Multiple energy sources and metabolic strategies sustain microbial diversity in Antarctic desert soils

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Numerous diverse microorganisms reside in the cold desert soils of continental Antarctica, though we lack a holistic understanding of the metabolic processes that sustain them. Here, we profile the composition, capabilities, and activities of the microbial communities in 16 physicochemically diverse mountainous and glacial soils. We assembled 451 metagenome-assembled genomes from 18 microbial phyla and inferred through Bayesian divergence analysis that the dominant lineages present are likely native to Antarctica. In support of earlier findings, metagenomic analysis revealed that the most abundant and prevalent microorganisms are metabolically versatile aerobes that use atmospheric hydrogen to support aerobic respiration and sometimes carbon fixation. Surprisingly, however, hydrogen oxidation in this region was catalyzed primarily by a phylogenetically and structurally distinct enzyme, the group 1l [NiFe]-hydrogenase, encoded by nine bacterial phyla. Through gas chromatography, we provide evidence that both Antarctic soil communities and an axenic Bacteroidota isolate (*Hymenobacter roseosalivarius*) oxidize atmospheric hydrogen using this enzyme. Based on ex situ rates at environmentally representative temperatures, hydrogen oxidation is theoretically sufficient for soil communities to meet energy requirements and, through metabolic water production, sustain hydration. Diverse carbon monoxide oxidizers and abundant methanotrophs were also active in the soils. We also recovered genomes of microorganisms capable of oxidizing edaphic inorganic nitrogen, sulfur, and iron compounds and harvesting solar energy via microbial rhodopsins and conventional photosystems. Obligately symbiotic bacteria, including Patescibacteria, Chlamydiae, and predatory Bdellovibrionota, were also present. We conclude that microbial diversity in Antarctic soils reflects the coexistence of metabolically flexible mixotrophs with metabolically constrained specialists.

Antarctica | actinobacteria | trace gas | metabolic water | hydrogen

Continental Antarctica is a relatively pristine but oligotrophic wilderness (1, 2). Terrestrial life on the continent is adapted to bioavailability of water, organic carbon, and nitrogen together with extreme temperatures, salt accumulation, and light/dark seasonality (1–3). These cumulative pressures exclude most macroscopic biota. Instead, microorganisms constitute most of the continent's biodiversity and biomass (4). While historical observational surveys indicated that few microorganisms exist in terrestrial Antarctica, subsequent molecular studies have uncovered rich microbial communities, especially in the continent's ice-free regions (5–9). Antarctic soil communities are comparable to mesophilic soils at the phylum level, with Actinobacteriota, Acidobacteriota, Chloroflexota, and Proteobacteria often predominant (2, 7, 8, 10, 11). These communities are highly specialized at lower taxonomic levels (6, 7, 12), however, and have unique functional traits (10, 13). Complementary culture-based studies have also isolated a growing number of bacteria from the continent, although only from seven phyla (predominantly Proteobacteria, Actinobacteriota, Bacteroidota, and Firmicutes) (14–17). Most community members are assumed to be extremely slow-growing or adopt

Significance

Diverse microbial life has been detected in the cold desert soils of Antarctica once thought to be barren. Here, we provide metagenomic, biogeochemical, and culture-based evidence that Antarctic soil microorganisms are phylogenetically and functionally distinct from those in other soils and adopt various metabolic and ecological strategies. The most abundant community members are metabolically versatile aerobes that use ubiquitous atmospheric trace gases to potentially meet energy, carbon, and, through metabolic water production, hydration needs. Lineages capable of harvesting solar energy, oxidizing edaphic inorganic substrates, or adopting symbiotic lifestyles were also identified. Altogether, these findings provide insights into microbial adaptation to extreme water and energy limitation and will inform ongoing efforts to conserve the unique biodiversity on this continent.

Author contributions: M.O., S.L.C., I.D.H., D.A.C., and C.G. conceived the study; D.A.C. and C.G. supervised the study; M.O., P.M.L., D.A.C., and C.G. designed experiments; P.M.L., G.S., and T.J. performed experiments; M.O., P.M.L., G.S., T.J., P.A.N., R.G., D.A.C., and C.G. analyzed data; M.O., P.M.L., R.G., D.A.C., and C.G. wrote the manuscript with input from all authors; I.D.H. and D.A.C. were responsible for the original sampling campaign; M.O. and D.A.C. were responsible for preliminary metagenomic analysis; P.M.L. and C.G. were responsible for metagenomic sequencing, assembly, and annotation; P.M.L., G.S., S.K.B., and C.G. were responsible for community analysis; P.M.L. was responsible for Bayesian divergence analysis; M.O., P.M.L., S.K.B., D.A.C., and C.G. were responsible for enzyme phylogenetic analysis; P.M.L., R.G., and C.G. were responsible for genetic analysis; R.G. and C.G. were responsible for molecular modeling; G.S., T.J., and Z.F.I. were responsible for pure culture experiment; P.M.L., G.S., and C.G. were responsible for biogeochemical analysis; P.M.L., P.A.N., and C.G. were responsible for thermodynamic modeling; P.M.L., M.W.V.G., S.K.G., and C.G. were responsible for geographical and physicochemical analysis; and M.W.V.G., K.J., S.V., and T.P.M. provided theoretical and logistical support.

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dormant states to adapt to the physicochemical conditions of the continent (18). In turn, the formation of a microbial "seed bank" may provide a means to maintain biodiversity (19, 20).

An enduring question is what metabolic strategies enable soil microorganisms to meet energy and carbon needs on this continent (2). Even in dormant states, cells still require a net energy input to maintain cellular integrity, repair damaged macromolecules, and generate a basal membrane potential (21, 22). Conventionally, it was thought that Cyanobacteria and microalgae are the major primary producers in Antarctic soils and that they produce the organic carbon that sustains organoheterotrophic bacteria (2, 10). However, oxygenic photoautotrophs are typically in low abundance (<1% of total bacterial community) outside lithic niches (10, 23) and hence are unlikely to produce sufficient organic carbon to sustain the energy and carbon needs of the dominant community members. More recently, some soil bacteria in the Windmill Islands region of Antarctica were shown to conserve energy and acquire carbon independently of photoautotrophs (11). Combined genome-centric metagenomic and ex situ biogeochemical studies revealed that bacteria from several phyla, including Actinobacteriota, consume molecular hydrogen (H_2) and carbon monoxide (CO) from the atmosphere. By liberating electrons from these ubiquitous and diffusible trace gases, these bacteria can sustain aerobic respiration and fix carbon even when preferred organic substrates are limiting (11, 24). However, this study was limited in geographical scope, focused on a low number of metagenome-assembled genomes (23 MAGs), and did not profile metagenomic short reads (11). As a result, it was not possible to profile the geographic extent, taxonomic diversity, and relative abundance of trace gas oxidizers compared to other chemosynthetic and photosynthetic microorganisms in Antarctic soils. Several molecular and biogeochemical studies have detected signatures of carbon fixation through the Calvin–Benson–Bassham (CBB) cycle within the continent, though it is unclear whether this originates primarily from activities of photoautotrophs or lithoautotrophs (13, 25–28). Molecular evidence also suggests that some Antarctic soil bacteria can also conserve energy through other means, including methanotrophy, nitrification, and rhodopsin-based light harvesting (13, 16, 29–31), though we lack a systematic understanding of the mediators, distribution, and contribution of these processes to the ecosystem energetics of the continent.

Here, we build on these initial findings to test the hypothesis that trace gas oxidation is a common process across Antarctic soils systems and to fully contextualize it in the context of the overall suite of processes and mediators controlling energy conservation and primary production in these environments. We profiled 16 soils with distinct physicochemical properties from the Mackay Glacier region, a cold hyper-arid region in Victoria Land that harbors the largest ice-free area in Antarctica, which is both geographically distant and environmentally distinct (32) from the Windmill Islands from where trace gas oxidation was originally reported. Soil microbial communities in this region are adapted to average annual temperatures of −20 °C, annual precipitation below 50 mm (33), and profound limitation for organic carbon (~0.1%) and nitrogen (~0.02%) (34). Through deep metagenomic sequencing, we generated a resource of 451 MAGs, covering all major microbial lineages in the region. Dominant community members are predicted to be predominantly descendants of ancient lineages uniquely assembled in Antarctic environments. The most abundant bacteria in the region are autochthonous mixotrophs that meet energy, carbon, and hydration needs by scavenging atmospheric trace gases, lending strong support to the recent hypothesis that survival in desert soils depends on continual harvesting of alternative energy sources (18). Nevertheless, these metabolically versatile bacteria coexist with microorganisms that adopt a wide range of other nutritional and ecological strategies, including apparent obligate parasites and predators.

Results and Discussion

Phylogenetically Diverse and Unique Bacteria Coexist across the Mackay Glacier Region. We analyzed surface soils collected from 16 glacial and mountainous sites sampled north of the Mackay Glacier region in South Victoria Land, Antarctica (refer to the map in *SI Appendix*, Fig. S1 and sampling details in *SI Appendix*, *Extended Materials and Methods*). Physicochemical analysis confirmed that the soils varied in key properties (e.g., pH, salinity, micronutrients, and texture), but in common with previously characterized soils from continental Antarctic regions (7, 35, 36), all contained exceptionally low organic carbon content (0.02 to 0.25%) (*SI Appendix*, Fig. S1 and Dataset S1). In line with other recent studies (5, 8), these soils nevertheless supported a substantial number of bacteria and archaea (Fig. 1*A*) (1.7×10^6 to 2.9×10^7 16S ribosomal RNA [rRNA] gene copies per gram dry soil; five- to 100-fold lower than in temperate surface soils) (37). Based on high-resolution 16S rRNA gene amplicon sequencing (*SI Appendix*, Fig. S2), diverse bacteria and archaea live in these soils (observed richness = 832 ± 258 amplicon sequence variants [ASVs], Shannon index = 5.27 ± 0.31) (Fig. 1*A* and *SI Appendix*, Fig. S2). Beta diversity analysis showed that sampled communities significantly vary between regions and with geographic distance (exponential distance decay; $P = 0.001$) and suggested that salinity related variables are key predictors of community composition (*SI Appendix*, Fig. S2).

To determine the community composition of the samples, we retrieved and classified shotgun metagenomic reads of the universal single-copy ribosomal protein gene *rplP* (Dataset S2). The most abundant community members were from seven bacterial phyla known to predominate in soil ecosystems (38, 39). Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexota, Gemmatimonadota, Verrucomicrobiota, and Bacteroidota were particularly abundant (Fig. 1*B*) in agreement with other Antarctic surveys (2, 18). Cyanobacteria were scarce in most soils except for Pegtop Mountain and Cliff Nunatak, accounting for an average of 0.50% in the soil communities. Likewise, Archaea were minor members of this ecosystem (average 0.88%) and mainly comprised the ammonia-oxidizing order Nitrososphaerales (Fig. 1*B*). Surprisingly, bacterial phyla that predominantly adopt a predatory (Bdellovibrionota) (40), intracellular parasitic (Dependentiae and Chlamydiae) (41, 42), or obligately symbiotic (Patescibacteria) (43, 44) lifestyle were prevalent and sometimes highly abundant (e.g., comprising 17% of the community at Mount Murray), suggesting that various symbiotic interactions occur in communities once thought to harbor minimal trophic structure (2). Similar community profiles were observed through 16S rRNA gene amplicon sequencing (*SI Appendix*, Fig. S2 and Dataset S3).

These inferences on the composition and metabolic capabilities of the microbial communities were supported by genome-resolved analysis. From the 99.5 gigabases of sequencing data (Dataset S4), we reconstructed a dereplicated set of 101 high-quality and 350 medium-quality (45) MAGs. The recovered genomes span 18 different phyla (including the first Antarctic soil genomes for 10 phyla) and have a relative composition that reflects the community structure patterns observed in the *rplP* and 16S rRNA gene analysis (Fig. 1*B*). In turn, they capture all major microbial lineages (present at > 1% in all samples) and map to an average of 26% of reads in each metagenome (Dataset S5). Bayesian divergence analysis suggests that the dominant community members have ancient origins (Dataset S6). Most Antarctic MAGs within the 24 analyzed bacterial orders clustered into uniquely monophyletic clades distinct

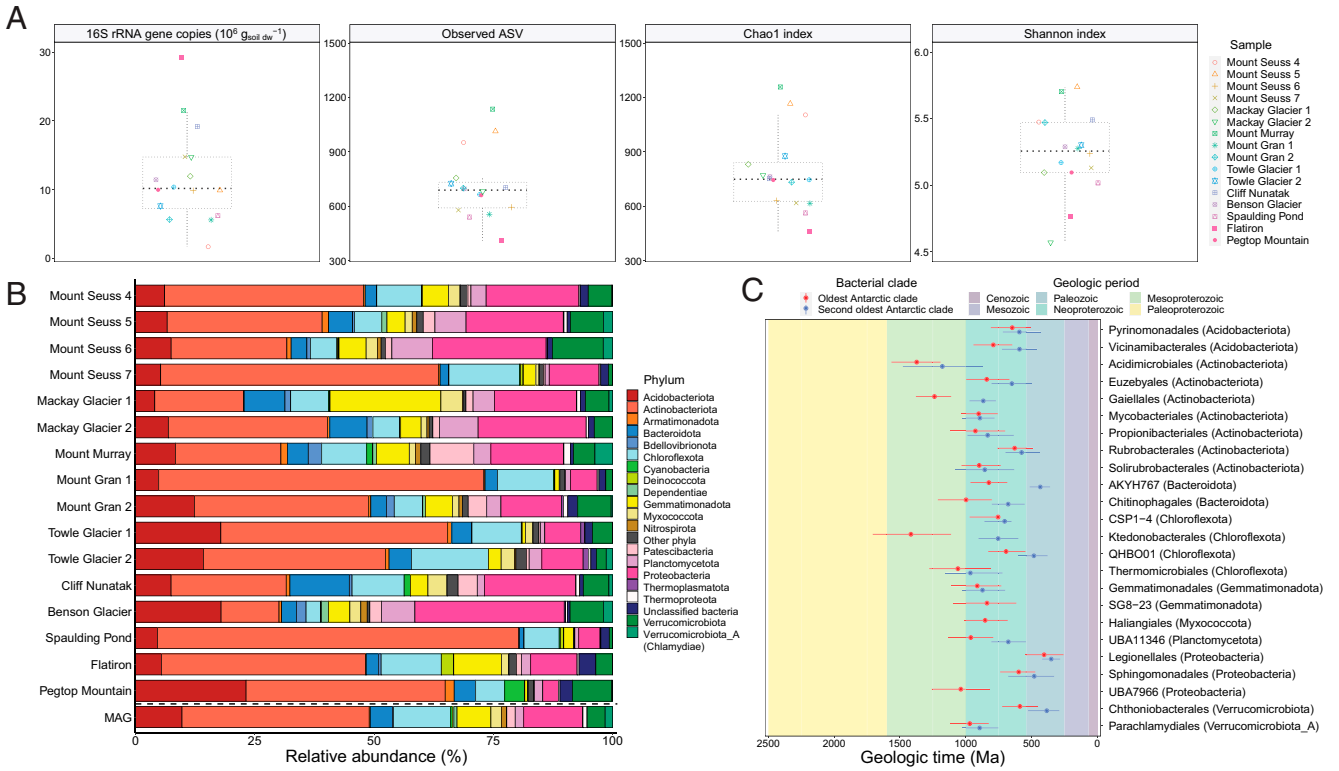

Fig. 1. Abundance, diversity, composition, and phylogenetic divergence of the microbial communities from the Mackay Glacier region. (*A*) Boxplot showing the estimated abundance of bacterial and archaeal taxa based on 16S rRNA gene copy number determined by qPCR and alpha diversity (Observed richness, Chao1 estimated richness, Shannon index) of microbial communities based on 16S rRNA gene amplicon sequence variants. (*B*) Stacked bar chart showing phylum-level community composition based on metagenomic reads of the single-copy marker gene *rplP* and metagenome-assembled genomes. Bacterial and archaeal taxonomy is based on the Genome Taxonomy Database (GTDB) release 05-RS95. Phyla with less than 1% abundance in the sample were grouped to "Other phyla." (*C*) Bayesian divergence estimates (in mega-annum) of uniquely Antarctic clades of metagenome-assembled genomes from 24 bacterial orders. Dots and bars show means and credible intervals of the divergence time estimate of the oldest and second-oldest Antarctic clades.

from genomes obtained outside Antarctica, including from the most abundant orders such as Pyrinomonadales (Acidobacteriota), Solirubrobacterales (Actinobacteriota), Thermomicrobiales (Chloroflexota), Gemmatimonadales (Gemmatomonadota), and Chthoniobacterales (Verrucomicrobiota) (Dataset S11). In agreement with recent divergence time estimates of Antarctic endolithic bacteria (12), the most ancient Antarctic clades appear to have diverged from other known taxa during the pre-Cambrian (541 Ma). The oldest and the second oldest clades are estimated on average to split at 880 and 700 million years ago, respectively (Fig. 1*C*). As elaborated in *SI Appendix*, Supplementary Note 1, this result indicates unique bacteria have natively adapted and recruited in Antarctic soils.

Most Abundant Lineages Encode Enzymes Supporting Trace Gas Oxidation, Including a Divergent Family of [NiFe]-Hydrogenases. We sought to understand which metabolic strategies support the numerous bacteria in these highly oligotrophic soils. We profiled the distribution and affiliation of 52 conserved marker genes representing different energy conservation and carbon acquisition pathways in both the metagenomic short reads (Dataset S7) and MAGs (Dataset S5) and compared their relative abundance with our recently analyzed global soil dataset comprised of 40 metagenomes and 757 MAGs (37) (*SI Appendix*, Supplementary Note 2 and Fig. S3). As expected, almost all community members encoded genes for aerobic organotrophic respiration (Fig. 2), whereas capacity for anaerobic respiration and fermentation was low (*SI Appendix*, Fig. S4). In support of recent findings in the Windmill Islands region (11), the other most-abundant markers included the catalytic subunits of group 1 [NiFe]-hydrogenases (present in average of 90% community members), form I carbon monoxide dehydrogenases (*coxL*; 32%), and RuBisCO (*rbcL*; 27%) (Fig. 2). Phylogenetic analysis revealed that most binned sequences of these enzymes were most closely related to clades that support atmospheric H_2 oxidation (46–49) (Fig. 3*A*), atmospheric CO oxidation (11, 50–52) (*SI Appendix*, Fig. S5), and chemosynthetic CO_2 fixation (11, 53–55) (*SI Appendix*, Fig. S6). Culture-based studies have shown that energy liberated by atmospheric H_2 and CO oxidation supports bacterial persistence during carbon starvation and, in some cases, mixotrophic growth (52, 56–61). This process may also enable continual energy harvesting when diffusion and uptake of nongaseous substrates is restricted by water limitation. Thus, the ability of bacteria to harvest these trace gases may confer a major selective advantage in the carbon- and water-depleted soils of Antarctica. Moreover, in extension of findings made in the Windmill Islands region (11), over a quarter of the community may fix carbon via the CBB cycle, providing a means to generate biomass independent of photoautotrophy (Fig. 2). Consistent with these inferences, uptake hydrogenase and RuBisCO genes were the most abundant genes that were significantly enriched in the Mackay Glacier soils compared to the global dataset ($P < 0.0001$; *SI Appendix*, Fig. S3). These results suggest that atmospheric trace gas oxidation and chemosynthesis is likely a universal microbial process across continental Antarctica.

Genome-resolved analysis confirmed trace gas oxidation genes were encoded by the most abundant and widespread

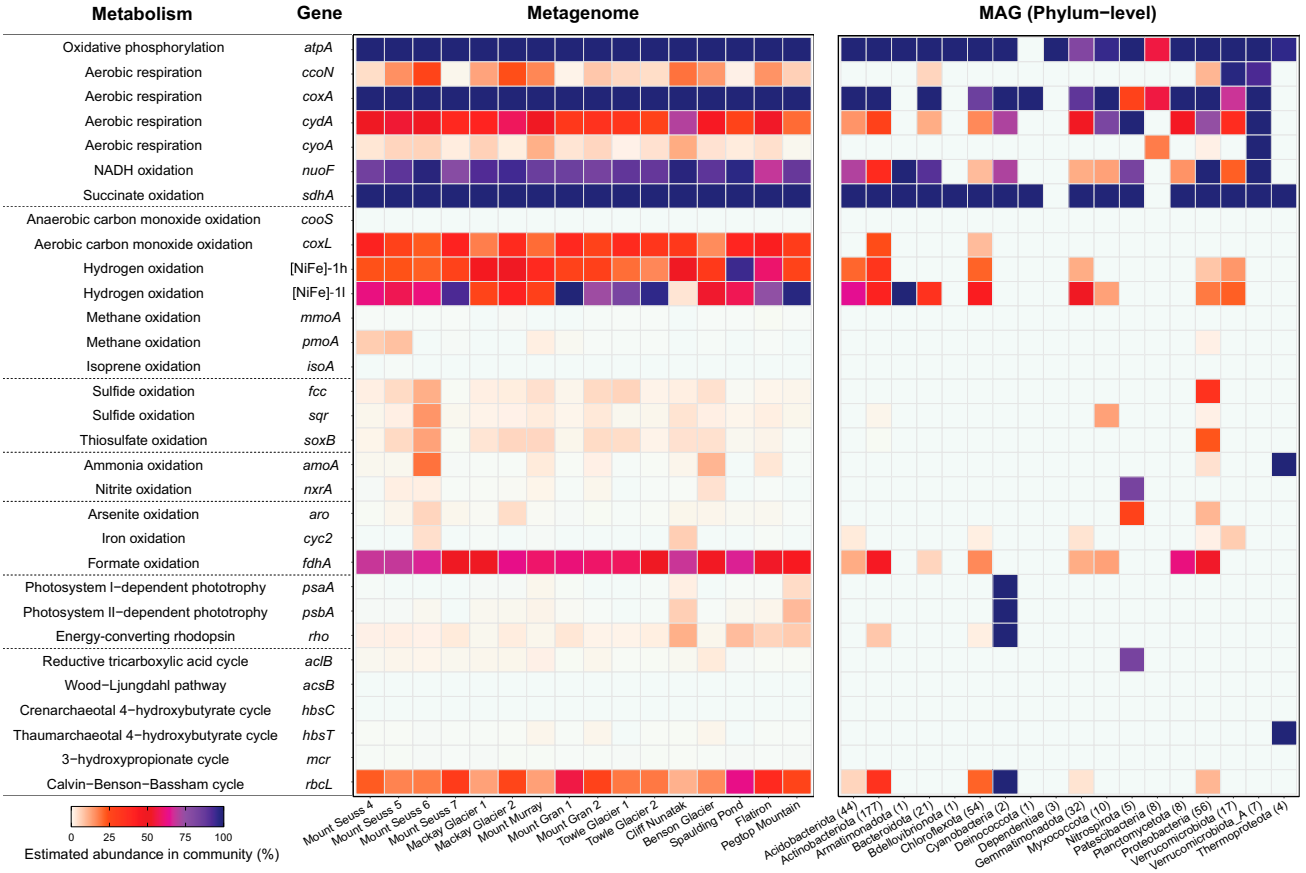

Fig. 2. Metabolic potential of the microbial communities to use inorganic compounds, organic compounds, and light for energy and carbon acquisition. Homology-based searches were used to identify signature genes encoding enzymes associated with (from top to bottom): oxidative phosphorylation, trace gas oxidation, sulfur compound oxidation, nitrification, other oxidative processes, photophosphorylation, and carbon fixation. The left heatmap shows the percentage of total community members predicted to encode each signature metabolic gene. To infer abundance, read counts were normalized to gene length and the abundance of single-copy marker genes. The right heatmap shows the presence of these genes across the 451 metagenome-assembled genomes spanning 18 phyla. Abundance was normalized by predicted MAG completeness.

community members. Uptake hydrogenases were encoded by MAGs from the seven dominant phyla, Myxococcota, and Armatimonadota (Figs. 2 and 3*A*), whereas CO dehydrogenases were confined to Actinobacteriota and Chloroflexota (*SI Appendix*, Fig. S5). Indeed, 17 of the 20 most-abundant Actinobacteriota and Chloroflexota MAGs encoded one or both enzymes (Dataset S5). RuBisCO (*SI Appendix*, Fig. S6 and Dataset S8) were frequently coencoded with hydrogenases (64%) and CO dehydrogenase (25%) in MAGs (Fig. 2 and Dataset S5), suggesting some community members mediate hydrogenotrophic, carboxydotrophic, or mixotrophic growth. This association was pronounced in the abundant uncultivated classes Ellin6529 (Chloroflexota) and UBA4738 (Actinobacteriota) (Dataset S7). These classes are predicted to couple atmospheric H_2 and CO oxidation to carbon fixation via their respective chemosynthetic type IC and IE RuBisCO enzymes (*SI Appendix*, Fig. S6 and Dataset S8). These traits in turn may contribute to their enrichment in the oligotrophic soils of Antarctica (Dataset S2) and other regions (15, 62–65). Given their abundance and genetic potential for atmospheric chemosynthesis (i.e., carbon fixation using atmospheric electron donors) (11, 24), we hypothesize that both classes are major primary producers in these Antarctic soils. As detailed in *SI Appendix*, Supplementary Note 3, we propose replacing the placeholder names UBA4738 with *Candidatus* Aridivitia and Ellin6529 with *Candidatus* Edaphomicrobia, as per recent taxonomic recommendations (66, 67).

Most bacteria in the Mackay Glacier region encoded a hydrogenase family that is phylogenetically and structurally distinct from known lineages (Fig. 2). We generated a maximum-likelihood tree of the conserved catalytic subunits of group 1 [NiFe] hydrogenases using amino acid sequences retrieved from 176 MAGs. All hydrogenase sequences form two major lineages that share less than 40% sequence identity with each other and were supported by robust bootstrapping (Fig. 3*A*). One branch is associated with characterized group 1 h [NiFe] hydrogenases from multiple bacterial isolates (46–48, 51, 60) and was also previously detected in Antarctic MAGs from the Windmill Islands region (11). The other divergent branch, herein the group 1l [NiFe]-hydrogenase, includes the previously unreported sole hydrogenase of the McMurdo Dry Valleys isolate *Hymenobacter roseosalivarius* (68). Group 1l is the most-abundant hydrogenase family within the Mackay Glacier region (2.3 times higher than group 1 h; Dataset S5) and is encoded by all nine hydrogenase-bearing phyla, including the two candidate classes. Moreover, the relative abundance of the marker gene for this hydrogenase was 19-fold higher in the Mackay Glacier metagenomes compared to those of non-Antarctic soils ($P = 2 \times 10^{-8}$; *SI Appendix*, Fig. S3). As elaborated in *SI Appendix*, Supplementary Note 4, structural modeling shows that this enzyme shares common structural features with previously characterized group 1 h [NiFe]-hydrogenase (69, 70) but contains large sequence insertions and a substitution in a residue ligating the proximal iron–sulfur cluster (Fig. 3*C* and *SI*

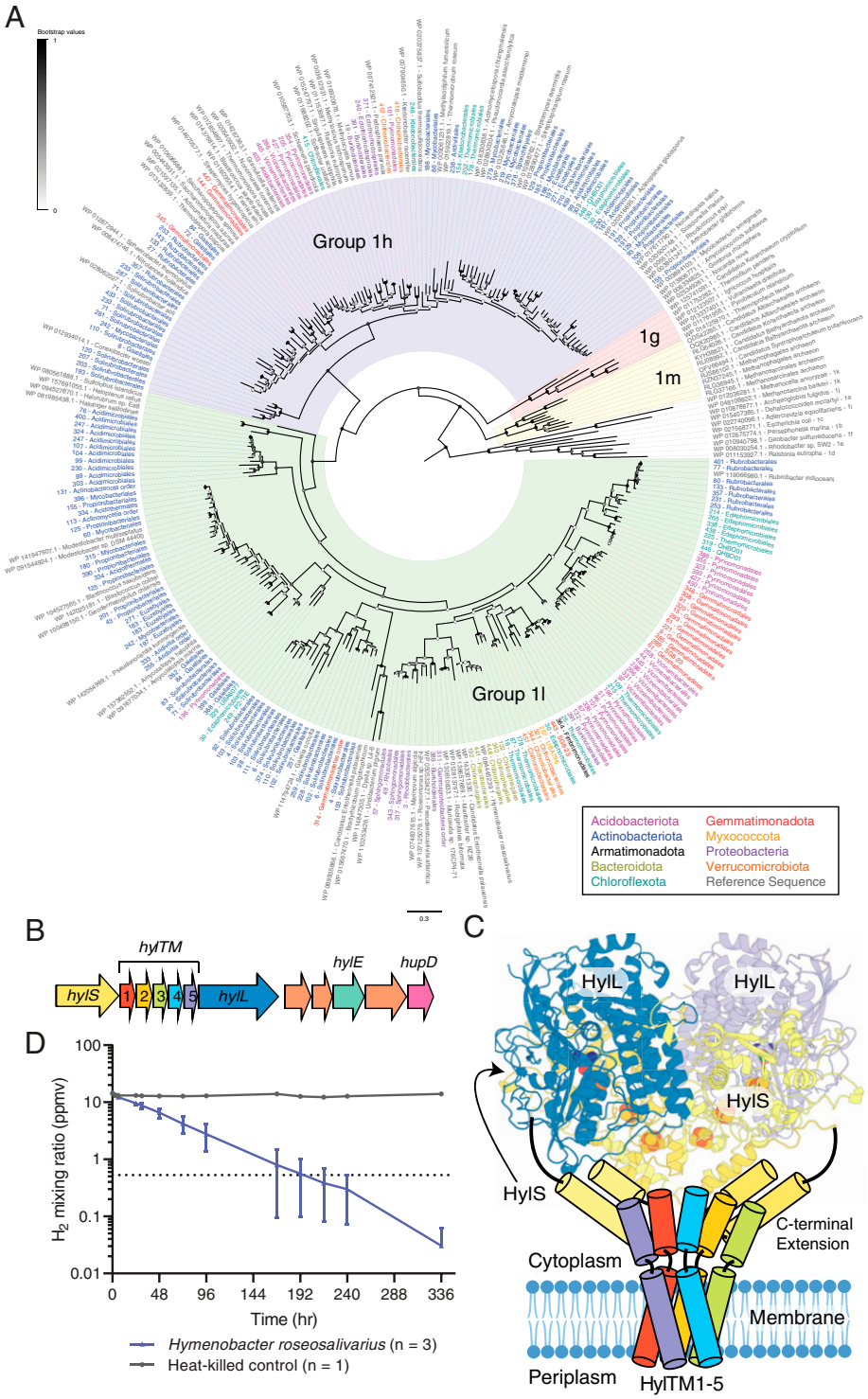

Fig. 3. Identification of the active group 1l family of [NiFe] hydrogenases widespread in the Antarctic soil bacterial communities. (*A*) Maximum-likelihood phylogenetic tree showing the sequence divergence of group 1 [NiFe] hydrogenases identified in MAGs from this study. Amino acid sequences retrieved from the reconstructed genomes were aligned against reference sequences (bootstrapped with 50 replicates). Branches of group 1 [NiFe] hydrogenases are shaded according to the subgroup classification, and tips are colored based on phylum-level affiliation of the sequence. All sequences from MAGs of the Mackay Glacier region clustered with the catalytic subunit sequences of either the well-characterized group 1 h [NiFe]-hydrogenases (HhyL) or the previously unreported group 1l [NiFe]-hydrogenases (HylL). All amino acid sequences used to generate this phylogenetic tree are included in Dataset S5. (*B*) Representative genetic organization of group 1l [NiFe] hydrogenase gene cluster derived from the Antarctic bacterium *H. roseosalivarius*. This shows the predicted open reading frames for the large (*hylL*) and small (*hylS*) hydrogenase subunits, the five interposing, short, predicted transmembrane proteins (*hylTM1-5*), a predicted electron-relaying Rieske-type protein (*hylE*), and a maturation endopeptidase (*hupD*). Conserved open reading frames with no predicted function are shown but not labeled. (*C*) Three-dimensional model of the group 1l [NiFe] hydrogenase. This shows a structural homology model of a heterotetramer of HylL and HylS subunits as a ribbon representation and a cartoon of a speculative complex between the hydrogenase and genetically associated HylTM proteins. (*D*) H_2 oxidation by pure stationary-phase cultures of the Antarctic isolate *H. roseosalivarius*. Autoclaved cells were included as a negative control. Error bars show the SD of three biological replicates, and the dotted line denotes the average atmospheric mixing ratio of H_2 (0.53 ppmv).

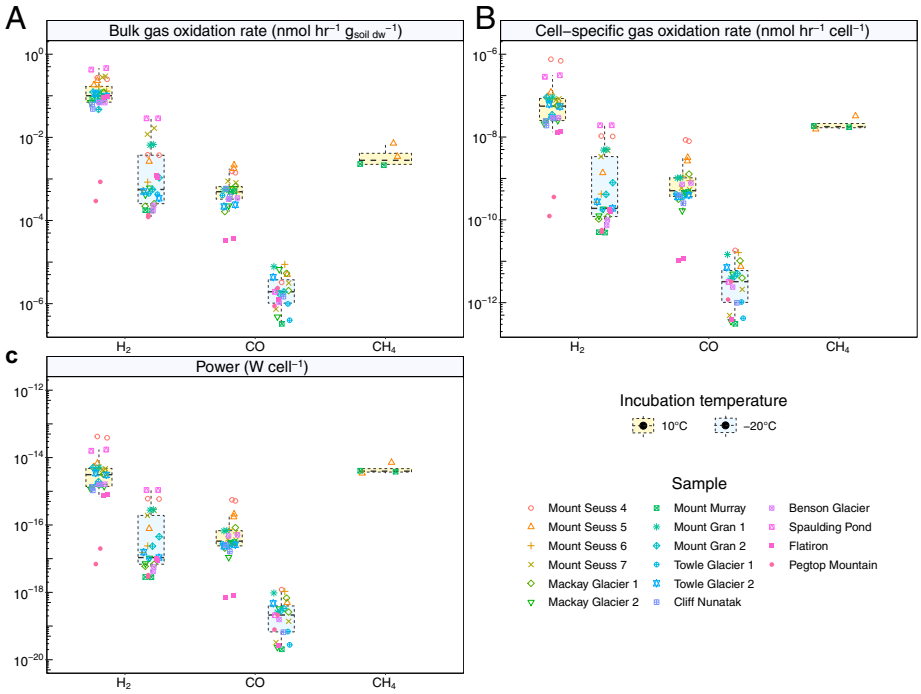

Fig. 4. Rates and energy yield of atmospheric trace gas oxidation by soils sampled from the Mackay Glacier region. Boxplots show rates of oxidation of atmospheric H_2, CO, and CH_4 for each soil in duplicate soil microcosms at 10 °C and −20 °C, based on gas chromatography measurements. Only rates for samples with detectable gas oxidation are shown. (*A*) Atmospheric gas oxidation rates for each microcosm normalized to dry weight of soil. (*B*) Cell-specific reaction rates for each microcosm. Cell-specific rates were estimated by dividing the estimated soil cell abundance and proportion of gas oxidizers based on quantitative qPCR and metagenome short-read analysis (*hhyL* and *hylL* abundance for H_2, *coxL* abundance for CO, and *pmoA* and *mmoA* abundance for CH_4). (*C*) Energy yield per cell from oxidation of each trace gas at mean atmospheric concentrations based on thermodynamic modeling.

Appendix, Fig. S8). The genomic organization of the hydrogenase-encoding region is also unusual, with five small open reading frames predicted to encode transmembrane proteins separating the small and large core structural subunits (Fig. 3*B* and *SI Appendix*, Fig. S7 and Dataset S8). To test activity of this hydrogenase lineage, we showed that an axenic stationary-phase culture of *H. roseosalivarius* oxidized H_2 below atmospheric concentrations (Fig. 3*D*). The group 1l [NiFe]-hydrogenase likely accounts for this activity given the bacterium encodes no other known enzymes capable of H_2 metabolism, though further genetic or biochemical studies would be required to definitively confirm this. In turn, this observation extends the phenomenon of atmospheric H_2 oxidation to a seventh microbial phylum (Bacteroidota) (48, 51, 59, 60, 71) and likely a fourth hydrogenase family (47, 72, 73). Integrating these findings, we predict that the group 1l [NiFe]-hydrogenase is a bona fide high-affinity, membrane-associated enzyme that relays electrons derived from atmospheric H_2 through the respiratory chain. The broad distribution and predominance of this hydrogenase suggests that it is the primary mediator of H_2 oxidation in these soils. Moreover, given the strong positive correlation between this hydrogenase and RuBisCO based on the MAGs and metagenomic short reads ($R^2 = 0.68$, $P = 0.002$) (*SI Appendix*, Fig. S9 and Dataset S10), electrons yielded by this enzyme may support carbon fixation.

Trace Gas Consumption Occurs at Sufficient Rates to Meet Energy Needs and Support Hydration of Mackay Glacier Region Bacteria. Our metagenomic analyses suggest that the most abundant soil bacteria across the Mackay Glacier region conserve energy and fix carbon by oxidizing atmospheric H_2 and CO. To test whether soil communities mediate these activities, we set up soil microcosms in which ambient air headspaces were amended with 10 parts per million (ppmv) of these gases and used high-sensitivity gas chromatography to measure their consumption at 10 °C and −20 °C over time. In line with predictions, H_2 was oxidized by soils from all 16 sites, and all but three soils consumed CO at 10 °C (Fig. 4*A*). Of these, all soils except Pegtop Mountain consumed H_2 to below atmospheric concentrations (0.53 ppmv) (74), and 10 soils consumed atmospheric CO (0.09 ppmv) (75) during the time course of our experiments (*SI Appendix*, Fig. S10). The subatmospheric thresholds confirm that these microbial communities can harvest energy from the atmosphere, a virtually unlimited source of diffusive and energy-rich reduced gases (76, 77). Remarkably, soils incubated at −20 °C mediated sustained H_2 oxidation and sometimes CO oxidation (*SI Appendix*, Fig. S11), providing unprecedented evidence of continual microbial activity in Antarctic soils at in situ temperatures. The average rate of atmospheric H_2 oxidation (139 and 4.1 pmol h^{-1} $g_{soil\ dw}{}^{-1}$ at 10 °C and −20 °C, respectively) was much faster than for atmospheric CO oxidation (0.63 and 0.0028 pmol h^{-1} $g_{soil\ dw}{}^{-1}$) (Dataset S9). Given ex situ incubations potentially disturb soils from their native states, field-based flux measurements will be necessary to quantify the true reaction rates in situ; however, the lower incubation temperature used reflects natural conditions and previous studies have shown very strong concordance between in situ and ex situ H_2 oxidation rates across a range of environments (37). These findings together with the higher abundance of putative H_2 oxidizers in the soil communities (Fig. 2) suggest that atmospheric H_2 is likely to be the predominant energy source sustaining these communities. As elaborated in *SI Appendix*, Supplementary Note 5, considerable variations in bulk and normalized oxidation rates were measured for both gases, which were significantly correlated with several measured physicochemical variables (*SI Appendix*, Fig. S9 and Dataset S10).

Cell-specific rates were calculated by normalizing bulk rates against soil microbial abundance and the proportion of trace

gas oxidizers. Cell-specific atmospheric H_2 oxidation rates were high and approximately two orders of magnitude higher than those of CO (Fig. 4*B*). This rate of atmospheric H_2 consumption yields an average of 5.9 and 0.16 fW of power per cell at 10 °C and −20 °C, respectively (Fig. 4*C*), which considerably exceeds the theoretical maintenance requirements of trace gas oxidizers at the temperatures tested (78–80) and is sufficient to support some biomass formation (*SI Appendix*, Supplementary Note 6). A caveat is that these calculations assume a consistent oxidation rate between cells, given that it is not possible to measure atmospheric H_2 uptake into cells, as the gas is not directly incorporated into biomass. In reality, some cells may be more active than others and may generate sufficient power to sustain slow growth. It should also be noted that metabolic water is the major end-product of the aerobic respiration of atmospheric H_2 ($2\,H_2 + O_2 \rightarrow 2\,H_2O$). Given the reported cytosolic orientation of high-affinity hydrogenases and terminal oxidases (61), the water produced would be retained in the cytosol, including as a solvent for macromolecules. Thus, H_2 oxidation may serve a simple but hitherto overlooked mechanism for microorganisms to stay hydrated in the hyper-arid deserts of Antarctica. Based on cell-specific rates of atmospheric H_2 oxidation at 10 °C, a theoretical average of 1.1 million water molecules would be produced per cell each minute. For a cell with an expected 1-μm^3 volume and 70% water content (81, 82), such production rates would be sufficient to replace all cellular water over a 15-d period (Dataset S9). We therefore propose that the metabolic water continuously generated by trace gas oxidation is a quantitatively significant source of hydration in this environment with minimal precipitation (33). Further in situ and ex situ studies should be conducted to thoroughly test this hypothesis, which constitutes a significant change to understanding of the significance of water limitation in Antarctic continental ecosystems (3).

Metabolically Constrained Phototrophs, Lithotrophs, and Organotrophs Coexist with Versatile Mixotrophs in Antarctic Soils. While the most abundant taxa in the Mackay Glacier region appear to be versatile mixotrophs, the genome compendium revealed that these ecosystems also harbor diverse bacteria and archaea with specialist strategies for energy and carbon acquisition. Multiple chemolithoautotrophs were present, including those capable of oxidizing the trace amounts of ammonium, sulfur, and iron detected in the soils (Dataset S1). Ammonium and nitrite oxidizers comprised an average of 2.9% and 1.0% of the communities but together comprised 23% and 15% of the community in Mount Seuss 6 and Benson Glacier samples, respectively (Fig. 2 and Dataset S7). Phylogenetic analysis indicated that Nitrososphaerales (archaea) and Burkholderiales (bacteria) were the dominant ammonium oxidizers (*SI Appendix*, Fig. S12), in line with previous reports for McMurdo Dry Valley soils (29), whereas Nitrospirota were the main nitrite oxidizers (*SI Appendix*, Fig. S13). These nitrifiers also respectively encoded the signature enzymes to fix carbon through the archaeal 4-hydroxybutyrate cycle (*SI Appendix*, Fig. S14), proteobacterial CBB cycle (*SI Appendix*, Fig. S4), and nitrospirotal reverse tricarboxylic acid cycle (*SI Appendix*, Fig. S15), suggesting that multiple chemosynthetic primary production strategies sustain biodiversity in these oligotrophic soils. The marker genes for sulfide and thiosulfate oxidation (*sqr*, *fcc*, and *soxB*) were each encoded by 1 to 4% of community members in most soils (Fig. 2 and Dataset S5), including multiple Burkholderiales MAGs and several other lineages (*SI Appendix*, Figs. S16–S18). The genes to oxidize ferrous iron via the c-type cytochrome *cyc2* were widespread in Mount Seuss 6 (4.7%) and Cliff Nunatak samples (7.3%) and present in select MAGs from five major phyla (*SI Appendix*, Fig. S19). Thus, atmospheric and edaphic inorganic compounds alike are major energy sources for Antarctic soil communities, though their relative importance varies across the physicochemically diverse soils from the region.

Our metagenomic analysis suggests that light energy supports few photoautotrophs but numerous photoheterotrophs in the region. Reflecting cyanobacterial distributions across the region (Fig. 1*B* and *SI Appendix*, Fig. S2), photosystems responsible for oxygenic photosynthesis were encoded by few community members except in the Pegtop Mountain and Cliff Nunatak samples (Fig. 2). Such observations agree with previous amplicon-based inferences that water deficiency constrains photoautotroph abundance in Antarctic soils (2, 7, 8, 27). We nevertheless detected some photosystem II sequences affiliated with proteobacterial anoxygenic photoheterotrophs (*SI Appendix*, Fig. S20). In contrast, energy-converting microbial rhodopsins were prevalent and abundant across the region (Fig. 2). These light-powered proton pumps are well characterized for their role in energy conservation in marine ecosystems (83–86), though they have been scarcely studied in desert environments (87). As outlined by the "continual energy harvesting hypothesis," sunlight (like atmospheric trace gases) is a relatively dependable energy source during the polar summer and hence lineages that harvest it may have a selective advantage in energy-poor desert soils (18). In line with this theory, energy-converting rhodopsin genes were significantly more abundant in the Mackay Glacier metagenomes than those of global soils ($P = 3.6 \times 10^{-13}$; *SI Appendix*, Fig. S3). Rhodopsins were present in several of the most dominant orders of Actinobacteriota and Chloroflexota in these soils (Dataset S5). They were also present in both cyanobacterial MAGs, thereby providing a means for photoautotrophs to conserve energy when water for oxygenic photosynthesis is limiting. Phylogenetic analysis confirmed the binned and unbinned sequences fell into diverse clades (*SI Appendix*, Fig. S21), including two clades that were most closely related (<50% sequence identity) to the biochemically characterized, energy-converting rhodopsins of halophilic archaea (bacteriorhodopsins) (88) and *Pantoea* species (pantorhodopsins) (89).

A total 20 MAGs were also recovered for the phyla known to adopt obligately symbiotic lifestyles, namely Patescibacteria, Chlamydiae, Dependentiae, and Bdellovibrionota (Dataset S5). All four phyla appear to be obligate organoheterotrophs that lack alternative pathways for energy conservation or carbon acquisition (Fig. 2). Based on previous reports, all characterized Bdellovibrionota predate bacterial species (40), whereas Chlamydiae and Dependentiae are likely to be parasites of protist or arthropod species (41, 42, 90), such as populations of springtails (Collembola) identified within the same sampling area (91). Signature genes associated with the symbiotic lifestyles of each MAG were detected, for example, host-targeted peptidoglycan metalloendopeptidases and self-protection proteins that Bdellovibrionota use to invade cells of bacterial prey (92, 93) as well as ankyrin repeat and WD40 repeat proteins implicated in modulation of eukaryotic hosts by Dependentiae (42, 90) (Dataset S5). Also in line with an obligately symbiotic lifestyle, several lineages have ultra-small genomes when adjusted for completeness, namely, the eight Patescibacteria MAGs (average 1.3 Mbp), three Dependentiae MAGs (average 1.8 Mbp), and a Rickettsiaceae MAG (1.3 Mbp) (Dataset S5), and are predicted to be auxotrophic for multiple amino acids. Building on the discovery of unexpected symbionts in Antarctic lakes (94, 95), we provide evidence for existence of previously overlooked symbiotic strategies and food web complexity in terrestrial Antarctica and confirm oxic niches for phyla such as Patescibacteria that have primarily been studied in anoxic ecosystems (43, 96, 97).

Finally, we obtained genomic and biogeochemical evidence that atmospheric methane oxidation occurs in nonmaritime Antarctic soils. Based on methane monooxygenase levels in short reads, aerobic methanotrophs are members of the rare biosphere in most of the sampled Antarctic soils but are present in very high levels in three soils, including Mount Seuss 5 (9.4%) (Fig. 2 and Dataset S5). Concordantly, during microcosm incubations, two of

these soils oxidized methane at high cell-specific rates to subatmospheric levels at 10 °C but not −20 °C; derived power was of the same magnitude as H_2 oxidation at 10 °C (average 4.6 fW cell^{-1}), suggesting capacity for slow methanotrophic growth (Fig. 4 and *SI Appendix*, Fig. S8). Genome-resolved analysis suggested that this activity is primarily mediated by a single bacterial species within the gammaproteobacterial order UBA7966, which encodes a particulate methane monooxygenase clustering distantly with sequences from the atmospheric methane-oxidizing clade USCγ (*SI Appendix*, Fig. S22). While this bacterium has a restricted distribution, based on read mapping, it is the most abundant single taxon across the entire region (Dataset S5). Thus, by adopting a relatively specialist lifestyle dependent on assimilating a widely available but catalytically demanding atmospheric substrate, this bacterium fills a distinct ecological niche. Importantly, although methanotroph genomes have previously been reported in Antarctic soils (11, 31), our experimental results confirm that such bacteria are biogeochemically active.

Conclusions

Altogether, these results demonstrate a remarkable diversity of both microbial lineages and metabolic strategies in the resource-poor soils of Antarctica. We show that the most abundant and prevalent bacterial lineages in the Mackay Glacier region are free-living mixotrophs capable of conserving energy and fixing carbon by scavenging the atmospheric trace gases H_2 and CO (i.e., "living on air") (98). In addition to confirming previous findings regarding atmospheric H_2 oxidation (11), we make five additional findings: 1) 90% of bacteria spanning at least nine phyla have the capacity to oxidize H_2 in Mackay Glacier soil, 2) a hydrogenase lineage mediates atmospheric H_2 oxidation, 3) rates of H_2 oxidation at environmentally relevant temperatures are sufficient for bacterial maintenance and potentially low-level growth, 4) H_2-derived metabolic water production may be a quantitatively significantly source of hydration, and 5) an Antarctic isolate and the phylum Bacteroidota can scavenge atmospheric H_2. Several bacteria and archaea also achieve high abundances in specific soils through more specialist strategies, spanning atmospheric methanotrophy, oxygenic photosynthesis, and lithoautotrophic growth on trace edaphic substrates. This environment has in turn selected for a range of as-yet-uncultivated bacterial lineages such as *Ca.* Edaphomicrobia, *Ca.* Aridivitia, and UBA7966 that are predicted to grow using atmospheric substrates as well as previously unreported gene families such as the group 1l [NiFe]-hydrogenases and potential microbial rhodopsins. In addition, a significant minority of community members gain resources through parasitism or predation of microorganisms. Through this combination of strategies, both free-living and symbiotic microorganisms can achieve stable niches in a polyextreme environment. In turn, the metabolic diversity of Antarctic microorganisms likely underlies their surprising phylogenetic diversity.

Additionally, the wealth of metagenomic sequencing data and 451 draft genomes generated by this study provides a valuable resource for two major areas of endeavor. First, these datasets support fundamental research and potentially inform decisions to secure Antarctica's environmental future, given forecasts of changing temperature and water availability (99–101). Thus, in line with one of the six priorities for Antarctic science (102), this resource will provide insights into how life has evolved and adapted on this microbially dominated continent and in turn may respond to forecast change. Second, these findings also contribute to considerations of what processes may sustain life on other cold, dry planets such as Mars. Antarctica has long been considered a potential analog for life elsewhere in the solar system (103). Our work brings that picture into sharper resolution.

Materials and Methods

Detailed descriptions of the abbreviated methods below are provided in *SI Appendix*. *SI Appendix* also provides all details on 16S rRNA gene amplicon sequencing and analysis, hydrogenase sequence analysis and homology modeling, culture-based analysis, and thermodynamic modeling.

Metagenomic Analysis. Soils used for this study were sampled from 16 glacier- or mountain-associated sites in an ∼3,500 km^2 region north of the Mackay Glacier region, South Victoria Land, Antarctica, during January 2015 as previously described (34, 36). Community DNA for metagenomic sequencing was extracted from 0.5 g of soil and sequenced together with an extraction blank control at the Australian Centre for Ecogenomics, University of Queensland. Community profiles in metagenomes were generated using SingleM. Raw metagenomic sequences were subjected to quality and length filtering using BBTools, and processed reads from each sample were assembled individually with metaSPAdes (104) and collectively with MEGAHIT (105). Genome binning was performed using CONCOCT (106), MaxBin2 (107), and MetaBAT2 (108), and resulting bins were dereplicated using the Deplication, Aggregation and Scoring Tool (DAS_Tool) (109), refined using RefineM, and consolidated to a nonredundant set of 451 medium or high-quality MAGs using dRep (110) and CheckM (111). MAG taxonomy was assigned with the Genome Taxonomy Database Toolkit (GTDB-Tk) (112) and open reading frames predicted with Prodigal (113). Bayesian divergence estimation of Antarctic MAGs was performed using the program Bayesian Evolutionary Analysis Sampling Trees (BEAST) (114). For metabolic annotation, metagenomes and derived genomes were searched against 52 custom protein databases of representative metabolic marker genes using DIAMOND (115). The proportion of community members that encode each gene was estimated by dividing the read count for the gene (in reads per kilobase million [RPKM]) by the mean of the read counts of 14 universal single-copy ribosomal marker genes (in RPKM). Maximum-likelihood phylogenetic trees were constructed to verify the presence and visualize the evolutionary history of key metabolic genes in the MAGs and assemblies.

Biogeochemical Analysis. Physicochemical analysis of soils was performed at the Environmental Analysis Laboratory (EAL), Southern Cross University. Soil microcosms were used to determine the capacity of soil microbial communities to oxidize H_2, CO, and CH_4. For each sample from 16 sites, soil was placed in a 120-mL serum vial and incubated at 10 °C (2 g soil used) or −20 °C (4 g soil used) in technical duplicate. The ambient air headspace was amended with ultra-pure H_2, CO, and CH_4 to give starting mixing ratios of ∼10 ppmv for each gas. A total of 2 mL of headspace gas was sampled at regular intervals and measured using a VICI gas chromatograph with a pulsed discharge helium ionization detector and autosampler as previously described (51). Pooled autoclaved soils were prepared as negative controls. Bulk atmospheric gas oxidation rates for each sample were calculated by fitting first-order reaction rate models and correcting against the mean values of negative controls. Soil cell abundance was estimated using 16S rRNA gene copy number from qPCR corrected to the reported average number of 16S rRNA gene copies per genome (i.e., 4.2) (116). Cell-specific gas oxidation rates were then inferred by dividing estimated soil cell abundance and the proportion of corresponding gas oxidizers from metagenomic data. For each of the 16 samples, a two-tailed, all-versus-all Spearman correlation matrix was generated of gas oxidation rates, gas oxidation gene abundances, and soil physicochemical variables.

Data Availability. All amplicon sequencing data, raw metagenomes, metagenomic assemblies, and metagenome-assembled genomes were deposited to the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the BioProject accession no. PRJNA630822. All other study data are included in the article and/or supporting information.

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