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A Contemporary Microbially Maintained Subglacial Ferrous "Ocean"

Jill A. Mikucki,^{1*} Ann Pearson,¹ David T. Johnston,² Alexandra V. Turchyn,³ James Farquhar,⁴ Daniel P. Schrag,¹ Ariel D. Anbar,⁵ John C. Priscu,⁶ Peter A. Lee⁷

An active microbial assemblage cycles sulfur in a sulfate-rich, ancient marine brine beneath Taylor Glacier, an outlet glacier of the East Antarctic Ice Sheet, with Fe(III) serving as the terminal electron acceptor. Isotopic measurements of sulfate, water, carbonate, and ferrous iron and functional gene analyses of adenosine 5'-phosphosulfate reductase imply that a microbial consortium facilitates a catalytic sulfur cycle. These metabolic pathways result from a limited organic carbon supply because of the absence of contemporary photosynthesis, yielding a subglacial ferrous brine that is anoxic but not sulfidic. Coupled biogeochemical processes below the glacier enable subglacial microbes to grow in extended isolation, demonstrating how analogous organic-starved systems, such as Neoproterozoic oceans, accumulated Fe(II) despite the presence of an active sulfur cycle.

Subglacial environments represent a largely unexplored component of Earth's biosphere (1). In the McMurdo Dry Valleys, Antarctica, an iron-rich subglacial outflow (Blood Falls) flows from the Taylor Glacier (Fig. 1A), providing unique access to a subglacial ecosystem. The likely fluid source to Blood Falls is a pool of marine brine of unknown depth trapped underneath the glacier ~4 km from the glacier snout where the overlying ice is ~400 m thick (2). Pliocene surface uplift of the Taylor Valley floor, and the associated recession of the Ross Sea Embayment, isolated this pocket of brine (3). Before isolation from direct contact with the atmosphere, the brine was cryoconcentrated (4, 5), resulting in hypersalinity (~1375 mM Cl⁻). This brine has been isolated for at least 1.5 million years (My), when the Taylor Glacier last advanced over the area (6). Although the brine at

present is anoxic and highly ferrous and the pH is circumneutral (Table 1), activity and DNA sequence data reveal that it supports a metabolically active, largely marine microbial assemblage (7).

Taylor Glacier is frozen to its bed, and surface-derived water does not penetrate to the base (8). Poorly understood hydrologic controls result in episodic release of brine. The data in this study are from one of these active discharge events. Salts and the iron mineral goethite rapidly precipitate upon contact of the outflow with the oxidizing atmosphere (5). Although regelation as the glacier slides over the bedrock and brine may add trace amounts of meteoric gases including O₂ (9), multiple geochemical measurements reveal no quantitatively significant contributions. Radiocarbon data confirm that dissolved inorganic carbon (DIC) is old [$\Delta^{14}\text{C}_{\text{DIC}} = -993 \pm 1$ (SE) per mil (‰) (10)]. Presumably, interactions with air at the point of outflow collection are responsible for the small amount of ¹⁴C_{DIC} in our samples (¹⁴C-free would be -1000‰). No dissolved O₂ was detected in the brine [reduction potential (*E_h*) = 90 mV], and iron was 97% Fe(II), indicating that inputs from ice-bound atmospheric O₂ are minimal.

Heterotrophic activity was measured by using ³H-thymidine incorporation (Table 1) (10). Although the most abundant electron acceptor in the brine is SO₄²⁻, isotope data suggest that SO₄²⁻ is not terminally reduced in quantities sufficient to affect isotopes of sulfur. Values of $\delta^{34}\text{S}_{\text{SO}_4}$ and $\Delta^{33}\text{S}_{\text{SO}_4}$ in the brine (21.0‰ and

range = 20.8 to 21.7‰ for ³⁴S_{SO₄}; 0.08‰ and range = 0.06 to 0.09‰ for ³³S_{SO₄}; *n* = 6) were similar to measurements of seawater SO₄²⁻ from the past 15 My measured in marine barites (11). In contrast, values of $\delta^{18}\text{O}_{\text{SO}_4}$ (3.3‰; range = 2.7 to 4.9‰; *n* = 6) were up to 7‰ depleted compared with that of seawater $\delta^{18}\text{O}_{\text{SO}_4}$ from marine barites over the Pliocene [10.4 ± 1.6‰ (12)]. Because values of both $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ in SO₄²⁻ are influenced independently by microbial sulfur metabolism, including sulfate reduction, disproportionation, and reoxidation reactions (13, 14), we expect that both oxygen and sulfur isotopes would be affected. For example, dissimilatory sulfate reduction to sulfide would cause preferential enrichment of ³⁴S (SO₄²⁻ → H₂S, fractionation factor (ϵ) = 20 to 40‰ for natural populations) (15) because ³⁴S-depleted S²⁻ is sequestered in iron sulfides. This would increase the $\delta^{34}\text{S}_{\text{SO}_4}$ value of the remaining sulfate pool.

Our isotope data indicate that incorporation of ¹⁸O-depleted brine water oxygen into sulfate has occurred (Fig. 1B and table S3). During glacial advancement, meltwater mixing with the remaining seawater decreased the $\delta^{18}\text{O}$ value of the brine from a marine value to its current composition ($\delta^{18}\text{O}_{\text{H}_2\text{O}_{\text{brine}}} = -39.5\text{‰}$; Table 1). The depleted value of $\delta^{18}\text{O}_{\text{SO}_4}$ cannot be explained by abiotic oxygen isotope exchange between SO₄²⁻ and water. Such equilibration would take tens of millions of years at subglacial temperatures and pH (16) (Table 1). However, oxygen isotope exchange between sulfite (SO₃²⁻) and water occurs rapidly [50% exchange in <5 min (17)], and the reduction of SO₄²⁻ to SO₃²⁻ is biologically mediated (7). Complete equilibration of SO₄²⁻ via cycling through SO₃²⁻ and exchanging all oxygen atoms with water would result in a 30.9‰ offset from brine water or a value for $\delta^{18}\text{O}_{\text{SO}_4}$ of -8.6‰ (Fig. 1B). This offset represents the temperature-adjusted equilibrium fractionation factor of ¹⁸O in SO₃²⁻ relative to brine H₂O ($\epsilon = 30.9\text{‰}$ at -5.2°C). Given the measured values of $\delta^{18}\text{O}_{\text{SO}_4}$ (2.7‰ to 4.9‰), isotopic mass balance requires that 30 to 40% of the SO₄²⁻ pool has exchanged its O atoms with water, likely through equilibration with SO₃²⁻.

Characterization of the Blood Falls microbial assemblage has revealed taxa that could participate in active sulfur cycling, including autotrophs and heterotrophs (table S1). Sulfate is biologically reduced to SO₃²⁻ by using (phospho)-adenosine 5'-phosphosulfate-reductase by assimilatory [3'-phosphoadenosine 5'-phosphosulfate (PAPS)]

¹Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138 USA. ²Department of Organismic and Evolutionary Biology, Harvard University, Cambridge MA, 02138 USA. ³Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, UK. ⁴Department of Geology, University of Maryland, College Park, MD 20742, USA. ⁵School of Earth and Space Exploration and Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA. ⁶Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, USA. ⁷Hollings Marine Laboratory, College of Charleston, Charleston, SC 29412, USA.

*Present address: Department of Earth Sciences, Dartmouth College, Hanover, NH 03755, USA. To whom correspondence should be addressed. E-mail: jill.a.mikucki@dartmouth.edu

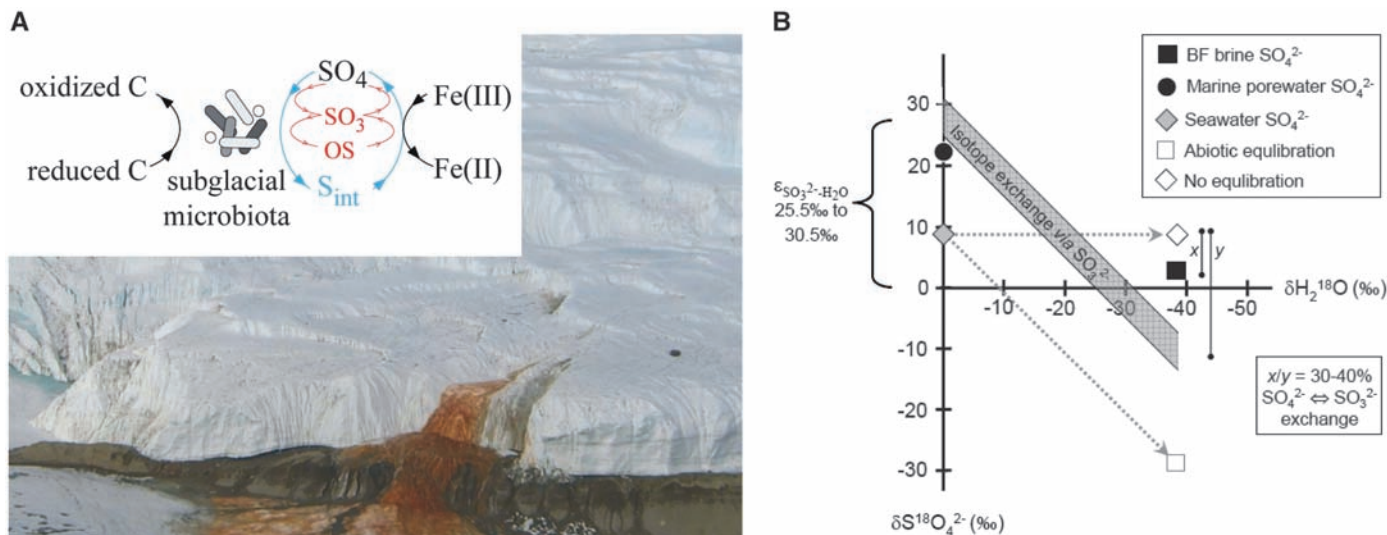


Fig. 1. (A) Blood Falls at the snout of the Taylor Glacier (77°72'S 162°27'E). (Inset) Conceptual model for the possible modes of redox cycling of iron, sulfur, and organic material in the Blood Falls brine based on data from this study. Red arrows indicate assimilatory pathways (via the cell), and blue arrows indicate an alternate pathway via catalytic sulfur cycling mediated by APS reductase. (B) Values of $\delta^{18}\text{O}_{\text{SO}_4^{2-}}$ relative to $\delta\text{H}_2^{18}\text{O}$, given different reaction scenarios. Sulfate and water will have equilibrated by no more than 1‰ within 50 My, therefore no net change would be expected in the $\delta^{18}\text{O}_{\text{SO}_4^{2-}}$ of a seawater brine mixed with ^{18}O -depleted glacier water (gray diamond). Where SO_4^{2-} reduction occurs, SO_3^{2-} equilibrates with the in situ water, $\epsilon = 25$ to 30.5‰, resulting in $\delta^{18}\text{O}_{\text{SO}_4^{2-}}$ predicted by the shaded gray line (15, 24). In marine sediments (32) (black diamond), complete equilibration of SO_3^{2-} with in situ water is observed, indicating quantitative reduction of SO_4^{2-} . If SO_4^{2-} reduction occurred to completion in Blood Falls, values of $\delta^{18}\text{O}_{\text{SO}_4^{2-}}$ between -8 and -10 ‰ (25 to 30.5‰ isotopically heavier than the in situ water) would be expected. Because the data (3.3‰, black circle) plot above this line of complete equilibration, only a portion (30 to 40%) of the total SO_4^{2-} pool has equilibrated.

or dissimilatory (APS) metabolisms, although APS also has been identified in sulfur oxidizers and certain organisms that only assimilate sulfate (18). The majority of APS genes detected in Blood Falls brine (Fig. 2) clustered with APS sequences of known dissimilatory and sulfur-disproportionating species (group 1). Sequences closely related to the APS gene from *Desulfocapsa sulfexigens* are consistent with the presence of a relative of this species among the 16S ribosomal RNA clones (7). Clone APS_20 (group 2) shares relations with sulfur-oxidizing isolates and *Thermacetogenium phaeum*, a syntrophic acetate-oxidizing bacterium that can also reduce sulfate (19). We cannot eliminate the possibility of trace production of H_2S because one sequence (clone B_11) showed distant relation to groups known to mediate complete sulfate reduction (e.g., *Desulfovibrio* spp.). However, we were unable to detect dissimilatory sulfite reductase (*dsrA*) genes across several methods and attempts, and the sulfur isotope data are inconsistent with measurable quantities of sulfide formation.

The presence of metabolically active cells requires a small supply of assimilated sulfur because sulfur composes $\sim 0.1\%$ of cell biomass (15). We estimated a doubling time for heterotrophs of ~ 300 days (Table 1) (10) equaling $\sim 10^6$ generations over 1.5 My of isolation. If all cellular organic sulfur requirements were derived each generation by de novo assimilatory reduction, an upper limit of $\sim 35\%$ of the SO_4^{2-} pool would

Table 1. Biogeochemical parameters of Blood Falls outflow during a brine discharge event. Outflow samples collected in December 2004. nd indicates none detected. The first six parameters and sulfate and chloride concentrations are from (33); total iron is from (7).

Temperature	-5.2°C
pH	6.2
E_h	90 mV
Dissolved oxygen	nd
DIC	55 mM
Dissolved organic carbon (DOC)	420 μM
Dissolved inorganic nitrogen (DIN)	94 μM (100% as NH_4^+)
Total iron	3.45 mM [$>97\%$ as Fe(II)]
Sulfate	50 mM
Chloride	1375 mM
Total cells	$6 \times 10^4 \text{ ml}^{-1}$
Thymidine (Tdr) incorporation rate	$1.9 \times 10^{-4} \text{ nM Tdr day}^{-1}$ ($\pm 2 \times 10^{-5}$)
$\Delta^{14}\text{C}_{\text{DIC}}$	$-993 \pm 1\text{‰}$
$\delta^{34}\text{S}_{\text{Sulfate}}$	$21.0 \pm 0.4\text{‰}$
$\Delta^{33}\text{S}_{\text{Sulfate}}$	0.08‰ (range = 0.06 to 0.09‰)
$\delta^{18}\text{O}_{\text{Sulfate}}$	$3.3 \pm 0.8\text{‰}$
$\delta^{18}\text{O}_{\text{H}_2\text{O}}$	$-39.5 \pm 0.1\text{‰}$
$\delta^{56}\text{Fe}$	$-2.60 \pm 0.5\text{‰}$

have been used. This result is consistent with our estimate of 30 to 40% SO_4^{2-} turnover but is unlikely to provide the full explanation for sulfur cycling in the brine. Initially the system would have included reduced organic nutrients (N, S, and P), including S-containing amino acids, in stoichiometric proportion to the initial supply of organic matter. Availability of this additional pool of biological metabolites could decrease the

effective assimilatory SO_4^{2-} demand substantially. Additionally, the presence of diverse sulfur-cycling microbes (7) and several groups of APS genes indicate the presence of metabolic processes beyond assimilation strictly for biomass.

How then is the additional sulfate cycled? The unchanged values of $\delta^{34}\text{S}_{\text{SO}_4}$ indicate that there is insignificant loss of sulfur to sedimentary pyrite. This implies that the reduction of SO_4^{2-} to

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Recursive Processes in Self-Affirmation: Intervening to Close the Minority Achievement Gap

Geoffrey L. Cohen,^{1*} Julio Garcia,¹ Valerie Purdie-Vaughns,² Nancy Apfel,³ Patricia Brzustoski³

A 2-year follow-up of a randomized field experiment previously reported in *Science* is presented. A subtle intervention to lessen minority students' psychological threat related to being negatively stereotyped in school was tested in an experiment conducted three times with three independent cohorts ($N = 133, 149, \text{ and } 134$). The intervention, a series of brief but structured writing assignments focusing students on a self-affirming value, reduced the racial achievement gap. Over 2 years, the grade point average (GPA) of African Americans was, on average, raised by 0.24 grade points. Low-achieving African Americans were particularly benefited. Their GPA improved, on average, 0.41 points, and their rate of remediation or grade repetition was less (5% versus 18%). Additionally, treated students' self-perceptions showed long-term benefits. Findings suggest that because initial psychological states and performance determine later outcomes by providing a baseline and initial trajectory for a recursive process, apparently small but early alterations in trajectory can have long-term effects. Implications for psychological theory and educational practice are discussed.

Whether and how psychological interventions produce lasting positive consequences are critical questions for scientists and policy-makers. This report presents evidence of how interventions, even brief or subtle, can produce lasting benefit when targeted at important psychological processes. It does so by focusing on the long-term impact of a psychological intervention designed to reduce the racial achievement gap through the lessening of academic underperformance.

The achievement gap between academically at-risk minority students and European American students has long concerned the educational community (1). In a society where economic success depends heavily on scholastic accomplishment, even partial remediation of this gap would be consequential. This is especially true for low-achieving students, given the societal, institutional, and personal costs of academic failure.

Research shows the importance of psychological factors in intellectual achievement (2–4).

¹Department of Psychology, University of Colorado, Muenzinger Psychology Building, Boulder, CO 80309–0345, USA. ²Department of Psychology, Columbia University, 405 Schermerhorn Hall, New York, NY 10027, USA. ³Department of Psychology, Yale University, 2 Hillhouse Avenue, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: cohen.geoff@gmail.com

Situations where one could be judged or treated in light of a negative stereotype can be stressful and thus undermine performance (5–7). For African Americans in school, the concern that they or another African American could be seen as confirming a negative stereotype about their group's intelligence can give rise to stress and depress performance (5–8).

Findings of two randomized field experiments addressing this psychological threat in the classroom were reported in *Science* (8). These tested a values-affirmation intervention. Beginning early in seventh grade, students reflected on an important personal value, such as relationships with friends and family or musical interests, in a series of structured writing assignments. Such self-affirmations reduce psychological threat and stress (9–11) and can thus improve performance. The intervention should benefit students from groups subjected to threat pervasive enough to undermine their average performance—in this case, negatively stereotyped minority students. As predicted, relative both to a control group and to historical norms, one or two administrations of the intervention improved the fall-term grades of African Americans and lowered the psychological availability of the stereotype. European Americans were unaffected (8).

A 2-year follow-up is now reported. We assess whether the affirmation buffers minority

students from the effects of psychological threat over the long term, leading to academic benefits beyond the short-term ones of a single academic term previously found. Generally, psychological processes and their consequences are examined for relatively brief periods, often in experimental studies lasting 30 min or an hour. By contrast, because the present study spans 2 years, its findings speak to how an apparently brief psychological intervention triggers processes that affect performance and psychological outcomes over considerable periods of time. Given the multitude of factors that could mute the effects of such processes in the classroom, the findings address the longevity and real-world significance of these processes. This is particularly important given that the effects of interventions and psychological manipulations often decay and may even reverse over time for reasons that are little understood (12, 13).

Because chronic evaluation is a key aspect of school and work environments, performance in these settings can be self-reinforcing. A recursive cycle, where psychological threat lowers performance, increasing threat and lowering performance further, in a repeating process, can magnify early performance differences among students (14). Early outcomes set the starting point and initial trajectory of a recursive cycle and so can have disproportional influence. For instance, the low self-confidence of students who experience early failure, even by chance, is surprisingly difficult to undo (15). A well-timed intervention could provide appreciable long-term performance benefits through early interruption of a recursive cycle.

Results encompass the original two student cohorts and a third cohort run after the original two experiments. The cohorts were observed for a period running from the first term of seventh grade to the end of eighth grade, typically covering ages 12 to 14. Although the period involves the last 2 years of middle school, for clarity these will henceforth be referred to as Year 1 and 2, respectively. Individual students were randomly assigned to the affirmation condition or the control condition. The former completed affirmation exercises, the latter neutral exercises. The treatment consisted of variations on the original affirmation exercise in which students wrote about the personal importance of a self-defining value (16). The control exercises consisted of variations on the original control exercise in which students wrote about an unimportant value or a similarly