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RESEARCH ARTICLE

Adaptation to Environmental Extremes Structures Functional Traits in Biological Soil Crust and Hypolithic Microbial Communities

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ABSTRACT Biological soil crusts (biocrusts) are widespread in drylands and deserts. At the microhabitat scale, they also host hypolithic communities that live under semitranslucent stones. Both environmental niches experience exposure to extreme conditions such as high UV radiation, desiccation, temperature fluctuations, and resource limitation. However, hypolithic communities are somewhat protected from extremes relative to biocrust communities. Conditions are otherwise similar, so comparing them can answer outstanding questions regarding adaptations to environmental extremes. Using metagenomic sequencing, we assessed the functional potential of dryland soil communities and identified the functional underpinnings of ecological niche differentiation in biocrusts versus hypoliths. We also determined the effect of the anchoring photoautotroph (moss or cyanobacteria). Genes and pathways differing in abundance between biocrusts and hypoliths indicate that biocrust communities adapt to the higher levels of UV radiation, desiccation, and temperature extremes through an increased ability to repair damaged DNA, sense and respond to environmental stimuli, and interact with other community members and the environment. Intracellular competition appears to be crucial to both communities, with biocrust communities using the Type VI Secretion System (T6SS) and hypoliths favoring a diversity of antibiotics. The dominant primary producer had a reduced effect on community functional potential compared with niche, but an abundance of genes related to monosaccharide, amino acid, and osmoprotectant uptake in moss-dominated communities indicates reliance on resources provided to heterotrophs by mosses. Our findings indicate that functional traits in dryland communities are driven by adaptations to extremes and we identify strategies that likely enable survival in dryland ecosystems.

IMPORTANCE Biocrusts serve as a keystone element of desert and dryland ecosystems, stabilizing soils, retaining moisture, and serving as a carbon and nitrogen source in oligotrophic environments. Biocrusts cover approximately 12% of the Earth's terrestrial surface but are threatened by climate change and anthropogenic disturbance. Given their keystone role in ecosystem functioning, loss will have wide-spread consequences. Biocrust microbial constituents must withstand polyextreme environmental conditions including high UV exposure, desiccation, oligotrophic conditions, and temperature fluctuations over short time scales. By comparing biocrust communities with co-occurring hypolithic communities (which inhabit the ventral sides of semitranslucent stones and are buffered from environmental extremes), we identified traits that are likely key adaptations to extreme conditions. These include DNA damage repair, environmental sensing and response, and intracellular competition. Comparison of the two niches, which differ primarily in exposure levels to extreme conditions, makes this system ideal for understanding how functional traits are structured by the environment.

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Biological soil crusts (biocrusts) are communities anchored by primary producers such as cyanobacteria, mosses, algae, and lichens, which are accompanied by diverse bacteria, archaea, and fungi (1). In deserts and drylands, biocrusts occupy the first few millimeters of the soil surface, where they perform multiple functions, including nutrient capture and erosion control (2, 3). Globally, biocrusts cover approximately 12% of the Earth's terrestrial surface (4) and contribute significantly to soil stability, hydrology, and carbon and nitrogen cycling at ecosystem scales (1). At the microhabitat scale, drylands sometimes support hypolithic niches on the ventral side of semitranslucent stones (usually quartz) embedded in the soil surface (5). Hypoliths can occur in hyper extreme habitats too harsh to support exposed biocrusts (6–9), but they are also found as dispersed microsites within areas supporting more extensive biocrusts (10).

Dryland soil organisms are physiologically specialized for survival in polyextreme environments characterized by challenges such as high (and low) temperatures, desiccation, intense UV radiation, and nutrient limitation (11). Though both biocrusts and hypoliths experience extreme conditions, the environment in hypolithic microhabitats is buffered compared to biocrusts. In the Mojave Desert, quartz stones reduce light transmission by ~98%, decrease daytime high temperatures by ~2°C, and increase relative humidity by nearly 100% (12). To survive environmental extremes, organisms in the community are typically poikilohydric, capable of equilibrating to the ambient relative humidity of their environment and suspending all metabolic activity in a dried and quiescent state. Once water is reintroduced, poikilohydric organisms resume metabolic activity almost instantaneously through a combination of cellular protective mechanisms deployed during drying (e.g., reactive oxygen species (ROS) scavenging, compatible solutes, mRNPs) and repair mechanisms initiated upon rehydration (13–16). For larger biocrust organisms (e.g., mosses) that may require extensive cellular repair upon rewetting from the desiccated state, the process of rehydration is energetically costly and creates a carbon deficit that must be recovered through a period of photosynthetic activity (17, 18). Thus, while biocrusts are physiologically specialized for environments with low precipitation, they are sensitive to the frequency, timing, and duration of hydration events (19).

In habitats where biocrusts occur, drying events happen quickly relative to the time required for poikilohydric organisms to launch extensive cellular protective processes. Thus, biocrust organisms tend to rely heavily on cellular repair during rehydration as their strategy for tolerating desiccation (20). Although these repair mechanisms are highly efficient (21) larger biocrust organisms such as mosses lose some cellular contents during the process of membrane repair during rehydration, which in turn may provide a nutritional resource to support a diverse community of heterotrophic microbes, a phenomenon coined the 'bryotic pulse' (22).

Photoautotrophs (cyanobacteria, mosses, and lichens) anchor biocrust communities, both physically (i.e., soil aggregation, hydrological controls) and through primary production. Typically, biocrusts are dominated by one type of photoautotroph, which in turn influences the diversity and abundance of other organisms in the community (10, 23–25). The identity of the dominant photoautotroph also influences biocrust multifunctionality and community stability in the presence of climate perturbations (26–29).

The identity of the dominant photoautotroph and taxonomic composition of the rest of the community is at least partially dictated by predictable successional processes (30, 31). Bare soils are first colonized by filamentous cyanobacteria such as *Microcoleus*, which aggregates soil particles with its polysaccharide sheaths and generates organic carbon to support a diverse community of heterotrophic bacteria, including diazotrophs, within the cyanosphere (32). Later successional stages are characterized by darkly pigmented nitrogen-fixing cyanobacteria like *Scytonema*, followed eventually by mosses and/or lichens (23). While cyanobacteria are typically the major photoautotroph found in hypolithic

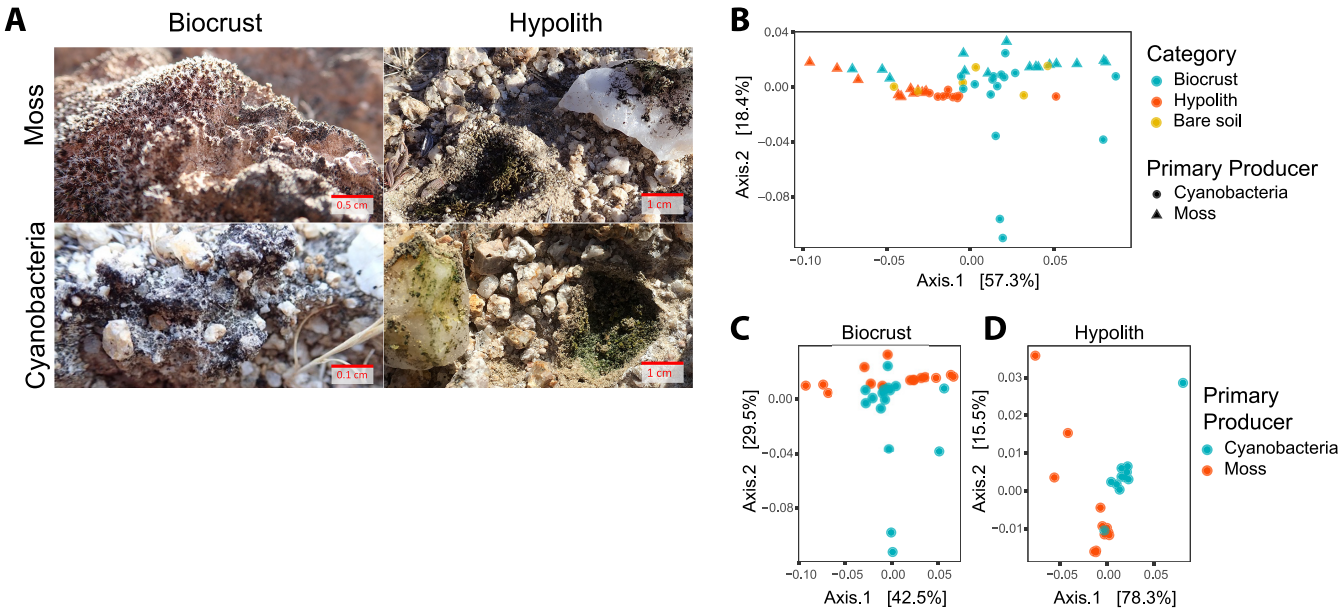

FIG 1 Examples of biocrust and hypolith environments with moss or cyanobacteria as the dominant photoautotroph anchor. (A) Principle Coordinate Analysis (PCoA) ordination plots of (B) all samples, (C) biocrust samples, and (D) hypolith samples. In panel B, samples are colored according to environmental niche (blue, biocrust; red, hypolith; yellow, bare soil) and shaped by dominant primary producer (circle, cyanobacteria; triangle, moss). Panel C shows biocrust samples, which are colored by primary producer (blue, cyanobacteria; red, moss). Panel D shows hypolith samples, which are colored by the same scheme as in panel B.

communities (5, 33), some hypoliths support mosses (5, 10, 34–36). Previous taxonomic work indicated some compositional overlap in microbial communities supported by hypoliths and moss-dominated biocrusts (10), but the extent to which hypolith communities may be functionally distinct from surrounding biocrusts is unknown.

Building on previous work demonstrating that biocrust photoautotrophs affect the taxonomic composition of their associated microbial communities (10, 23, 25) and biocrust ecophysiology/multifunctionality (23, 26, 27, 37), we investigated the degree to which niches (biocrust or hypolithic microsites) harbor communities with distinct functional repertoires using a comparative metagenomics approach. We also assessed the effect of dominant photoautotroph (moss, cyanobacteria) on microbial traits to assess the degree to which the photoautotroph anchor might support communities with distinct functional pathways. Specifically, we set out to test the following hypotheses: (1) hypolithic microsites within regions supporting biocrusts should harbor their own distinct microbial communities enriched in pathways reflective of lower levels of heat and desiccation stress relative to surrounding biocrusts, and (2) the presence of moss in biocrusts creates an important nutritional resource due to the 'leakiness' of gametophyte tissues, and moss biocrusts should support communities with pathways that reflect the utilization of diverse substrates provided by moss leakage in an oligotrophic environment. We sampled replicate cyanobacteria- and moss-dominated biocrusts and hypoliths from two distinct habitats in the Mojave Desert of California. Metagenomic sequence data generated from these samples were then analyzed to compare functional potential across different biocrust and primary producer types to identify adaptive strategies related to survival in extreme dryland environments.

RESULTS

Factors driving differences among sites. We performed metagenomic sequencing on 60 samples from biocrusts, hypoliths, and bare soil at an average depth of 9.06 Gb per sample for a total of 534 Gb (Table S1). Reads were annotated by comparison to the KEGG gene database (Table S3). Ordinations of KEGG gene count data revealed clear differences in genetic repertoire between biocrust and hypolith communities (Fig. 1B). When ordinations

TABLE 1 KEGG pathways significantly enriched in biocrust versus hypolith communities[a]

Pathway no.	Pathway name
ko02020	Two-component system***
ko00540	Flagellar assembly***
ko02040	Cell cycle–Caulobacter***
ko04112	Bacterial secretion system***
ko03070	Bacterial chemotaxis***
ko02030	Lipopolysaccharide biosynthesis***
ko00480	Glutathione metabolism***
ko05111	Biofilm formation–*Vibrio cholerae***
ko00550	Peptidoglycan biosynthesis**
ko03060	Protein export**
ko03440	Homologous recombination*

[a]***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

of biocrust and hypolith communities were plotted separately, samples clustered by the dominant primary producer (cyanobacteria or moss) (Fig. 1C and D).

To determine the effect of environmental niche (biocrust, hypolith, bare soil), dominant primary producer (cyanobacteria, moss), sampling location/season (Sheep Creek Wash/March versus UC Sweeny Granite Mountains Reserve/August), and sample depth (within the biocrust or hypolith versus below) on microbial functional potential, we performed permutational multivariate analysis of variance (PERMANOVA) tests on KEGG gene count data. Functional potential differed significantly between biocrust, hypolith, and bare soil samples ($F = 3.58$, $R^2 = 0.09$, $P = 0.005$) but no other single factor was significant. Environmental niche and primary producer interactions had the strongest effect on microbial community functional potential, explaining 11.4% of the variation in diversity ($F = 9.27$, $P = 0.001$). Primary Producer $\times$ Layer and Primary Producer $\times$ Collection site/month interactions had much smaller (but significant) effects on functional potential (Table S4).

Alpha diversity differed significantly among sample types (ANOVA, $F = 12.76$, $P = 7.29e\text{-}10$; Fig. S1). Diversity was highest in the moss-associated biocrusts and lowest in the samples from below moss-associated biocrusts and hypoliths. Samples where cyanobacteria were the dominant primary producer had similar diversity regardless of environmental niche (biocrust or hypolith) or layer (below or within).

Genes and pathways differentiating biocrust and hypolith communities. Because environmental niche had the strongest effect on gene relative abundances, we first focused our analyses on the genes (Table S5) and pathways (Tables 1 and 2) differing significantly between biocrust and hypolith communities. Those that were more abundant in biocrusts were largely related to survival in extreme environments; specifically, DNA damage repair, environmental sensing and response via the two-component regulatory system, biofilm formation, motility, and the ability to interact and compete via the bacterial secretion system (Table 1). Pathways significantly more abundant in hypolith communities were dominated by secondary metabolite synthesis, including antibiotic biosynthesis (Table 2).

TABLE 2 KEGG pathways significantly enriched in hypolith versus biocrust communities[a]

Pathway no.	Pathway name
ko01055	Biosynthesis of vancomycin group antibiotics***
ko01051	Biosynthesis of ansamycins***
ko00522	Biosynthesis of 12-, 14- and 16-membered macrolides***
ko01056	Biosynthesis of type II polyketide backbone***
ko03013	RNA transport***
ko00650	Butanoate metabolism***
ko00260	Glycine, serine, and threonine metabolism***
ko00680	Methane metabolism***
Ko00720	Carbon fixation pathways in prokaryotes**
ko00195	Photosynthesis**
ko00523	Polyketide sugar unit biosynthesis*
ko00630	Glyoxylate and dicarboxylate metabolism*

[a]***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

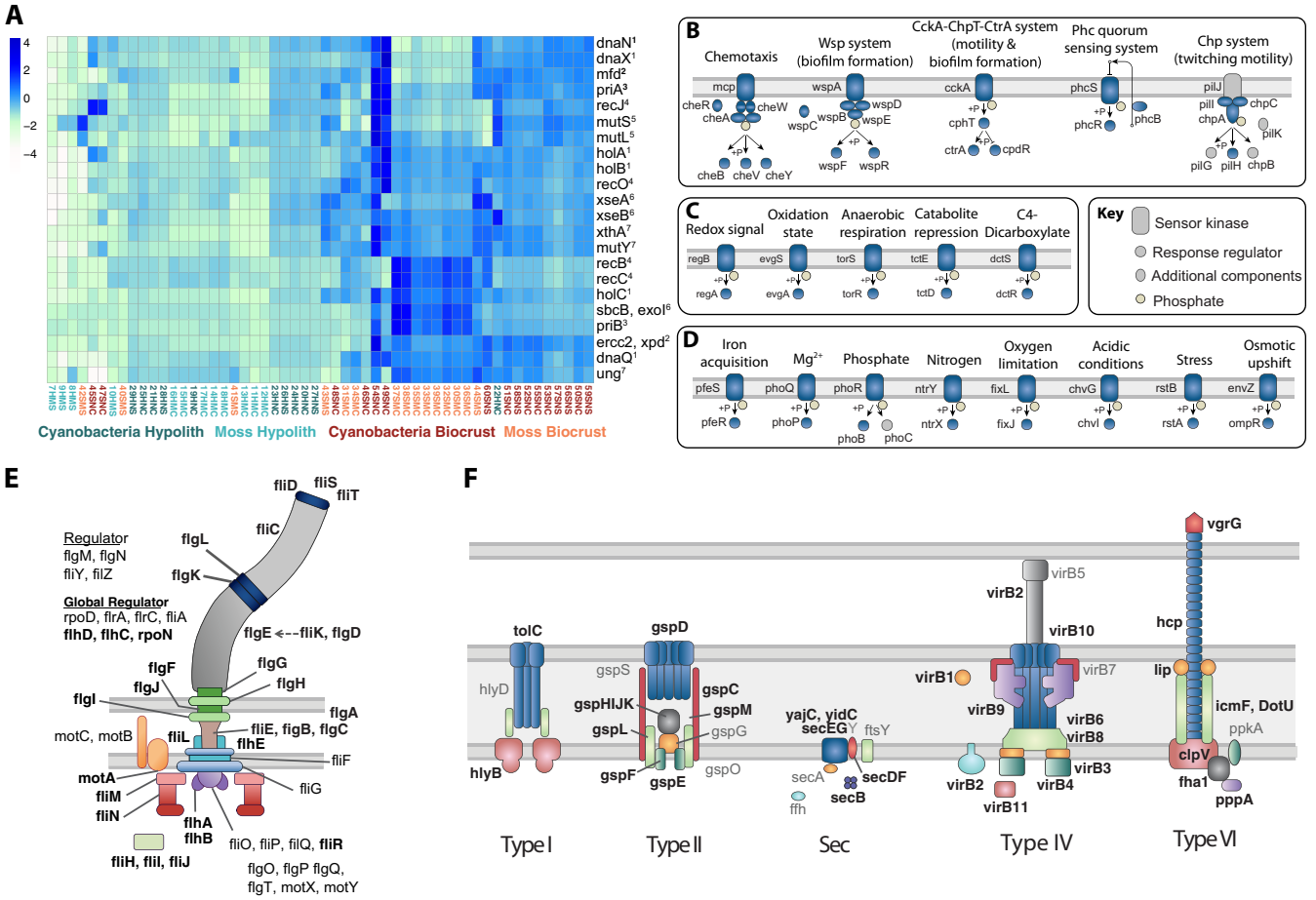

FIG 2 Selected genes and pathways enriched in biocrusts versus hypoliths. (A) Heatmap of DNA repair genes significantly more abundant in biocrust communities versus hypolith communities. Genes are shown in rows and samples are shown in columns. Heatmap colors show the relative abundances of genes scaled by row. Rows are labeled by gene names. Sample names are colored according to the dominant primary producer and environment. Hypolith samples are indicated by cool colors and biocrust samples are shown by warm colors (light blue, moss hypolith; dark blue, cyanobacterial hypolith; orange, moss biocrust; red, cyanobacterial biocrust). Columns and rows were clustered for visualization purposes using the complete linkage method. DNA repair related functions are indicated by superscripts after the gene names as follows: 1, DNA polymerase III; 2, nucleotide excision repair; 3, replication restart; 4, homologous recombination-based repair; 5, mismatch recognition; 6, endonuclease; and 7, base excision repair. A list of genes with the corresponding KEGG orthology numbers can be found in Table S6. (B–D) Selected two component systems enriched in biocrust crust communities. Two component systems canonically contain a sensor kinase (shown as rectangles within the membrane) and response regulator (shown as squares), which mediate downstream cellular responses when phosphorylated. Additional components specific to a particular system are shown in ovals. Phosphate (P) is indicated by yellow circles. Each protein is labeled by the name of the gene that encodes it. Blue indicates genes significantly more abundant in biocrust compared to hypolith communities ($P < 0.01$). Gray shows genes with P-values > 0.01. Panel B contains lifestyle-related two component systems, C contains two component systems related to redox signals and catabolites, and D contains systems related to nutrient limitation and environmental stressors. This figure highlights systems with strong evidence (i.e., multiple significant genes within the system) of being more abundant in biocrusts versus hypoliths. A complete list of genes within the KEGG two component system pathway that were significant is provided in Table S6. (E) Diagram of the KEGG flagellar assembly pathway. Genes significantly more abundant in biocrusts versus hypoliths ($P < 0.01$) are indicated in bold text. Genes where $P > 0.01$ are not bolded. KEGG orthology numbers corresponding to gene names are listed in Table S6. (F) Schematic of bacterial secretion systems significantly more abundant in biocrust compared with hypolith communities. Each component is labeled with a gene name. Names in bold represent significant genes ($P < 0.01$) and names in gray represent nonsignificant genes ($P > 0.01$). A full list of bacterial secretion system genes significantly more abundant in biocrusts versus hypoliths can be found in Table S6. The pathway schematic is based on the KEGG secretion system diagram, (89), and (49).

Genes and pathways enriched in biocrust versus hypolith communities. Commensurate with elevated environmental exposure to UV and desiccation, DNA repair gene frequencies were higher in biocrusts than in hypoliths. At the level of pathway, this was shown by a significant enrichment of the KEGG homologous recombination pathway ($P = 0.018$) and a nearly significant enrichment of the mismatch repair pathway ($P = 0.058$). These observations prompted us to investigate specific genes in both these and other DNA repair pathways that were more abundant in biocrust versus hypolith samples. We identified 22 significant DNA repair genes ($P < 0.01$) with a wide range of repair functions, including double strand break, single strand break, mismatch, base and nucleotide excision repair, and replication restart (Fig. 2A, Table S6). Six subunits of DNA polymerase III, which

is involved in the repair processes listed above and in DNA replication, were also significantly enriched in biocrusts.

The two-component regulatory system is the major means bacteria use to sense environmental signals and modify behavior or physiology accordingly (51). We found that the KEGG two-component system pathway was significantly more abundant in biocrusts compared with hypoliths ($P < 0.001$; Table 1). Significant genes ($P < 0.01$) within the pathway were grouped into three primary categories: lifestyle, redox signals and catabolites, and nutrient limitation and stress (Fig. 2B–D; Table S6). Within the lifestyle category, all genes for the chemosensory pathway of bacterial chemotaxis, the Wsp chemosensory pathway for biofilm formation, and the CckA-ChpT-CtrA phosphorelay system (potentially controlling motility and biofilm formation [52, 53]) were significant. Multiple genes from other chemosensory systems related to twitching motility, quorum sensing, and biofilm formation were also significant (Fig. 2B). In the redox signals and catabolites category (Fig. 2C), we found that both genes in the conserved RegB/RegA signal transduction system, which controls many energy-generating and energy-using processes (54), were significant. Other significant genes in this category include those for sensing oxidation states, anaerobic respiration, catabolite repression, and C4-Dicarboxylate transport. Finally, significant genes within the nutrient limitation and stress category indicate that biocrust community members are better able to sense and respond to low nutrient availability (particularly iron, phosphate, Mg^{2+}, and nitrogen) and certain stressors (osmotic stress, acidic conditions, and oxygen limitation) (Fig. 2D). We also note that RNA polymerase sigma-54 factor, which plays a role in stress response (55) and interacts with multiple two component systems, was significantly more abundant in biocrusts than in hypolith communities.

Providing additional evidence for an increased capacity for motility, the KEGG bacterial flagellar assembly pathway was significantly more abundant in biocrust versus hypolith communities ($P < 0.01$; Table 1). Thirty-six of 54 (66.7%) genes within the pathway were significant ($P < 0.01$, Fig. 2E; Table S6). When genes with a narrow phylogenetic distribution were excluded (H and T ring genes), 75% were significant. The KEGG chemotaxis pathway was also enriched in biocrusts ($P < 0.01$), which was expected because it includes many of the same two-component system genes described previously (Table S6). In the biofilm formation pathway (Table S6), significant genes included Type IV pilus formation and the Type II secretion system.

Bacterial secretion systems are sophisticated protein complexes that transport proteins, small molecules, and DNA into the extracellular milieu or into target cells. The bacterial secretion system KEGG pathway, which includes six secretion systems (types I through VI) and two accessory transport systems (sec and tat), was significantly more abundant in biocrusts than in hypoliths ($P < 0.001$). To determine which of the systems were enriched in biocrust communities, we identified the genes encoding secretion system components that were likewise significant (Fig. 2F; Table S6). We found that four of the six secretion systems (types I, II, IV, and VI) and the sec accessory system were enriched in biocrusts. Eight of the nine genes forming the Type VI secretion system (T6SS), which is one of the main weapons of interbacterial competition, were significantly more abundant in biocrusts versus hypoliths ($P < 0.01$). The Type I and Type II Secretions Systems (T1SS and T2SS, respectively) were also significantly more abundant in biocrusts (Fig. 2F). These, along with T2SS-associated secretion system, transport a large variety of protein substrates into the extracellular environment. Finally, Type IV (T4SS), which is most commonly involved in conjugation, was also enriched in biocrusts.

Genes and pathways enriched in hypolith versus biocrust communities. Five of the 12 pathways significantly more abundant in hypolith versus biocrust communities were related to the synthesis of antibiotics and secondary metabolites (vancomycin group antibiotics, ansamycin antibiotics, macrolides, polyketide backbones, and polyketide sugar units; Table 2). The biosynthesis of vancomycin group antibiotics is a complex process that requires the synthesis of nonstandard amino acids,

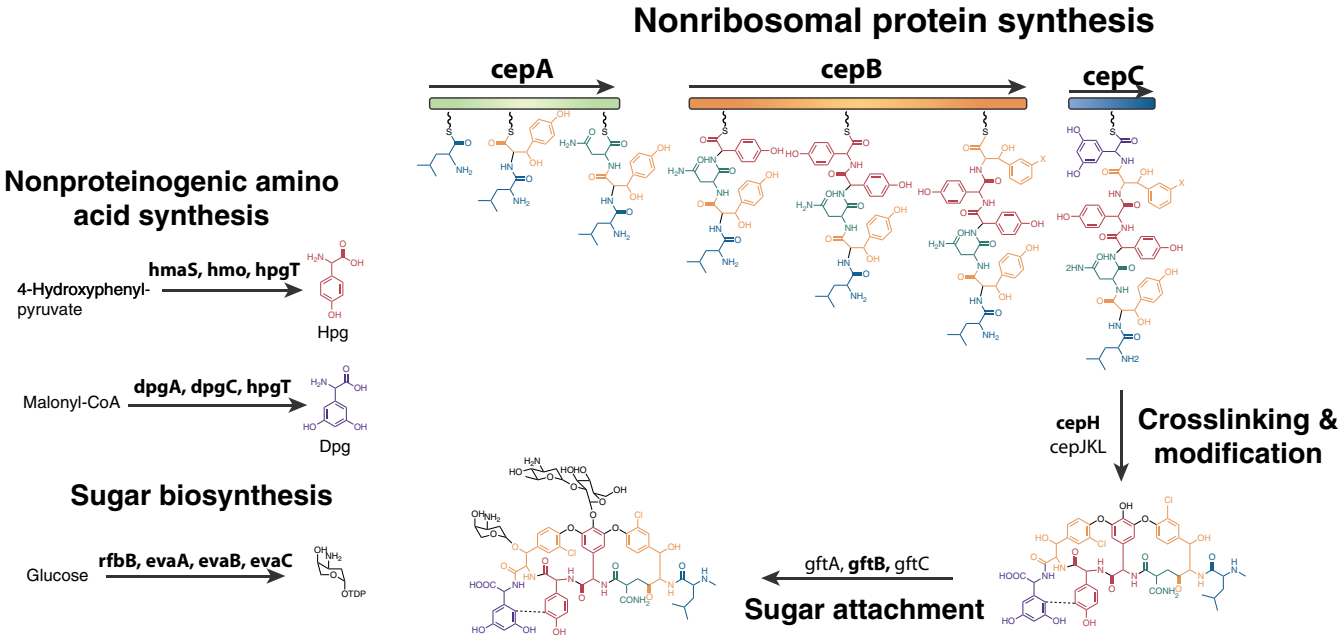

FIG 3 Overview of vancomycin biosynthesis pathway labeled with genes significantly more abundant in hypolith compared with biocrust microbial communities. Schematic represents all major steps in the pathway, including synthesis of nonproteinogenic amino acids 4-Hydroxyphenlglycine (Hpg) and 3,5-Dihydroxyphenylglycine (Dpg), sugar biosynthesis, assembly of the peptide backbone via nonribosomal proteins synthesis, cross-linking and modification, and sugar attachment. Genes significantly more abundant in hypoliths ($P < 0.01$) are indicated by bold text. KEGG orthology numbers corresponding to genes names are in Table S6.

assembly of both standard and nonstandard amino acids into a heptapeptide backbone through nonribosomal protein synthesis, chlorination, oxidative cross-linking, synthesis of sugar moieties, and attachment of sugars to the backbone. Six of the eight nonstandard amino acid synthesis genes in the KEGG pathway were enriched in hypolithic communities ($P < 0.01$), as were all three of the required genes for nonribosomal protein synthesis. Multiple genes for chlorination, sugar moiety biosynthesis, and sugar attachment were also significant (Fig. 3; Table S6).

Like vancomycin group antibiotics, ansamycin (including the antibiotic rifamycin) biosynthesis follows a long multi-step pathway. However, ansamycins differ in that the carbon framework is formed by a polyketide backbone. We found that multiple pathways for the synthesis of polyketides were significantly enriched in hypoliths compared to biocrusts ($P < 0.05$; Table 2). Within the KEGG ansamycin biosynthesis pathway, five genes encoding polyketide synthases (which assemble smaller subunits into the larger polyketide backbone) were significantly more abundant in hypoliths versus biocrusts ($P < 0.01$; Table S6). Other significant genes in the pathway encode proteins for modifying the polyketide backbone, synthesizing precursors, and postsynthesis modification (Table S6).

The methane metabolism pathway was significantly enriched in hypolithic communities versus biocrust communities ($P < 0.001$), which was primarily driven by methanotrophy and methylotrophy genes (Fig. S2; Table S6). All three subunits of particulate methane monooxygenase (*pmoABC*), which encodes the key enzyme required for aerobic oxidation of methane, were significant ($P < 0.01$) but substantially less abundant than other significant genes within the pathway suggesting that methylotrophy is more common than methanotrophy in hypolith communities. Other significant genes in the pathway suggest the use of two pathways for converting formaldehyde to formate (the H_4MPT dependent multistep pathway and direct conversion via glutathione-independent formaldehyde dehydrogenase), which is corroborated by the high abundance of genes related to the synthesis of coenzymes required in the H_4MPT pathways (specifically coenzyme F420). The data also indicate the use of the serine pathway for formaldehyde assimilation.

TABLE 3 KEGG pathways significantly enriched cyanobacteria-anchored versus moss-anchored communities[a]

Pathway no.	Pathway name
ko00195	Photosynthesis***
ko00196	Photosynthesis—antenna proteins***
ko04080	Glutathione metabolism***
ko00906	Carotenoid biosynthesis**
ko00860	Porphyrin and chlorophyll metabolism*
ko00130	Ubiquinone and other terpenoid-quinone biosynthesis*
ko03018	RNA degradation*

[a]***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Effects of dominant primary producer (moss versus cyanobacteria) on functional potential. We observed differences in functional potential in moss- versus cyanobacteria-dominated communities independent of the ecological niche (Table S7), though fewer pathways were significantly different in moss versus cyanobacterial comparisons (14 pathways) than in biocrust versus hypolith comparisons (23 pathways) (Tables 3 and 4). In cyanobacteria-dominated samples, most significantly enriched pathways (four of seven) were related to cyanobacterial metabolism — specifically photosynthesis, antenna proteins, carotenoid biosynthesis, and porphyrin and chlorophyll metabolism. An additional pathway (ubiquinone, and other terpenoid-quinone biosynthesis) was significant in cyanobacterial-dominated communities due to the high abundance of vitamin E (produced exclusively by photosynthetic organisms) synthesis genes.

In moss-dominated samples, the ATP-binding cassette (ABC) transporters pathway was significantly more abundant than in cyanobacterial communities. The ABC transport system couples ATP hydrolysis with the transport of substrates across the membrane. Transporters typically consist of multiple components, including transmembrane domains, ATP-hydrolyzing domains, and a substrate-binding protein. A total of 66 genes within the pathway were significant ($P < 0.01$; Fig. S3 and Table S6) and primarily encode monosaccharide and amino acid transporters. Genes related to the uptake of osmoprotectants (e.g., glycine betaine, proline, putrescine) and precursors for the synthesis of stress related molecules (arginine/ornithine) were also significant.

DISCUSSION

Our investigations revealed that the functional repertoire of surface communities in dryland ecosystems is strongly shaped by ecological niche (biocrust versus hypolith) and, to a lesser degree, dominant primary producer (moss versus cyanobacteria). Relative to niche and primary producer, location and season (Sheep Creek Wash collected in March and Granite Mountains Reserve collected in August) had a substantially reduced effect on functional potential. This observation suggests that the functions identified here likely play conserved roles in the ecology of the different environmental niches distributed in dryland soils. As hypothesized, genes and pathways enriched in biocrusts relative to hypoliths reflect adaptation to heat and desiccation stressors. These communities showed an increased capacity for

TABLE 4 KEGG pathways significantly enriched moss-anchored versus cyanobacteria-anchored communities[a]

Pathway no.	Pathway name
ko02010	ABC transporters***
ko01051	Biosynthesis of ansamycins***
ko00524	Neomycin, kanamycin, and gentamicin biosynthesis***
ko00630	Glyoxylate and dicarboxylate metabolism**
ko00650	Butanoate metabolism*
ko00450	Selenocompound metabolism*
ko00362	Benzoate degradation*

[a]***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

DNA repair, motility, environmental stimuli sensing and response, and interactions with other community members. On the other hand, hypolithic communities were enriched in antibiotic and secondary metabolite synthesis pathways. Moss-dominated samples showed an increased abundance of genes for the uptake of monosaccharides, amino acids, and osmoprotectants relative to cyanobacteria-dominated samples, which may reflect leakage of these substrates by moss gametophyte tissues (the "bryotic pulse") (22).

Given that severe prolonged water deprivation exerts extreme stress on microbial communities, we expected to find differences in samples collected in March, where rainfall had occurred within 2 to 3 days prior to collection, and August, where communities had experienced months of extreme heat without recent precipitation. We also predicted that geographic location might affect community functional potential due to climatic differences between sites. The Wrightwood site experiences cooler annual temperatures and higher precipitation (average high and low annual temperatures, 16.8°C and 1.7°C; average annual precipitation, 49.4 cm) compared to the Granite Mountains site (annual high and low temperatures, 26.5°C and 3.5°C; average annual precipitation, 22 cm) (Wrightwood Weather Station, NOAA National Climatic Data Center; Granites Weather Station, UC Natural Reserve System). Instead, collection month and location had minor effects compared to niche and dominant primary producers. This suggests that biocrust and hypolithic communities are resilient to the stressors imposed by environmental extremes and that these taxa have high degrees of physiological flexibility that enable them to maintain consistent abundances during seasonal fluctuations (56–58). This is opposed to a model where taxa adapted to specific seasonal environmental conditions (and their genes) change in abundance with yearly cycles. In the future, the resilience of these communities and their physiological plasticity could be further investigated by tracking concurrent changes in taxonomy, function, and functional potential across seasons. Our data also suggest that factors characteristic of the two niches we investigated are more important than geographic distance and broad climatic similarities in determining functional potential. This observation has played out on an even larger scale, where studies have demonstrated that hypolithic communities in cold and hot desert environments share more similarities with each other than with nonhypolithic soils (8).

DNA repair. Desiccation and high UV exposure induce multiple types of DNA damage, which is countered by a variety of repair mechanisms. Hypolithic communities colonize the ventral sides of semitranslucent stones, which filter UV radiation and increase moisture availability. Hypolithic communities also experience an attenuation of daily high and low temperature extremes (12), whereas biocrust communities must persist without this protective buffer. Our data show that biocrust communities have an increased capacity for DNA damage repair, likely to counteract the effects of UV and desiccation. We speculate that enrichment of DNA repair genes may be due to increased copy numbers in biocrust genomes. Previous work from Negev Desert biocrusts demonstrated that taxa highly specialized for the desert crust environment contained multiple copies of double-stranded break repair genes in their genomes (37), which may enhance expression or produce proteins with alternative activities or specificities. An analogous scenario holds for the genome of the common biocrust moss, *S. caninervis*, which contains a highly expanded repertoire of protective early light-induced protein (ELIP) genes (40), a signature of physiological desiccation and UV tolerance in land plants (60). The higher abundance of DNA repair genes may also be because biocrust taxa on average possess more repair mechanisms and pathways than hypolith taxa. Previous studies have shown uneven distributions of DNA repair pathways across taxa and suggest the number of repair systems may be related to desiccation and UV tolerance (61). Future work should enable further investigations into these explanations through genomes assembled from metagenomic sequence data.

Intercellular competition and antibiotic synthesis. The differential abundance of bacterial secretion systems, quorum sensing genes, biofilm formation genes, and antibiotic synthesis pathways suggests intercellular interactions play an important role in niche specialization in dryland communities. Competition for finite resources through eliminating competitors appears to be crucial in both biocrust and hypolith communities (62), but the strategies

for doing so have diverged. Biocrusts have a greater capacity to use the T6SS as a weapon of interbacterial competition, whereas hypoliths have an increased ability to produce multiple classes of antibiotics. T6SSs deliver toxic effector proteins to the cytoplasm of target cells through a tubular device that extends to puncture the cell envelope (63). Such interactions require direct cell-to-cell contact, suggesting higher encounter rates between cells. This is consistent with previous observations that T6SS-bearing cells are more abundant in environments with closer cell proximities (64). Conversely, the production of antibiotics may reflect a more open system and higher moisture content, enabling metabolites to diffuse away from cells. The greater abundance of biofilm-related genes in biocrusts than hypoliths is consistent with increased opportunities for direct cellular interactions. Cells are packed densely in biofilms (65), which may facilitate the direct contact necessary for the T6SS to deliver toxins to neighboring cells. In hypoliths, increased moisture availability via condensation and slower rates of evaporation (12) may facilitate the diffusion of compounds between cells, favoring the use of antibiotics. To a lesser degree, we also observed a significant enrichment of antibiotic synthesis pathways in samples containing moss as the primary producer. The association of antibiotic synthesis with the presence of moss might also reflect the increased availability of moisture to enable diffusion of antibiotics, as mosses possess morphological features (e.g., leaf and branch architecture, leaf papillae, and leaf hair points) that are specialized for the sequestration, transport, and retention of external water (66, 67). We note that the enrichment of antibiotic synthesis pathways in hypoliths compared with biocrusts does not imply biocrusts lack this ability. Indeed, biocrusts and other arid soils may contain more of these genes and pathways that other soil types (68, 69). Biocrusts were previously found to harbor a diversity of biosynthetic gene clusters, which were crucial for niche differentiation and maintenance (70).

Canonically, bacterial antibiotic production has been viewed as a weapon in competitive interactions (71), but alternative hypotheses suggest that subinhibitory concentrations may play a role in intercellular signaling (72, 73). Recent studies designed to distinguish between the hypotheses strongly indicate that antibiotics act as weapons of interbacterial competition (74, 75). Regardless, the diversity and abundance of antibiotic synthesis genes suggest this environment is a large potential untapped resource that could aid in addressing the mounting public health crisis of widespread antibiotic resistance in pathogens. Bacterial soil isolates represent a major source of modern antibiotics and other metabolites useful in medicine and biotechnology. However, the use of environmental bacteria for antibiotic discovery has slowed due to high rates of antibiotic rediscovery and because only a small fraction of isolates produce useful metabolites (76, 77). Here, deep sequencing of the uncultured majority provides a resource that could be used to overcome this hurdle either through targeted cultivation or synthetic biology, potentially revealing novel compounds useful in the clinic and beyond (76, 78).

Members of the phylum Actinomyceota (formerly Actinobacteria) are known for their ability to produce a diversity of secondary metabolites, including antibiotics. A previous study that surveyed community composition based on 16S rRNA amplicons at one of our sample sites (Sheep Creek Wash) found that four Actinomyceota genera (*Solirubrobacter*, *Rubrobacter*, *Conexibacter*, and *Angustibacter*) were significantly more abundant in in hypoliths versus biocrusts (10). None of these are particularly notable for producing secondary metabolites (79). The apparent disconnect might be explained by more limited knowledge of these genera compared with known antibiotic producers such as *Streptomyces* (80, 81), differences between the studies (e.g., one study site rather than two and the use of different samples), or a distribution of biosynthetic genes in taxa that extends beyond well-known secondary metabolite producers. Further investigation using assembly and binning methods will likely shed light on this topic revealing the taxa harboring these pathways, their phylogenetic distribution, and potentially other factors such as the relationships between genome size and antibiotic production (taxa with larger genomes tend to have more biosynthetic gene clusters [82]).

Environmental sensing and response via the two-component regulatory system. Two component systems are found in nearly all bacterial genomes, but those that inhabit rapidly changing or diverse environments typically encode a large number of two component system genes, suggesting that organisms expand their repertoire to adapt to environmental challenges (51, 83). The high prevalence in biocrust communities, which are exposed to more extremes than hypolith communities, is consistent with this observation suggesting that sensing and responding to local conditions and stressors play a key role in adaptation to the biocrust ecological niche. Since the input signal and cellular response of a given system can often be predicted based on DNA sequence, the specific two-component system genes that are enriched in a particular environment can be used to infer which environmental stimuli microbial communities are attuned to and the subsequent downstream response (49). In the case of biocrust communities, these adaptations include motility and chemotaxis, surface adhesion and biofilm formation, redox conditions, nutrient limitation, and environmental stressors, all of which may be particularly important for organisms inhabiting oligotrophic environments with transient pulses of nutrient availability and metabolic activity (58, 84, 85). Biocrusts are poikiloydric communities with the ability to desiccate completely during extended dry periods and quickly resume metabolic activity when moisture becomes available (1). Heterotrophic organisms in these communities must thus maintain the capacity to respond quickly to changes in their environment and exploit resources generated by primary producers during brief pulses of hydration and metabolic activity. Moss-dominated biocrusts produce vertical strata, with gradients of light, moisture, UV exposure, and nutrients lost from moss leaf cells during rehydration (22). It is likely critical for microorganisms to optimize and maintain their position relative to the spatial distribution of these variables within the moss biocrust. Similarly, the microbial community associated with the common early-successional cyanobacterium *Microcoleus* (the "cyanosphere," [32]) is also likely to harbor adaptations to chemotaxis and motility, as *Microcoleus* moves vertically within the soil surface in response to moisture availability (86).

Methylotrophy, photosynthesis, and CO_2 fixation in hypolithic communities. The enrichment in genes related to methylotrophy in hypoliths compared to biocrust communities could reflect a higher abundance of moss-associated Methylobacteria in hypoliths. Methylobacteria are known to live as epiphytes on plants, including mosses, where they utilize methanol emitted as a by-product of pectin degradation in cell walls during cell division and growth (87). We note that if the moss-associated methylotrophs were the sole explanation for the high abundance of methylotrophy genes in hypoliths, we should also have observed a corresponding enrichment of the methane metabolism pathway in moss- versus cyanobacteria-dominated samples, which was not apparent at the conservative threshold we used to identify differentially abundant genes ($P < 0.01$). However, when a threshold of $P < 0.05$ was applied, most of the methotrophy genes that were enriched in hypoliths versus biocrusts were also enriched when comparing moss versus cyanobacterial samples. This suggests the enrichment observed in hypoliths may be due to higher abundances of moss-associated methylotrophs in hypolithic microsites plus other yet unknown metabolic processes resulting the availability of C1 substrates to methylotrophic communities. In hypolithic communities, we also found that photosynthesis and CO_2 fixation pathways were more abundant than in biocrusts, which might be explained by a higher abundance of cyanobacteria relative to other microbial taxa in hypoliths (10).

Other factors controlling functional potential in hypolith and biocrust communities. Previous studies have observed that differences in carbon and nitrogen content affect microbial communities in biocrusts at varying successional states, with pH playing a weaker role (23, 59). At our sites, pH values were weakly acidic to neutral (6.15 to 7.52) and differed between primary producer (cyanobacteria, 7.08; moss, 6.37), niche (hypolith, 6.48; biocrust, 6.96), and sample site (Wrightwood, 6.49; Granite Mountains, 6.96) (Table S2). Though pH levels differed between niches, the magnitude of difference between primary producers and sample sites was roughly the same. Because sample site and primary producer had a reduced effect on functional potential compared to niche, it is likely that pH gradients may influence functional potential but play a secondary role compared to other environmental factors. Further investigations

including other physicochemical analyses and additional pH measurements should shed light on this issue.

We note that we did not observe substantial differences between samples and bare soil controls (Fig. 1), which is contrary to our expectations and prior observations (59). We posit three explanations. First, we sampled the surface (1–2 cm) in areas directly adjacent to biocrusts (within 1 m). It is likely that we captured transient communities seeded by the surrounding biocrusts/hypoliths. Second, it plausible that bare soil contains a subset of biocrust/hypolith communities given the shared stressors and environmental conditions. Finally, we collected many fewer bare soil controls compared with samples. Additional controls may have improved our ability to detect differences.

Approaches to analyzing metagenomes. This study represents an overview of the functional potential of biocrust and hypolith microbial communities. By using reads rather than assembled data, we were able to capture sequences from low abundance taxa and avoid biases introduced by assembling and binning metagenomic sequence data (88), thus providing quantitative information on gene relative abundances. We mitigated issues associated with read-based analyses such as mis-annotations due to short read length by using an extremely conservative approach, setting a low *P*-value threshold ($P > 0.01$) and requiring that many genes within a pathway reach significance.

Unlike 16S rRNA amplicon data or assembly approaches, our analysis strategy yields limited information regarding taxonomy or the phylogenetic distribution of traits. The exception is instances where taxonomy and functional genes are tightly linked, as shown by the enrichment of cyanobacteria metabolic pathways in samples where cyanobacteria are the dominant primary producer. Ongoing work that includes assembly and constructing metagenome assembled genomes will provide complementary information, revealing how functions are partitioned among community members and enabling us to address questions generated by read-based analyses such as whether enhanced DNA repair abilities in biocrust communities are due to copy number variation, more DNA repair pathways, or a combination of the two.

Concluding remarks. In dryland regions where hypoliths and biocrusts are intermingled on the soil surface, both niches share superficial similarities, such as primary producers, which motivated us to question how the microbial communities associated with these proximate yet distinct microsites might differ. We found that niche (biocrust or hypolith) had a stronger influence on functional pathway differences than primary producers (moss or cyanobacteria). The importance of environmental stressors such as desiccation, extreme daily temperature fluctuations, and UV exposure was reflected in the significant enrichment of pathways associated with responding to and mitigating these stresses in biocrusts as opposed to hypoliths. Contrasting strategies for competition that we observed in our comparisons may reflect different conditions promoted by niche and primary producers and highlight hypoliths and moss biocrusts as potential sources of novel antibiotics. Notably, the functional signal generated by niche and primary producers greatly overshadowed the influence of spatial (collection site) or temporal (season) variations, highlighting the deterministic nature of these communities. The consistency of functional pathways across divergent environmental conditions suggests that the communities we sampled may be relatively stable, relying on physiological plasticity and/or intermittent quiescence (dormancy) for survival as opposed to compositional turnover.

MATERIALS AND METHODS

Field site and sample collection. Soil and biocrust samples were collected on March 25, 2018 from the Sheep Creek Wash near Wrightwood, CA. The Sheep Creek Wash site is located at the northern base of the San Gabriel Mountains (34°22′33.85″N, 117°36′34.59″W) and the western edge of the Mojave Desert at an elevation of 1800 m (12). A second site in the UC Sweeny Granite Mts. Reserve (34°47′08.3″N 115°39′36.2″W; 1,280 m elevation), located along the southwestern edge of the Mojave National Preserve, was visited for collection on Aug 3–4, 2018. Both sites were chosen based on the cooccurrence of biocrusts and hypoliths containing the moss *Syntrichia caninervis* as the dominant photosynthetic anchor species within the same restricted ($\sim$ 3 m^2) area (12) (Fig. 1A; Table S1). *S. caninervis* was identified based on characteristics such as hair points on the apices of leaves, leaf morphology, and colony pigmentation. Seven replicate samples were collected for each of the following microsite types: hypolith with moss; hypolith without moss (cyanobacteria only); moss biocrust; cyanobacterial biocrust; soil ca. 1 cm below each of

these microsite types; and non-biocrust surface soil. A sterile spatula (surface sterilized with 70% isopropyl alcohol between samples) was used to collect of 5 to10 g soils and biocrusts, and each sample was placed individually into a sterile Nasco Whirl Pak bag (Fort Atkinson, WI). For hypolithic samples, quartz rocks (often with visible adhered microbial biomass) were collected along with soil and associated organisms. Soil pH was measured using a LabQuest2 handheld field unit (Vernier Inc., Beaverton, OR) (Table S2). All samples were stored on ice during field collection and transported back to the lab, where they were stored at −20°C until DNA extraction.

DNA extraction and sequencing. Quartz samples were crushed with a UV sterilized hammer to obtain biological matter adhered to the rock samples. Smaller rocks were scraped using a sterile scalpel to gather biological materials for DNA extraction. For samples containing moss biocrusts, ca 5 stems of moss were first submerged for several seconds (using sterile forceps) in the buffer used during the cell disruption step of the DNA extraction protocol to remove some of the adhered soil and biocrust material for subsequent DNA extractions. Care was taken to remove all traces of moss after submersion. The prepared samples then underwent DNA extraction using a FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions (with the addition of the moss-washing step noted above). Quantification readings were taken immediately after DNA extraction using a Qubit 4 fluorometer (Invitrogen, Carlsbad, CA). Libraries were prepared at the Department of Energy (DOE) Joint Genome Institute (JGI) and sequenced (2×150) on the Illumina NovaSeq platform (Illumina Inc., San Diego, CA) (GOLD Study ID: Gs0136120; GOLD Sequencing Project IDs: Gp0356221–Gp0356280). Raw reads were filtered and trimmed using the DOE JGI metagenome workflow (38) and downloaded from the Integrated Microbial Genomes and Microbiomes system (39). Additional accession numbers are in Table S1.

Annotation and statistical analyses. Reads derived from *S. caninervis* (40), the dominant moss species, were removed using bbduk.sh (version updated October 10 2020) from the BBtools suite of programs (41). Functional annotation of reads was performed by comparison to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (42) using DIAMOND (v0.9.30.131) (43) with an E-value cutoff of 1e-6. Reads were assigned a KEGG Orthology (KO) number according to the top hit. The resulting KO annotation data were summarized in a gene count matrix. We applied a low abundance filter, where genes observed more than 100 times in at least 10% of the samples were retained. Relationships between samples were visualized using Principle Coordinate Analysis based on Bray-Curtis dissimilarities using the phyloseq package (44) in R (45). Alpha diversity (Shannon index) was also calculated using phyloseq. Differences in alpha diversity among samples were evaluated using ANOVA followed by a Tukey *post hoc* test. Permutational Multivariate Analysis of Variance (PERMANOVA) was performed using the adonis function in the vegan package in R (46). Genes whose abundances differed between groups were identified using a quasi-likelihood negative binomial generalized linear model implemented in the edgeR package (47). For individual genes, we used a conservative adjusted *P*-value threshold of $P < 0.01$. Heatmaps were generated using the R pheatmap package (48). Rows and columns within the heatmap were clustered using the complete linkage method for the purpose of visualization.

We identified differentially abundant pathways using a method we developed previously (49). Briefly, we placed genes onto KEGG pathway maps and counted the number of genes in each pathway that were significantly more abundant in one category versus another (i.e., biocrust versus hypolith, hypolith versus biocrust, moss- versus cyanobacteria-dominated, and cyanobacteria- versus moss-dominated). For each of the sample category comparisons, we then randomly assigned *P*-values to each gene from the observed set of *P*-values. A total of 10,000 permutations were performed, generating a null distribution of the number of significantly different genes expected in each pathway. Pathways differing significantly between sample categories were identified by assigning *P*-values based on how often the number of significant genes in the permutations exceeded the observed number of significant genes in each pathway. The resulting *P*-values were corrected using the false discovery rate (50). Significant pathways were manually inspected and removed if the majority of significant genes were broadly distributed across many pathways. We implemented this conservative measure so that pathways reaching significance due to a high abundance of "promiscuous" genes, rather than enrichment of the specific pathway, were not considered in downstream analyses.

Data availability. Sequence data are available at the Integrated the Microbial Genomes & Microbiomes (IMG/M) database under the GOLD Study ID number Gs0136120 and GOLD Sequencing Project ID numbers Gp0356221 to Gp0356280.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, EPS file, 0.8 MB.
FIG S2, EPS file, 0.7 MB.
FIG S3, EPS file, 1.3 MB.
TABLE S1, XLSX file, 0.01 MB.
TABLE S2, DOCX file, 0.01 MB.
TABLE S3, XLSX file, 2.6 MB.
TABLE S4, DOCX file, 0.01 MB.
TABLE S5, XLSX file, 0.2 MB.
TABLE S6, XLSX file, 0.02 MB.
TABLE S7, XLSX file, 0.1 MB.

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We declare no competing interests.

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