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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. Lee[7]

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 O_2 (*9*), multiple geochemical measurements reveal no quantitatively significant contributions. Radiocarbon data confirm that dissolved inorganic carbon (DIC) is old [$\Delta^{14}C_{DIC}$ = –993 ± 1 (SE) per mil (‰) (*10*)]. Presumably, interactions with air at the point of outflow collection are responsible for the small amount of $^{14}C_{DIC}$ in our samples (^{14}C-free would be –1000‰). No dissolved O_2 was detected in the brine [reduction potential (E_h) = 90 mV], and iron was 97% Fe(II), indicating that inputs from ice-bound atmospheric O_2 are minimal.

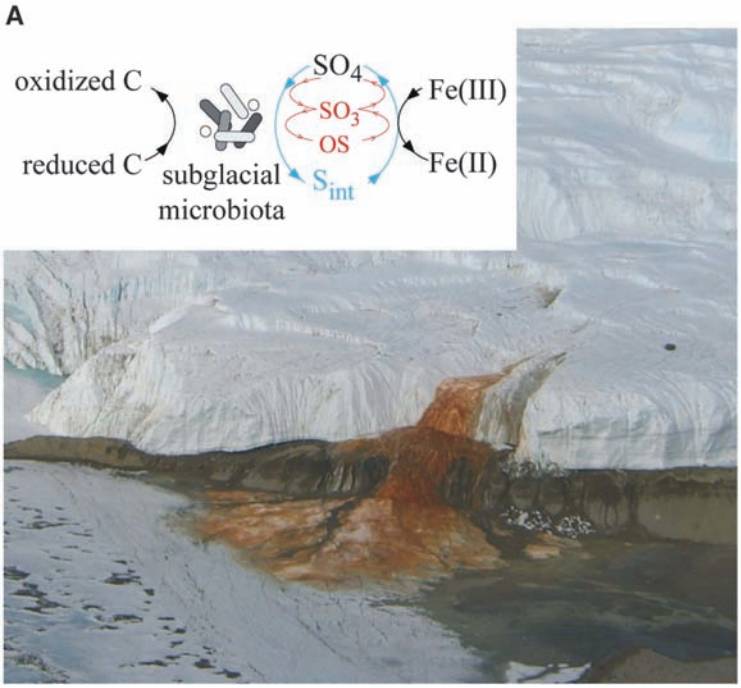
Heterotrophic activity was measured by using ^{3}H-thymidine incorporation (Table 1) (*10*). Although the most abundant electron acceptor in the brine is SO_4^{2-}, isotope data suggest that SO_4^{2-} is not terminally reduced in quantities sufficient to affect isotopes of sulfur. Values of $\delta^{34}S_{SO4}$ and $\Delta^{33}S_{SO4}$ in the brine (21.0‰ and range = 20.8 to 21.7‰ for $^{34}S_{SO4}$; 0.08‰ and range = 0.06 to 0.09‰ for $^{33}S_{SO4}$; n = 6) were similar to measurements of seawater SO_4^{2-} from the past 15 My measured in marine barites (*11*). In contrast, values of $\delta^{18}O_{SO4}$ (3.3‰; range = 2.7 to 4.9‰; n = 6) were up to 7‰ depleted compared with that of seawater $\delta^{18}O_{SO4}$ from marine barites over the Pliocene [10.4 ± 1.6‰ (*12*)]. Because values of both $\delta^{34}S$ and $\delta^{18}O$ in SO_4^{2-} 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 ^{34}S ($SO_4^{2-} \rightarrow H_2S$, fractionation factor (ε) = 20 to 40‰ for natural populations) (*15*) because ^{34}S-depleted S^{2-} is sequestered in iron sulfides. This would increase the $\delta^{34}S_{SO4}$ value of the remaining sulfate pool.

Our isotope data indicate that incorporation of ^{18}O-depleted brine water oxygen into sulfate has occurred (Fig. 1B and table S3). During glacial advancement, meltwater mixing with the remaining seawater decreased the $\delta^{18}O$ value of the brine from a marine value to its current composition ($\delta^{18}H_2O_{Brine}$ = –39.5‰; Table 1). The depleted value of $\delta^{18}O_{SO4}$ cannot be explained by abiotic oxygen isotope exchange between SO_4^{2-} 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 (SO_3^{2-}) and water occurs rapidly [50% exchange in <5 min (*17*)], and the reduction of SO_4^{2-} to SO_3^{2-} is biologically mediated (*7*). Complete equilibration of SO_4^{2-} via cycling through SO_3^{2-} and exchanging all oxygen atoms with water would result in a 30.9‰ offset from brine water or a value for $\delta^{18}O_{SO4}$ of –8.6‰ (Fig. 1B). This offset represents the temperature-adjusted equilibrium fractionation factor of ^{18}O in SO_3^{2-} relative to brine H_2O (ε = 30.9‰ at –5.2°C). Given the measured values of $\delta^{18}O_{SO4}$ (2.7‰ to 4.9‰), isotopic mass balance requires that 30 to 40% of the SO_4^{2-} pool has exchanged its O atoms with water, likely through equilibration with SO_3^{2-}.

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 SO_3^{2-} by using (phospho-)adenosine 5′-phosphosulfate–reductase by assimilatory [3′-phosphoadenosine 5′-phosphosulfate (PAPS)]

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



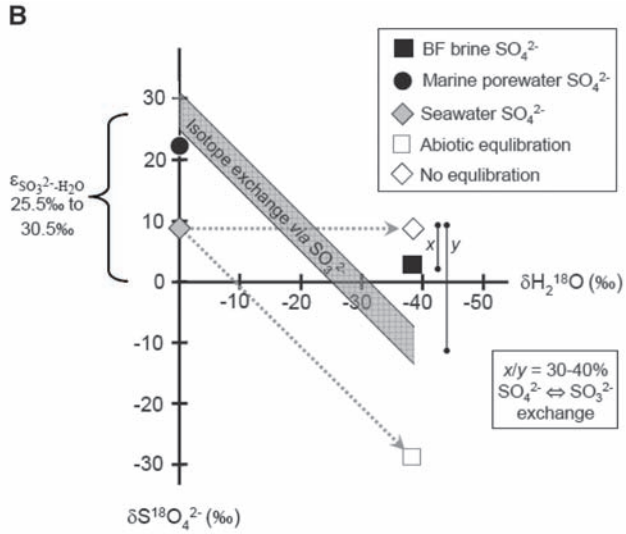


Fig. 1. (**A**) Blood Falls at the snout of the Taylor Glacier (77°72′S 162°27′E). (Inset) Conceptual model for the possible modes of redox cycling of iron, sulfur, and organic material in the Blood Falls brine based on data from this study. Red arrows indicate assimilatory pathways (via the cell), and blue arrows indicate an alternate pathway via catalytic sulfur cycling mediated by APS reductase. (**B**) Values of $\delta^{18}O_{SO4}$ relative to $\delta H_2{}^{18}O$, 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 $\delta^{18}O_{SO4}$ 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, ε = 25 to 30.5‰, resulting in $\delta^{18}O_{SO4}$ 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 $\delta^{18}O_{SO4}$ 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 16*S* 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 measureable quantities of sulfide formation.

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	6×10^4 ml^{-1}
Thymidine (Tdr) incorporation rate	1.9×10^{-4} nM Tdr day^{-1} ($\pm 2 \times 10^{-5}$)
$\Delta^{14}C_{DIC}$	−993 ± 1‰
$\delta^{34}S_{Sulfate}$	21.0 ± 0.4‰
$\Delta^{33}S_{Sulfate}$	0.08‰ (range = 0.06 to 0.09‰)
$\delta^{18}O_{Sulfate}$	3.3 ± 0.8‰
$\delta^{18}O_{H2O}$	−39.5 ± 0.1‰
$\delta^{56}Fe$	−2.60 ± 0.5‰

The presence of metabolically active cells requires a small supply of assimilated sulfur because sulfur composes ~0.1% of cell biomass (*15*). We estimated a doubling time for heterotrophs of ~300 days (Table 1) (*10*) equaling ~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 ~35% of the SO_4^{2-} pool would 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 $\delta^{34}S_{SO4}$ indicate that there is insignificant loss of sulfur to sedimentary pyrite. This implies that the reduction of SO_4^{2-} to

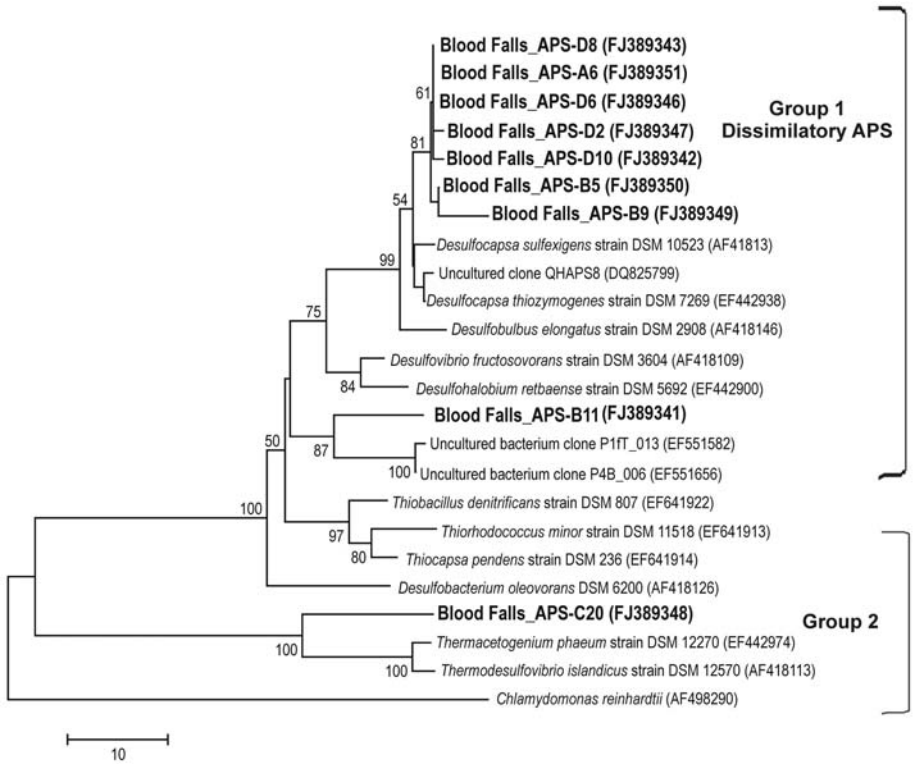


Fig. 2. Neighbor-joining phylogenetic tree of APS-reductase genes cloned from Blood Falls genomic DNA. Results of bootstrap analysis with 1000 replications are noted. The scale bar represents 10 nucleotide substitutions per sequence position. Clones from Blood Falls are in bold. GenBank accession numbers are listed in parentheses.

SO_3^{2-} does not proceed all the way to H_2S, which is consistent with the observation of ferruginous and not euxinic conditions (no detectable H_2S). To achieve these isotopic values, the net metabolism in the brine requires two remarkable properties: (i) SO_4^{2-} must recycle through sulfur intermediates, and (ii) these sulfur intermediates must be quantitatively reoxidized to SO_4^{2-}.

We advocate that sulfur is catalytically cycled to facilitate the oxidation of organic matter in a system in which Fe(III) is the terminal acceptor. Both assimilatory and dissimilatory reduction of SO_4^{2-} proceed enzymatically through intermediate species (*15*). To date, no cultured dissimilatory organism has been described in which SO_4^{2-} reduction does not ultimately terminate in H_2S. The initial steps of $SO_4^{2-} \rightarrow SO_3^{2-} \rightarrow S_2O_3^{2-}$ are endergonic, but progression to S^0 when coupled to common electron donors [e.g., acetate and lactate (*10*)] does yield free energy (table S2). Coupling of any of the subsequent reoxidation reactions to the reduction of Fe(III) would also yield free energy (table S2). Additionally, numerous examples suggest that microbes use carrier molecules, including sulfur compounds, as electron shuttles between cells and iron oxides (*20*–*22*). Cycling sulfur as an electron shuttle to catalyze iron reduction would also be a feasible route to reduced sulfur species in this system.

Such a catalytic cycle would preserve the total dissolved sulfur concentration (Fig. 1, inset) while progressively accumulating Fe(II) to a level controlled by the solubility product constant of siderite. Although the mechanism by which recycling of sulfur species mediates Fe(III) reduction is not fully understood (*23*), in contemporary marine pore waters at depths below O_2 penetration values of $\delta^{18}O_{SO4}$ show SO_4^{2-} regeneration perhaps in association with Fe and Mn oxides (*24*). The net thermodynamics of such a system are favorable and could be described as syntrophic.

Ferric iron likely is mobilized by the scouring action of the glacier over the basement complex of metamorphic rocks intruded by granodioritic and granitic plutons (*25*). The redox state of these rocks is minimally $Fe^{2.4+}$ in the contacts and $Fe^{2.2+}$ in the basement sills and diabase sheets (*26*). A nonzero $\delta^{56}Fe$ signature in Fe(II) dissolved in the brine indicates iron redox cycling, and the strong negative fractionation (–2.6‰; Table 1) implies dissimilatory iron reduction (*27*). Given the elemental abundance of iron in Earth's crust, Fe(III) may be an important electron acceptor for many subglacial microbial ecosystems. Most subglacial environments do not contain entrapped marine salts, but all subglacial biomes interact with their underlying bedrock (*28*), and many contain low concentrations of dissolved organic carbon (*29*). Consequently, glaciers may be important in delivery of soluble Fe(II). Below Taylor Glacier, the subglacial supply of Fe(III) appears to exceed the supply of electron donors such as reduced sulfur and organic matter. Thus, the system is paradoxically rich in electron acceptors relative to electron donors, despite being anaerobic; the result is the absence of euxinia.

During periods of diminished net photosynthesis, such as Neoproterozoic Snowball Earth episodes (*30*, *31*), a decrease in organic flux from reduced photosynthetic production would drive the ocean away from SO_4^{2-} reduction, analogous to the Blood Falls system. When organic matter became sufficiently limited, euxinia would cease and Fe(II) would accumulate. This respiratory source of Fe(II) would add to the flux of hydrothermal Fe to the deep ocean (*13*), and both could lead to an episodically ferruginous ocean (*32*). Importantly, our model is independent of the size of the marine SO_4^{2-} reservoir because it depends only on the ratio of Fe(III) to labile organic matter. The brine below the Taylor Glacier provides a contemporary, natural example of an active, catalytic sulfur cycle, and it uniquely allows for the study of the long-term persistence of life and associated bioenergetics under ice.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/324/5925/397/DC1
Materials and Methods
Fig. S1
Tables S1 to S4

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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 Brzustoski[3]

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, 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*). 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

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

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