath polar ice sheets, are beginning to be recognized as an important part of Earth's biosphere. However, except for indirect indications of microbial assemblages in subglacial Lake Vostok, Antarctica, no sub-ice sheet environments have been shown to support microbial ecosystems. Here we report 16S rRNA gene and isolate diversity in sediments collected from beneath the Kamb Ice Stream, West Antarctic Ice Sheet and stored for 15 months at 4°C. This is the first report of microbes in samples from the sediment environment beneath the Antarctic Ice Sheet. The cells were abundant (~10^7 cells g^{-1}) but displayed low diversity (only five phylotypes), likely as a result of enrichment during storage. Isolates were cold tolerant and the 16S rRNA gene diversity was a simplified version of that found in subglacial alpine and Arctic sediments and water. Although *in situ* cell abundance and the extent of wet sediments beneath the Antarctic ice sheet can only be roughly extrapolated on the basis of this sample, it is clear that the subglacial ecosystem contains a significant and previously unrecognized pool of microbial cells and associated organic carbon that could potentially have significant implications for global geochemical processes.

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Introduction

Reports of microbial life beneath nearly 4 km of ice in Lake Vostok were initially thought to be an example of a highly unusual environment on Earth (Priscu *et al*., 1999; 2008; Christner *et al*., 2006). However, a basic requirement for life, water, is relatively abundant beneath the Antarctic ice sheet, where basal melting occurs over more than half of its area (Llubes *et al*., 2006) and subglacial till provides mineral substrata for microbial growth. Hence, subglacial microbial habitats in Antarctica may be spatially more extensive and variable in physical characteristics than those represented by subglacial lakes alone and may influence continental weathering processes (Skidmore *et al*., 2000).

The abundance of subglacial water is a key factor in the dynamics of ice sheets and for isolated life in an environment beneath hundreds to thousands of meters of ice (Vogel *et al*., 2005; Fricker *et al*., 2007). Seismic and borehole evidence indicate that regions of fast-moving ice, known as ice streams, move over a layer of unconsolidated, water-saturated sediments. These ice streams are the primary route for mass loss from the West Antarctic Ice Sheet (WAIS), with about half of the ice mass flow feeding into the Ross Ice Shelf (Oppenheimer, 1998). Water flows along the hydrological potential gradient towards the coastal ocean forming a hydrological system with lakes, freshwater-saturated sediments ('wetlands') and subglacial drainage pathways (Fricker *et al*., 2007; Priscu *et al*., 2008). While studies of accretion ice indicate that life may be present in subglacial lakes like Lake Vostok (Karl *et al*., 1999; Priscu *et al*., 1999; Christner *et al*., 2001; Bulat *et al*., 2004), no direct evidence has been described for the presence of microbial communities in other parts of the Antarctic subglacial hydrological system. Here we present such evidence from subglacial sediments recovered from beneath the Kamb Ice Stream (KIS), one of the six major West Antarctic ice streams draining into the Ross Ice Shelf.

Results and discussion

A 43-cm-long sediment core was recovered from the base of KIS during a 2000/01 expedition by Kamb and colleagues with the goal of understanding geophysical aspects of the sediment with respect to motion of the ice stream (Vogel *et al*., 2005). Based on the ice stratigraphy, we estimate an age of ≥ 20 000 and up to 100 000 years

Table 1. Identification of isolates.

Group	No. of isolates	% of total isolates	Nearest neighbour	Phylogenetic group	Similarity
A	43	68.3	Clone RA13C6/*Polaromonas vacuolata*	*Betaproteobacteria*	98%
B	11	17.5	*Afipia* genom. 14	*Alphaproteobacteria*	98%
C	9	14.2	*Microbacterium phyllosphaerae*	*Actinobacteria*	97%

for the ice at the base of KIS (Siegert and Payne, 2004; Catania *et al*., 2005). This location was also most likely ice covered and isolated from the atmosphere for at least the past 400 000 years (Scherer *et al*., 1998).

We subsampled this sediment core ~15 months after it was collected. DAPI direct cell counts were $2.74 \pm 0.13 \times 10^7$ and $1.50 \pm 0.05 \times 10^7$ cells g^{-1} wet sediment in the upper and lower halves of the core respectively. These numbers are extremely high compared with samples from other subglacial environments that were not stored prior to sampling, i.e. $2.3–7.4 \times 10^6$ cells g^{-1} at the Fox and Franz Josef Glaciers in New Zealand and $2.1–5.3 \times 10^5$ cells g^{-1} at two Svalbard glaciers (Foght *et al*., 2004; Kastovska *et al*., 2007). Thus, we assume that significant growth occurred during storage. Additionally, it is likely that this growth was biased towards groups capable of surviving the transient exposure to high temperatures the sample experienced during transport as well as other conditions introduced by transport and storage. Based on cell-specific growth rates from Lake Bonney in the McMurdo Dry Valleys, Antarctica (Takacs and Priscu, 1998), which is an Antarctic environment with a similar thermal regime, we estimate that the organisms within the KIS samples may have undergone a maximum of 4.1 doublings. Thus, we estimate that the *in situ* cell abundance in the sub-ice sheet sedimentary environment is likely to be within the range $2–4 \times 10^5$ cells g^{-1}: similar values to those seen in the Svalbard subglacial sediments, and less than those observed for the New Zealand glaciers. However, growth rates in Lake Bonney are likely to be significantly higher than in the more isolated, oligotrophic KIS subglacial environment. Therefore, the *in situ* abundance estimated here should be considered a minimum. It should be noted that without direct measures of growth rate or cell abundance over time, these numbers are highly speculative and should be considered estimates only.

Only a small fraction of the microbes were culturable on the relatively low organic content medium R2A, which was previously shown to be optimal for isolation of microorganisms from ice cores (Christner *et al*., 2001). Approximately 0.005% of cells ($1.5 \pm 1.1 \times 10^3$ cells g^{-1}) from the upper half of the sediment core were culturable during a 5-week aerobic incubation at room temperature. Incubation at 4°C for greater than 3 months increased culturability to *c*. 0.2% and 0.1% ($5.6 \pm 0.8 \times 10^4$ and $1.8 \pm 0.9 \times 10^4$ cells g^{-1}) in the upper and lower halves respectively. The higher culturability at 4°C is consistent with adaptation to growth at the low, stable temperatures in the subglacial environment or to adaptation during storage at 4°C. No growth was observed at either temperature under anaerobic conditions on R2A, indicating either a lack of viable heterotrophic anaerobic organisms or insufficient electron acceptors in the medium. A lack of anaerobic microorganisms is consistent with models of Lake Vostok that show supersaturation with atmospheric gasses (including molecular oxygen) in the subglacial environment resulting from melting of ice of aeolian origin and subsequent exclusion of dissolved gasses during subsequent basal freeze-on (McKay *et al*., 2003), although it is unclear how well this model would apply in a system with moving water, such as the KIS. No direct measurements of redox state were made for this sample, as long-term storage made such measurements highly suspect.

Sixty-three isolates randomly chosen from the aerobic 4°C plates all grew well at 9°C; 42% of these also grew at 25°C, while the other 58% were unable to grow at this elevated temperature, further supporting cold tolerance and cold adaptation of these isolates. All isolates fell into three groups based on their 16S rRNA gene sequences (at > 98% similarity within each group). The most abundant isolate was related to *Betaproteobacteria* (designated *Comamonas* and relatives) environmental clones and isolates obtained from other subglacial and polar environments (Table 1) (Foght *et al*., 2004; Skidmore *et al*., 2005). These are also dominant organisms in subglacial systems in alpine environments in both the Northern and Southern hemispheres, the Arctic and recently deglaciated soils (Foght *et al*., 2004; Skidmore *et al*., 2005; Nemergut *et al*., 2007). The other two isolate groups were related to the *Alphaproteobacterium Afipia* spp. and the *Actinobacterium Microbacterium phyllosphaerae*. *Afipia* spp. are not commonly found in subglacial sediments but were found in Lake Vostok accretion ice (Priscu *et al*., 1999); *M. phyllosphaerae* has not been described in other subglacial or polar systems.

To examine the uncultivated diversity of the enriched microbial community, we extracted total DNA from the KIS sediments and performed 16S rRNA gene denaturing gradient gel electrophoresis (DGGE). Only six distinct bacterial DGGE bands were observed in both the upper and

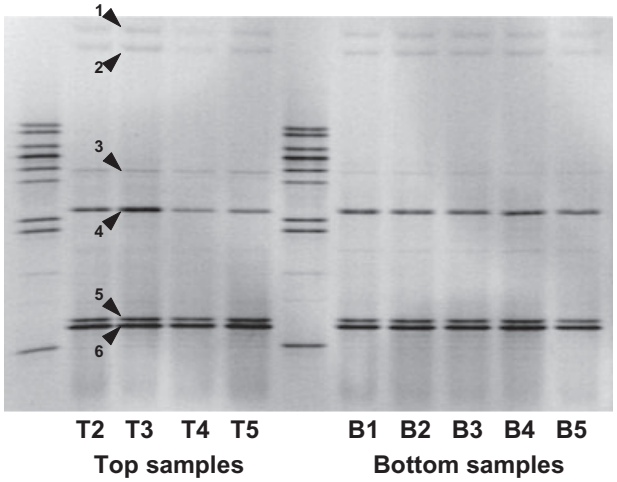

Fig. 1. 16S rRNA DGGE analysis of microbial community diversity in the top and bottom samples. Four replicate extractions (labelled T2–T5) are shown for top samples and five replicate extractions (labelled B1–B5) are shown for bottom samples. Unlabelled lanes are previously described markers (Gaidos *et al*., 2004). Bands are indicated with arrows and numbered; see text for more details.

lower halves of the sample (Fig. 1). The apparent diversity was even more restricted based on sequence analysis of the bands: sequences from bands 1, 2 and 4 were identical to each other, as were bands 5 and 6. Migration of the bands to different points in the gel despite having identical sequences was most likely due to artifacts of the DGGE method, such as heteroduplex formation (Muyzer *et al*., 1993). Thus, three distinct 16S rRNA genes could be detected by this method.

To more fully characterize the uncultivated diversity, clone libraries were constructed; 127 and 124 nearly full-length 16S rRNA gene clones for the upper and lower halves of the core were examined, respectively. No discernable differences were found between the clone libraries from the upper and lower samples in either group presence or group representation. Three sequence clusters, all within the *Betaproteobacteria* class, were observed in these clone libraries, and all were matched at $>98\%$ similarity to the sequences obtained from the DGGE bands (Fig. 2). Intriguingly, these sequences were closely related to groups of organisms (*Comamonas*, *Gallionella* and *Thiobacillus*) previously observed as the dominant groups in alpine and Arctic subglacial systems and proglacial soils (FJ, BG and JEG sequences in Fig. 2) (Foght *et al*., 2004; Skidmore *et al*., 2005; Nemergut *et al*., 2007). The dominant isolates obtained were also *Comamonas*–like organisms (Table 1, Group A). No isolates within the *Gallionella*-like and/or *Thiobacillus*-like groups were obtained; however, cultured *Gallionella* spp. are neutrophilic, chemolithoautotrophic iron oxidizers and cultured *Thiobacillus* spp. are acidiphilic, chemolithoautotrophic iron or sulfur oxidizers. If these sequences are derived from organisms with similar physiology to their closest cultivated relatives, they would not be isolated using R2A media, which specifically selects for heterotrophic organisms. No representatives of isolate groups B and C were observed in the clone library. Clearly, greater diversity is present in these samples than is indicated in the clone library and DGGE results; however, assuming no significant procedural biases, these results indicate the presence of only three dominant groups of microorganisms in these enriched samples; the remaining groups are presumably quite rare in this sample.

While it is difficult to extrapolate from a stored, enriched sample to the *in situ* environment, our microbial analysis suggests a relatively simple community in this enriched assemblage with both heterotrophs and chemolithoautotrophs. Iron and sulfide oxidation is compatible with the physiology of the closest cultured relatives of sequences from the clone libraries, i.e. neutrophilic iron oxidizers (i.e. *Gallionella*) and acidiphilic iron or sulfur oxidizers (i.e. *Thiobacillus*). Biologically driven sulfide oxidation has been proposed as a significant source of sulfate flux at the Bench Glacier in Alaska, an environment with a similar microbial community composition (Skidmore *et al*., 2005), and is a proposed energy source for Lake Vostok and Taylor Glacier Antarctic microbial communities (Christner *et al*., 2006; Mikucki and Priscu, 2007). If these groups are also prevalent *in situ*, such lithotrophic metabolism, combined with autotrophic carbon fixation, could explain the energetic basis of the community and its persistence over the extremely long time period this system has been isolated from direct interaction with the atmosphere. Furthermore, there is sufficient organic carbon of unknown origin in the sediments (0.5–1.5 wt%) to support heterotrophic microbial activity, perhaps carried out by other organisms observed in the clone libraries (e.g. *Comamonas*).

Priscu and colleagues (2008) have argued that, due to the presence of water-saturated sediments at the base of the ice sheet, the subglacial environment of Antarctica should be considered the Earth's largest wetland, containing between 10^4 and 10^6 km^3 of groundwater. Based on their calculations and the *in situ* cell abundance calculated here (which are highly speculative), we estimate the Antarctic subglacial sediments contain between 5×10^{26} and 2.5×10^{27} prokaryotic cells. Using a conversion factor of 11 (fg C) cell^{-1}, these values equate to 0.005 and 0.029 Pg of prokaryotic cell carbon respectively. This prokaryotic abundance is equivalent to between 2 and 11 times greater than the prokaryotic abundance estimated for all surface freshwaters (lakes and rivers) combined (Whitman *et al*., 1998). These numbers are lower than those reported by Priscu *et al*. as they used the actual cell abundance reported here and did not correct for growth during storage. These crude initial estimates must be refined by further sampling of the subglacial environment

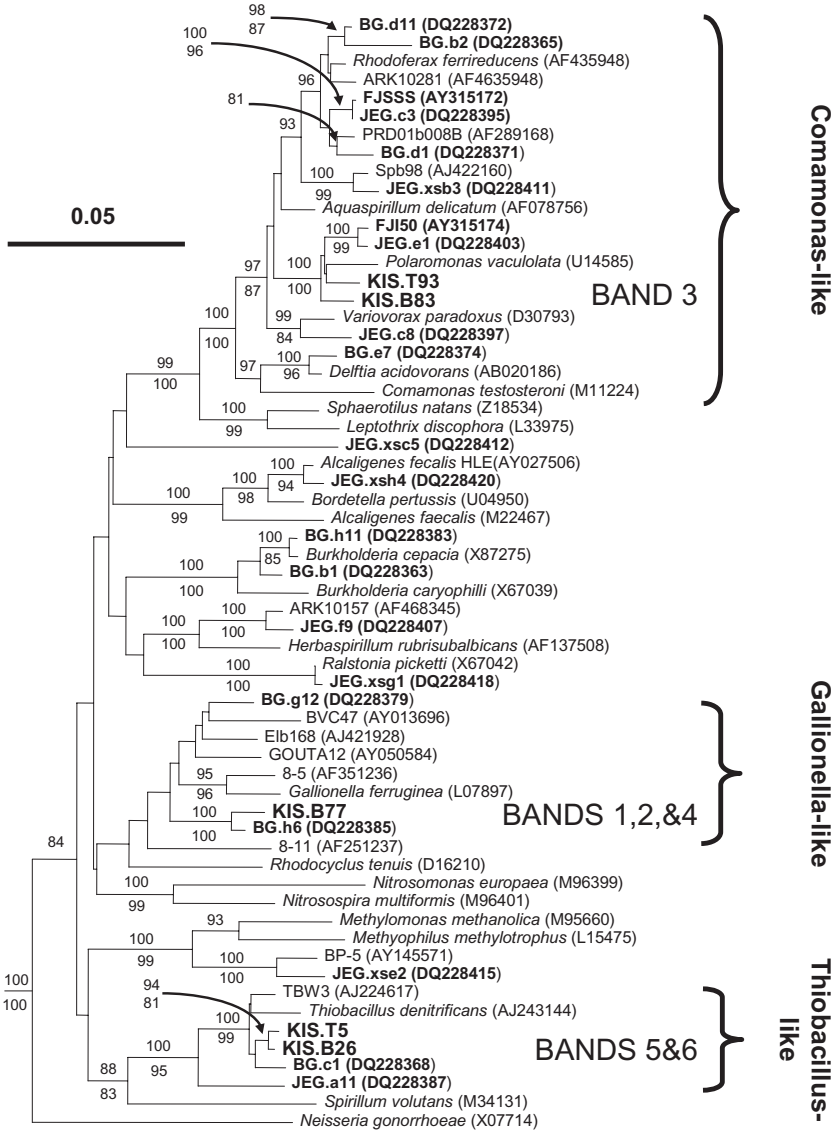

Fig. 2. Neighbour-joining phylogenetic tree showing the relationship of sequences obtained from KIS sediment samples (bold and labelled KIS) to sequences from other subglacial systems (bold) and other sequences from public databases. Characterized isolates names are italicized; uncultivated clone sequences or uncharacterized isolates are in plain text. GenBank accession numbers are included after the names. Sequences corresponding to DGGE bands (Fig. 1) are indicated. Bootstrap values (100 replications) generated by the neighbour-joining method are shown above relevant nodes, and those generated by maximum-parsimony analysis are shown below; only bootstrap values above 50 are shown. Scale bar indicates five conserved nucleotide changes per 100 base pairs. The tree is rooted with *Escherichia coli* (J01695).

and better estimation of the extent of water-saturated sediments at the base of the Antarctic ice sheets; however, even with the broad uncertainties expressed here, they clearly indicate that Antarctica contains a significant and previously unrecognized reservoir of prokaryotic carbon that should be considered when addressing issues concerning global carbon dynamics. Furthermore, such abundant microbes, if active, are likely to have a significant impact on subglacial weathering processes (Sharp *et al*., 1999; Tranter *et al*., 2002).

Due to growth in the sediments during storage, the diversity studies described above should be considered as characterizing a group of organisms enriched for members of the *in situ* bacterial assemblage well adapted to low-temperature, stable environments, rather than a direct characterization of the bacterial assemblage found in the subglacial environment. Nonetheless, the organisms found during this analysis are almost certainly present in the subglacial environment, although perhaps not at the same relative abundances observed in this study. The low porosity and hydraulic conductivity of the sediments (Tulaczyk *et al*., 2001) would prevent contamination of the interior of the core where we sampled. Our contention that the observed community is endogenous to the subglacial environment is supported by the extremely low diversity and the relationship of the observed community to previously characterized subglacial microbial communities (Foght *et al*., 2004; Skidmore *et al*., 2005; Nemergut *et al*., 2007). In addition, the observed community lacks commonly observed laboratory contaminants and no PCR products were observed in procedural controls (Tanner *et al*., 1998).

By analogy with other subglacial environments, our biological data indicate that Antarctic subglacial environ-

ments may support a microbial assemblage based on chemoautotrophic sulfide and/or iron oxidation by *Gallionella*- or *Thiobacillus*-like organisms. With significant caveats, the similarity in microorganisms in our enriched samples to those from Arctic and alpine subglacial environments from both the Northern and Southern hemispheres and in proglacial soils, we have identified components of a globally distributed 'subglacial microbial community' that may play an important role in global biogeochemical processes. These data provide new information on life beneath ice sheets and extends what is known about microbial biogeography on our planet, and indicate the presence of an abundant, overlooked microbial community in subglacial Antarctic sediments.

Experimental procedures

Sample collection and processing

The sediment core was obtained with a piston corer through a hot-water-drilled borehole in December 2000 (Vogel *et al*., 2003) and was stored in a plastic core liner until sampling in March 2002. The core was stored at non-freezing conditions during air shipment from Antarctica (~40 h) and experienced transient temperatures ranging from 4°C to ~25°C during this transition period. Sub-samples from the top 5 cm and the bottom 10 cm of the 43-cm-long core were taken for porosity studies in March 2001, during which time the cores were exposed to room temperature conditions for up to several hours. These periods of higher temperature may have placed a considerable selection pressure on members of this assemblage, which experience highly stable *in situ* temperatures at the pressure depressed freezing point of approximately −1.5°C. Otherwise, the core section was kept refrigerated at 4°C for the 15 months prior to sampling.

The core was fully hydrated (43–51% w/v water content) and showed no evidence of cracking at the time of sampling, providing conditions that should eliminate contamination of the inner portion of the core. Based on the hydraulic conductivity of the sediment (Tulaczyk *et al*., 2001), it would take 3.2 years for a water molecule to penetrate 1 cm into the core. Considering that bacterial cells have diameters significantly larger than water molecules and their motility would be inhibited by both physical tortuosity and adsorption to sediment particles, it is highly unlikely that outside contaminants would reach the inner portion of the core where our samples were taken (Huysman and Verstraete, 1993; Rebata-Landa and Santamaria, 2006).

For sampling, the core liner was cut lengthwise with a Dremmel tool. The outer 1.5 cm of the core was aseptically removed in a class 100 laminar flow hood (Labconco Purifier Vertical Clean Bench, Fisher Scientific) to eliminate possible contamination from core handling. Samples of the inner core were collected from the upper and lower halves of the core with heat-sterilized spatulas and placed in sterile 50 ml Falcon centrifuge tubes. The samples were homogenized by mixing and then split into two sterile centrifuge tubes. Sediment organic carbon content was determined by loss on ignition by standard methods (Franson *et al*., 1998). Witness plates of R2A medium present in the laminar flow hood during sample processing showed no growth after 6 months of incubation at 4°C and 25°C, and negative process controls (water blanks processed through the entire extraction and amplification procedure) gave no PCR products, indicating that the sampling procedure did not contaminate the samples.

Direct microscopic cell counts and estimation of in situ *abundance*

Cells were extracted from sediments and direct cell counts were determined by fluorescence microscopy following DAPI staining using standard methods (Bottomley, 1994). No effort was made to determine the efficiency of cell extraction using this approach; however, in other systems efficiency is > 50% (Bottomley, 1994; Frischer *et al*., 2000; and references therein).

To estimate the *in situ* abundance of cells, we had to estimate their growth rate. Takacs and Priscu (1998) measured the growth rate for prokaryotic cells as 1.0×10^{-10} (μg C) $cell^{-1}day^{-1}$ in the surface waters of the east lobe of the perennially ice covered Lake Bonney, McMurdo Dry Valleys, Antarctica (ELB). Assuming a conversion factor of 11 (fg C) $cell^{-1}$, the cell specific growth rate for ELB is 0.0091 day^{-1} (Takacs and Priscu, 1998). These values are similar to those of other cold aquatic environments, but are lower than mesophilic environments (Takacs and Priscu, 1998 and references therein). To determine the cell abundance at the time of sampling, we used the standard growth formula:

$$N_0 = N_t \times e^{-kt}$$

where N_t = number of cells at time t, N_0 = initial cell abundance, k = cell specific growth rate (estimated here based on the ELB values), and t = time (here, 450 days).

Plate counts and analysis of cultures

Ten-fold serial dilutions of sediments from 10^{-2}- to 10^{-7}-fold for the upper and lower halves of the core were plated in triplicate onto R2A media (Atlas, 1993) and incubated at room temperature (~22°C) or 4°C either aerobically for 5 weeks (room temperature) or 3 months (4°C) or under anaerobic conditions at the same temperatures for up to 6 months. Plate counts were determined by the mean of all plates showing between 20 and 200 colonies per plate. Randomly selected colonies were re-plated on R2A media and single colonies were then analysed for growth in liquid R2 medium. Liquid cultures were incubated at 9°C and 25°C and growth was assessed periodically for up to 3 months.

16S rRNA gene analysis

DNA was isolated from cultures and 16S rRNA gene sequences were determined as previously described (Lanoil *et al*., 2000; Skidmore *et al*., 2005). DNA was extracted from sediments with a FastPrep Soil DNA extraction kit as recommended by the manufacturer (MP Biomedicals, Solon, OH). DGGE and clone library analysis were performed as previously described (Gaidos *et al*., 2004; Skidmore *et al*., 2005). For details of the phylogenetic analysis, see the legend to Fig. 2.

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Author contributions

B.D.L. conceptualized and coordinated the project. M.S and B.D.L. performed culture work, and B.D.L., M.S., S.H. and W.F. did 16S rRNA gene clone library construction and analysis, gene sequencing and phylogenetic analysis. S.W.V., S.T. and H.E. collected and performed initial physical characterization of the samples. S.W.V and M.S. performed chemical analyses. J.C.P. calculated subglacial abundance and carbon content of prokaryotes. B.D.L., M.S., J.C.P., S.W.V. and S.T. prepared the manuscript.

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