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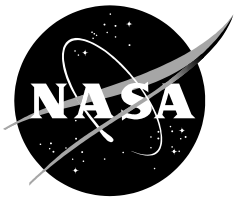

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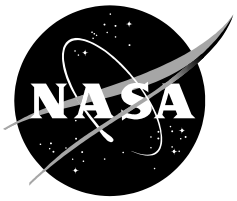

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Abstract

Biological soil crusts (biocrusts) are widespread in drylands and deserts. At the microhabitat scale, they also host hypolithic communities that live under semi-translucent 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 waging war using the Type VI Secretion System (T6SS) and hypoliths favoring 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 an 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 semi-translucent 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 ennvironment.

Introduction

Biological soil crusts (biocrusts) are communities anchored by primary producers such as cyanobacteria, mosses, algae, and lichens, and 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 semi-translucent 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).

Biocrust 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). To survive in these conditions, 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 re-introduced, poikilohydric organisms resume metabolic activity almost instantaneously through a combination of cellular protective mechanisms deployed during drying (e.g., ROS scavenging, compatible solutes, mRNPs) and repair mechanisms initiated upon rehydration (12–15). 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 (16, 17). Thus, while biocrusts are physiologically specialized for

84 environments with low precipitation, they are sensitive to the frequency, timing, and duration of
85 hydration events (18).

86 In habitats where biocrusts occur, drying events happen quickly relative to the time
87 required for poikilohydric organisms to launch extensive cellular protective processes. Thus,
88 biocrust organisms tend to rely heavily on cellular repair during rehydration as their strategy for
89 tolerating desiccation (19). Although these repair mechanisms are highly efficient (20) larger
90 biocrust organisms such as mosses lose some cellular contents during the process of membrane
91 repair during rehydration, which in turn may provide a nutritional resource to support a diverse
92 community of heterotrophic microbes, a phenomenon coined the 'bryotic pulse' (21).

93 Photoautotrophs (cyanobacteria, mosses, and lichens) anchor biocrust communities, both
94 physically (i.e., soil aggregation, hydrological controls) and through primary production.
95 Typically, biocrusts are dominated by one type of photoautotroph, which in turn influences the
96 diversity and abundance of other organisms in the community (10, 22, 23). The identity of the
97 dominant photoautotroph also influences biocrust multifunctionality and community stability in
98 the presence of climate perturbations (24–27).

99 The identity of the dominant photoautotroph and taxonomic composition of the rest of the
100 community is at least partially dictated by predictable successional processes (28, 29). Bare soils
101 are first colonized by filamentous cyanobacteria such as *Microcoleus*, which aggregates soil
102 particles with its polysaccharide sheaths and generates organic carbon to support a diverse
103 community of heterotrophic bacteria, including diazotrophs, within the cyanosphere (30). Later
104 successional stages are characterized by darkly pigmented nitrogen-fixing cyanobacteria like
105 *Scytonema*, followed eventually by mosses and/or lichens (22). While cyanobacteria are typically
106 the major photoautotroph found in hypolithic communities (5, 31), some hypoliths support

mosses (5, 10, 32–34). 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, 22) and biocrust ecophysiology/multifunctionality (22, 24, 25, 35), 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. **(2)** The presence of moss in biocrust 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.

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 (34º22'33.85"N, 117º36'34.59"W) and the western edge of the Mojave Desert at an elevation of 1800 m (36). A second site in the UC Sweeny Granite Mts. Reserve (34°47'08.3"N 115°39'36.2"W, 1280 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 co-occurrence of biocrusts and hypoliths containing the moss *Syntrichia caninervis* as the dominant photosynthetic anchor species within the same restricted (~ 3 m^2) area (36). *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 5-10 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. 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 Joint Genome Institute and sequenced (2x150) on the Illumina NovaSeq platform (Illumina Inc., San Diego, CA) (Walnut Creek, CA; GOLD Study ID: Gs0136120; GOLD Sequencing Project IDs: Gp0356221 - Gp0356280). Additional accession numbers are found in Supplemental Table 1.

Annotation and Statistical Analyses

Reads derived from *S. caninervis* (37), the dominant moss species, were removed using bbduk.sh (version updated October 10 2020) from the BBtools suite of programs (38). Functional annotation of reads was performed by comparison to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (39) using DIAMOND (v0.9.30.131) (40) 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 (41) in R (42). 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 (43). Genes whose abundances differed between groups were identified using a quasi-likelihood negative binomial generalized linear model implemented in the edgeR package (44). For individual genes, we used a conservative adjusted P-value threshold of $P < 0.01$. Heatmaps were generated using the R pheatmap package (45). 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 (46). 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. 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 (47). 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 actual functional enrichment were not considered in downstream analyses.

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 (Supplemental Table 1). Reads were annotated by comparison to the KEGG gene database (Supplemental Table 2). Ordinations of KEGG gene count data revealed clear differences in genetic repertoire between biocrust and hypolith communities (Figure 2A). When ordinations of biocrust and hypolith communities were plotted separately, samples clustered by the dominant primary producer (cyanobacteria or moss) (Figure 2B-C).

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 x Layer and

Primary Producer x Collection site/month interactions had much smaller (but significant) effects on functional potential (Supplemental Table 3).

Alpha diversity differed significantly among sample types (ANOVA, F=12.76, P=7.29e-10, Supplemental Figure 1). 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 (Supplemental Tables 4 & 5) and pathways (Tables 1 & 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).

Genes and pathways enriched in biocrust versus hypolith communities

Commensurate with elevated environmental exposure to UV and desiccation, DNA repair gene frequencies were higher 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 twenty-two 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 (Figure 3, Supplemental Table 6). 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 (48). 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 (Figure 4, Supplemental Table 7). 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 (49, 50)) were significant. Multiple genes from other chemosensory systems related to twitching motility, quorum sensing, and biofilm formation were also significant (Figure 4A). In the redox signals and catabolites category (Figure 4B), we found that both genes in the conserved RegB/RegA signal transduction system, which controls a large number of energy-generating and energy-using processes (51), 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) (Figure 4C). We also note that RNA polymerase sigma-54 factor, which plays a role in stress response (52) 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 ($P<0.01$, Figure 5, Supplemental Table 8). 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 (Supplemental Table 9). In the biofilm formation pathway (Supplemental Table 10), 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 IV) 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 (Figure 6, Supplementary Table 11). 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 (Figure 5). These, along with T2SS-associated Sec 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 twelve 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, 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 (Figure 7, Supplemental Table 12).

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

312 synthesis of polyketides were significantly enriched in hypoliths compared to biocrusts ($P<0.05$,
313 Table 2). Within the KEGG ansamycin biosynthesis pathway, five genes encoding polyketide
314 synthases (which assemble smaller subunits into the larger polyketide backbone) were
315 significantly more abundant in hypoliths versus biocrusts ($P<0.01$, Supplemental Table). Other
316 significant genes in the pathway encode proteins for modifying the polyketide backbone,
317 synthesizing precursors, and post-synthesis modification (Supplemental Table 13).
318 The methane metabolism pathway was significantly enriched in hypolithic communities
319 versus biocrust communities ($P<0.001$), which was primarily driven by methanotrophy and
320 methylotrophy genes (Figure 8, Supplemental Table 14). All three subunits of particulate
321 methane monooxygenase (*pmoABC*), which encodes the key enzyme required for aerobic
322 oxidation of methane, were significant ($P<0.01$) but substantially less abundant than other
323 significant genes within the pathway suggesting that methylotrophy is more common than
324 methanotrophy in hypolith communities. Other significant genes in the pathway suggest the use
325 of two pathways for converting formaldehyde to formate (the H_4MPT dependent multi-step
326 pathway and direct conversion via glutathione-independent formaldehyde dehydrogenase),
327 which is corroborated by the high abundance of genes related to the synthesis of coenzymes
328 required in the H_4MPT pathways (specifically coenzyme F420). The data also indicate the use of
329 the serine pathway for formaldehyde assimilation.
330
331 **Effects of dominant primary producer (moss versus cyanobacteria) on functional potential**
332 We observed differences in functional potential in moss- versus cyanobacteria-dominated
333 communities independent of the ecological niche (Supplemental Tables 15 & 16), though fewer
334 pathways were significantly different in moss versus cyanobacterial comparisons (14 pathways)

than in biocrust versus hypolith comparisons (23 pathways) (Tables 3 & 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 ($P<0.01$, Figure 9, Supplemental Table 17) 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 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") (21).

Given that severe prolonged water deprivation exerts extreme stress on microbial communities, we expected to find differences in samples collected March, where rainfall had occurred within 2-3 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 (53–55). 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 non-hypolithic 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 semi-translucent stones, which filter UV radiation and increase moisture availability. Hypolithic communities also experience an attenuation of daily high and low temperature extremes (36), 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 (35), 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 *ELIP* genes (37), a signature of physiological desiccation and UV tolerance in land plants (56). 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 (57). 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 (58), 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 (59). 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 (60). 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 (61), 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 (36) 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 (62, 63). We note that the enrichment of antibiotic synthesis pathways in hypoliths compared with biocrusts does not imply biocrusts lack this ability. Indeed, biocrusts may contain more of these genes and pathways that other soil types. Biocrusts were previously found to harbor a diversity of biosynthetic gene clusters, which were crucial for niche differentiation and maintenance (64).

Canonically, bacterial antibiotic production has been viewed as a weapon in competitive battles (65), but alternative hypotheses suggest that sub-inhibitory concentrations may play a role in intercellular signaling (66, 67). Recent studies designed to distinguish between the hypotheses strongly indicate that antibiotics act as weapons of interbacterial competition (68, 69). 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 (70, 71). 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 (70, 72).

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 (48, 73). 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 (46). 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 (55, 74, 75). 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 (21). 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", (30)) is also likely to harbor adaptations to chemotaxis and motility, as *Microcoleus* moves vertically within the soil surface in response to moisture availability (76).

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 byproduct of pectin degradation in cell walls during cell division and growth (77). 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).

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 (78), thus providing quantitative information on gene relative abundances. 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. 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.

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.

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Competing Interests

The authors declare no competing interests

References

1. Belnap J. 2003. The world at your feet: desert biological soil crusts. Front Ecol Environ 1:181–189.

2. Eldridge DJ, Reed S, Travers SK, Bowker MA, Maestre FT, Ding J, Havrilla C, Rodriguez-Caballero E, Barger N, Weber B, Antoninka A, Belnap J, Chaudhary B, Faist A, Ferrenberg S, Huber-Sannwald E, Malam Issa O, Zhao Y. 2020. The pervasive and multifaceted influence of biocrusts on water in the world's drylands. Glob Chang Biol 26:6003–6014.

3. Chamizo S, Rodríguez-Caballero E, Román JR, Cantón Y. 2017. Effects of biocrust on soil erosion and organic carbon losses under natural rainfall. Catena 148:117–125.

4. Rodriguez-Caballero E, Belnap J, Büdel B, Crutzen PJ, Andreae MO, Pöschl U, Weber B. 2018. Dryland photoautotrophic soil surface communities endangered by global change. Nature Geoscience.

5. Chan Y, Lacap DC, Lau MCY, Ha KY, Warren-Rhodes KA, Cockell CS, Cowan DA, McKay CP, Pointing SB. 2012. Hypolithic microbial communities: between a rock and a hard place. Environ Microbiol 14:2272–2282.

560 6. Makhalanyane TP, Valverde A, Lacap DC, Pointing SB, Tuffin MI, Cowan DA. 2013.
561 Evidence of species recruitment and development of hot desert hypolithic communities.
562 Environ Microbiol Rep 5:219–224.

563 7. Stomeo F, Valverde A, Pointing SB, McKay CP, Warren-Rhodes KA, Tuffin MI, Seely M,
564 Cowan DA. 2013. Hypolithic and soil microbial community assembly along an aridity
565 gradient in the Namib Desert. Extremophiles 17:329–337.

566 8. Le PT, Makhalanyane TP, Guerrero LD, Vikram S, Van de Peer Y, Cowan DA. 2016.
567 Comparative Metagenomic Analysis Reveals Mechanisms for Stress Response in Hypoliths
568 from Extreme Hyperarid Deserts. Genome Biol Evol 8:2737–2747.

569 9. Vikram S, Guerrero LD, Makhalanyane TP, Le PT, Seely M, Cowan DA. 2016.
570 Metagenomic analysis provides insights into functional capacity in a hyperarid desert soil
571 niche community. Environ Microbiol 18:1875–1888.

572 10. Fisher K, Jefferson JS, Vaishampayan P. 2020. Bacterial Communities of Mojave Desert
573 Biological Soil Crusts Are Shaped by Dominant Photoautotrophs and the Presence of
574 Hypolithic Niches. Frontiers in Ecology and Evolution 7:518.

575 11. Antoninka A, Bowker MA, Reed SC, Doherty K. 2016. Production of greenhouse-grown
576 biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restor
577 Ecol 24:324–335.

578 12. Wood AJ, Oliver MJ. 1999. Translational control in plant stress: the formation of messenger
579 ribonucleoprotein particles (mRNPs) in response to desiccation of Tortula ruralis
580 gametophytes. Plant J 18:359–370.

13. Oliver MJ, Velten J, Mishler BD. 2005. Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45:788–799.

14. Oliver MJ, Farrant JM, Hilhorst HWM, Mundree S, Williams B, Bewley JD. 2020. Desiccation tolerance: Avoiding cellular damage during drying and rehydration. Annu Rev Plant Biol 71:435–460.

15. Baubin C, Ran N, Siebner H, Gillor O. 2021. The response of desert biocrust bacterial communities to hydration-desiccation cycles.

16. Coe KK, Belnap J, Sparks JP. 2012. Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology 93:1626–1636.

17. Coe KK, Sparks JP. 2014. Physiology-based prognostic modeling of the influence of changes in precipitation on a keystone dryland plant species. Oecologia 176:933–942.

18. Reed SC, Coe KK, Sparks JP, Housman DC, Zelikova TJ, Belnap J. 2012. Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nat Clim Chang 2:752–755.

19. Tuba Z, Protor CF, Csintalan Z. 1998. Ecophysiological responses of homoiochlorophyllous and poikilochlorophyllous desiccation tolerant plants: a comparison and an ecological perspective. Plant Growth Regul 24:211–217.

20. Coe KK, Greenwood JL, Slate ML, Clark TA, Brinda JC, Fisher KM, Mishler BD, Bowker MA, Oliver MJ, Ebrahimi S, Stark LR. 2021. Strategies of desiccation tolerance vary across life phases in the moss Syntrichia caninervis. Am J Bot 108:249–262.

21. Slate ML, Sullivan BW, Callaway RM. 2019. Desiccation and rehydration of mosses greatly increases resource fluxes that alter soil carbon and nitrogen cycling. J Ecol 107:1767–1778.

22. Maier S, Tamm A, Wu D, Caesar J, Grube M, Weber B. 2018. Photoautotrophic organisms control microbial abundance, diversity, and physiology in different types of biological soil crusts. ISME J 12:1032–1046.

23. Maier S, Schmidt TSB, Zheng L, Peer T, Wagner V, Grube M. 2014. Analyses of dryland biological soil crusts highlight lichens as an important regulator of microbial communities. Biodivers Conserv 23:1735–1755.

24. Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Bowker MA, Ochoa V, Gozalo B, Berdugo M, Val J, Singh BK. 2016. Biocrust-forming mosses mitigate the negative impacts of increasing aridity on ecosystem multifunctionality in drylands. New Phytol 209:1540–1552.

25. Liu L, Liu Y, Hui R, Xie M. 2017. Recovery of microbial community structure of biological soil crusts in successional stages of Shapotou desert revegetation, northwest China. Soil Biol Biochem 107:125–128.

26. Maestre FT, Castillo-Monroy AP, Bowker MA, Ochoa-Hueso R. 2012. Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern. J Ecol 100:317–330.

27. Delgado-Baquerizo M, Maestre FT, Eldridge DJ, Bowker MA, Jeffries TC, Singh BK. 2018. Biocrust-forming mosses mitigate the impact of aridity on soil microbial communities in drylands: observational evidence from three continents. New Phytol 220:824–835.

623 28. Belnap J, Wilcox BP, Van Scoyoc MW, Phillips SL. 2013. Successional stage of biological
624 soil crusts: an accurate indicator of ecohydrological condition. Ecohydrology 6:474–482.

625 29. Weber B, Bowker M, Zhang Y, Belnap J. 2016. Natural recovery of biological soil crusts
626 after disturbance, p. 479–498. *In* Biological Soil Crusts: An Organizing Principle in
627 Drylands. Springer International Publishing, Cham.

628 30. Couradeau E, Giraldo-Silva A, De Martini F, Garcia-Pichel F. 2019. Spatial segregation of
629 the biological soil crust microbiome around its foundational cyanobacterium, Microcoleus
630 vaginatus, and the formation of a nitrogen-fixing cyanosphere. Microbiome 7:55.

631 31. Pointing SB. 2016. Hypolithic Communities, p. 199–213. *In* Weber, B, Büdel, B, Belnap, J
632 (eds.), Biological Soil Crusts: An Organizing Principle in Drylands. Springer International
633 Publishing, Cham.

634 32. Cowan DA, Khan N, Pointing SB, Cary SC. 2010. Diverse hypolithic refuge communities in
635 the McMurdo Dry Valleys. Antarct Sci 22:714–720.

636 33. de los Ríos A, Cary C, Cowan D. 2014. The spatial structures of hypolithic communities in
637 the Dry Valleys of East Antarctica. Polar Biol 37:1823–1833.

638 34. Cowan DA, Sohm JA, Makhalanyane TP, Capone DG, Green TGA, Cary SC, Tuffin IM.
639 2011. Hypolithic communities: important nitrogen sources in Antarctic desert soils. Environ
640 Microbiol Rep 3:581–586.

641 35. Meier DV, Imminger S, Gillor O, Woebken D. 2021. Distribution of Mixotrophy and
642 Desiccation Survival Mechanisms across Microbial Genomes in an Arid Biological Soil
643 Crust Community. mSystems 6.

644 36. Ekwealor JTB, Fisher KM. 2020. Life under quartz: Hypolithic mosses in the Mojave
645 Desert. PLoS One 15:e0235928.

646 37. Silva AT, Gao B, Fisher KM, Mishler BD, Ekwealor JTB, Stark LR, Li X, Zhang D,
647 Bowker MA, Brinda JC, Coe KK, Oliver MJ. 2021. To dry perchance to live: Insights from
648 the genome of the desiccation-tolerant biocrust moss Syntrichia caninervis. Plant J
649 105:1339–1356.

650 38. Bushnell B. BBTools.

651 39. Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. 2014. Data,
652 information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res
653 42:D199-205.

654 40. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using
655 DIAMOND. Nat Methods 12:59–60.

656 41. McMurdie PJ, Holmes S. 2013. phyloseq: an R package for reproducible interactive analysis
657 and graphics of microbiome census data. PLoS One 8:e61217.

658 42. R Core Team. 2021. R: A Language and Environment for Statistical Computing. R
659 Foundation for Statistical Computing, Vienna, Austria.

660 43. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M. 2007.
661 The vegan package. Community ecology package 10:719.

662 44. McCarthy DJ, Chen Y, Smyth GK. 2012. Differential expression analysis of multifactor
663 RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40:4288–
664 4297.

665 45. Kolde R. 2019. pheatmap: Pretty Heatmaps.

666 46. Mackelprang R, Burkert A, Haw M, Mahendrarajah T, Conaway CH, Douglas TA, Waldrop
667 MP. 2017. Microbial survival strategies in ancient permafrost: insights from metagenomics.
668 ISME J 11:2305–2318.

669 47. Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: A practical and
670 powerful approach to multiple testing. J R Stat Soc 57:289–300.

671 48. Capra EJ, Laub MT. 2012. Evolution of two-component signal transduction systems. Annu
672 Rev Microbiol 66:325–347.

673 49. Francez-Charlot A, Kaczmarczyk A, Vorholt JA. 2015. The branched CcsA/CckA-ChpT-
674 CtrA phosphorelay of Sphingomonas melonis controls motility and biofilm formation. Mol
675 Microbiol 97:47–63.

676 50. Zan J, Heindl JE, Liu Y, Fuqua C, Hill RT. 2013. The CckA-ChpT-CtrA phosphorelay
677 system is regulated by quorum sensing and controls flagellar motility in the marine sponge
678 symbiont Ruegeria sp. KLH11. PLoS One 8:e66346.

679 51. Elsen S, Swem LR, Swem DL, Bauer CE. 2004. RegB/RegA, a highly conserved redox-
680 responding global two-component regulatory system. Microbiol Mol Biol Rev 68:263–279.

681 52. Danson AE, Jovanovic M, Buck M, Zhang X. 2019. Mechanisms of σ54-Dependent
682 Transcription Initiation and Regulation. J Mol Biol 431:3960–3974.

683 53. Allison SD, Martiny JBH. 2008. Resistance, resilience, and redundancy in microbial
684 communities. Proceedings of the National Academy of Sciences.

685 54. Green TGA, Proctor MCF. 2016. Physiology of Photosynthetic Organisms Within
686 Biological Soil Crusts: Their Adaptation, Flexibility, and Plasticity, p. 347–381. *In* Weber,
687 B, Büdel, B, Belnap, J (eds.), Biological Soil Crusts: An Organizing Principle in Drylands.
688 Springer International Publishing, Cham.

689 55. Rajeev L, da Rocha UN, Klitgord N, Luning EG, Fortney J, Axen SD, Shih PM, Bouskill
690 NJ, Bowen BP, Kerfeld CA, Garcia-Pichel F, Brodie EL, Northen TR, Mukhopadhyay A.
691 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological
692 soil crust. ISME J 7:2178–2191.

693 56. VanBuren R, Pardo J, Man Wai C, Evans S, Bartels D. 2019. Massive Tandem Proliferation
694 of ELIPs Supports Convergent Evolution of Desiccation Tolerance across Land Plants. Plant
695 Physiol 179:1040–1049.

696 57. Cassier-Chauvat C, Veaudor T, Chauvat F. 2016. Comparative Genomics of DNA
697 Recombination and Repair in Cyanobacteria: Biotechnological Implications. Front
698 Microbiol 7:1809.

699 58. Ghoul M, Mitri S. 2016. The Ecology and Evolution of Microbial Competition. Trends
700 Microbiol 24:833–845.

59. Durand E, Nguyen VS, Zoued A, Logger L, Péhau-Arnaudet G, Aschtgen M-S, Spinelli S, Desmyter A, Bardiaux B, Dujeancourt A, Roussel A, Cambillau C, Cascales E, Fronzes R. 2015. Biogenesis and structure of a type VI secretion membrane core complex. Nature 523:555–560.

60. Kempnich MW, Sison-Mangus MP. 2020. Presence and abundance of bacteria with the Type VI secretion system in a coastal environment and in the global oceans. PLoS One 15:e0244217.

61. Xavier JB, Foster KR. 2007. Cooperation and conflict in microbial biofilms. Proc Natl Acad Sci U S A 104:876–881.

62. Giordano S, Colacino C, Spagnuolo V, Basile A, Esposito A, Castaldo-Cobianchi R. 1993. Morphological adaptation to water uptake and transport in the poikilohydric moss Tortula ruralis. G Bot Ital 127:1123–1132.

63. Pan Z, Pitt WG, Zhang Y, Wu N, Tao Y, Truscott TT. 2016. The upside-down water collection system of Syntrichia caninervis. Nat Plants 2:16076.

64. Van Goethem MW, Osborn AR, Bowen BP, Andeer PF, Swenson TL, Clum A, Riley R, He G, Koriabine M, Sandor L, Yan M, Daum CG, Yoshinaga Y, Makhalanyane TP, Garcia-Pichel F, Visel A, Pennacchio LA, O'Malley RC, Northen TR. 2021. Long-read metagenomics of soil communities reveals phylum-specific secondary metabolite dynamics. Communications Biology 4:1302.

65. Ratcliff WC, Denison RF. 2011. Microbiology. Alternative actions for antibiotics. Science 332:547–548.

722 66. Romero D, Traxler MF, López D, Kolter R. 2011. Antibiotics as signal molecules. Chem
723 Rev 111:5492–5505.

724 67. Linares JF, Gustafsson I, Baquero F, Martinez JL. 2006. Antibiotics as intermicrobial
725 signaling agents instead of weapons. Proc Natl Acad Sci U S A 103:19484–19489.

726 68. Abrudan MI, Smakman F, Grimbergen AJ, Westhoff S, Miller EL, van Wezel GP, Rozen
727 DE. 2015. Socially mediated induction and suppression of antibiosis during bacterial
728 coexistence. Proc Natl Acad Sci U S A 112:11054–11059.

729 69. Westhoff S, van Leeuwe TM, Qachach O, Zhang Z, van Wezel GP, Rozen DE. 2017. The
730 evolution of no-cost resistance at sub-MIC concentrations of streptomycin in Streptomyces
731 coelicolor. ISME J 11:1168–1178.

732 70. Hover BM, Kim S-H, Katz M, Charlop-Powers Z, Owen JG, Ternei MA, Maniko J, Estrela
733 AB, Molina H, Park S, Perlin DS, Brady SF. 2018. Culture-independent discovery of the
734 malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-
735 positive pathogens. Nat Microbiol 3:415–422.

736 71. Reddy BVB, Kallifidas D, Kim JH, Charlop-Powers Z, Feng Z, Brady SF. 2012. Natural
737 product biosynthetic gene diversity in geographically distinct soil microbiomes. Appl
738 Environ Microbiol 78:3744–3752.

739 72. Crits-Christoph A, Diamond S, Butterfield CN, Thomas BC, Banfield JF. 2018. Novel soil
740 bacteria possess diverse genes for secondary metabolite biosynthesis. Nature 558:440–444.

741 73. Galperin MY. 2005. A census of membrane-bound and intracellular signal transduction
742 proteins in bacteria: bacterial IQ, extroverts and introverts. BMC Microbiol 5:35.

74. Bowker MA, Belnap J, Davidson DW, Phillips SL. 2005. Evidence for Micronutrient Limitation of Biological Soil Crusts: Importance to Arid-Lands Restoration. Ecol Appl 15:1941–1951.

75. Swenson TL, Couradeau E, Bowen BP, De Philippis R, Rossi F, Mugnai G, Northen TR. 2018. A novel method to evaluate nutrient retention by biological soil crust exopolymeric matrix. Plant Soil 429:53–64.

76. Garcia-Pichel F, Pringault O. 2001. Microbiology. Cyanobacteria track water in desert soils. Nature 413:380–381.

77. Schauer S, Kutschera U. 2011. A novel growth-promoting microbe, Methylobacterium funariae sp. nov., isolated from the leaf surface of a common moss. Plant Signal Behav 6:510–515.

78. Nelson WC, Tully BJ, Mobberley JM. 2020. Biases in genome reconstruction from metagenomic data. PeerJ 8:e10119.

Figure legends

Figure 1. Examples of biocrust and hypolith environments with moss or cyanobacteria as the dominant photoautotroph anchor.

Figure 2. Principle Coordinate Analysis (PCoA) ordination plots of (A) all samples, (B) biocrust samples, and (C) hypolith samples. In panel A, 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 B shows biocrust samples, which are colored by primary producer (blue: cyanobacteria, red: moss). Panel C shows hypolith samples, which colored by the same scheme as in panel B.

Figure 3. 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. Heat map 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, 7: base excision repair. A list of genes with the corresponding KEGG orthology numbers can be found in Supplemental Table 6.

Figure 4. 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 ($P < 0.01$). Grey shows genes with p-values > 0.01. Panel A contains lifestyle-related two component systems, B contains two component systems related to redox signals and catabolites, and C 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 Supplemental Table 7.

Figure 5. 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 Supplemental Table 8.

Figure 6. 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 non-significant genes ($P>0.01$). A full list of bacterial secretion system genes significantly more abundant in biocrusts versus hypoliths can be found in Supplemental Table 11. The pathway schematic is based on the KEGG secretion system diagram, Costa et al. (2015), and (Mackelprang et al. 2017).

Figure 7: **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, crosslinking 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 Supplemental Table 12.

Figure 8. Overview of methanotrophy/methylotrophy pathways enriched in hypolith compared to biocrust communities. Significant genes ($P<0.01$) are indicated on the diagram. Genes with a p-value > 0.01 were omitted from the figure. Abbreviations are as follows. OAA: oxaloacetate, PEP: phosphenolpyruvate, EPPG: enolpyruvoyl-2-diphospho-5'-guanosine. A list of genes and the corresponding KEGG orthology numbers can be found in Supplemental Table 14.

Figure 9: Selected ABC transporters significantly more abundant in moss-dominated samples compared to cyanobacteria-dominated samples. Each system is labeled by the substrate transported. Each component is labeled with the gene name. Colored proteins are significant ($P<0.01$). Greyed proteins represent genes with a p-value > than 0.01. A complete list of ABC transporter genes that are significantly more abundant in moss-dominated samples are found in Supplemental Table 17.

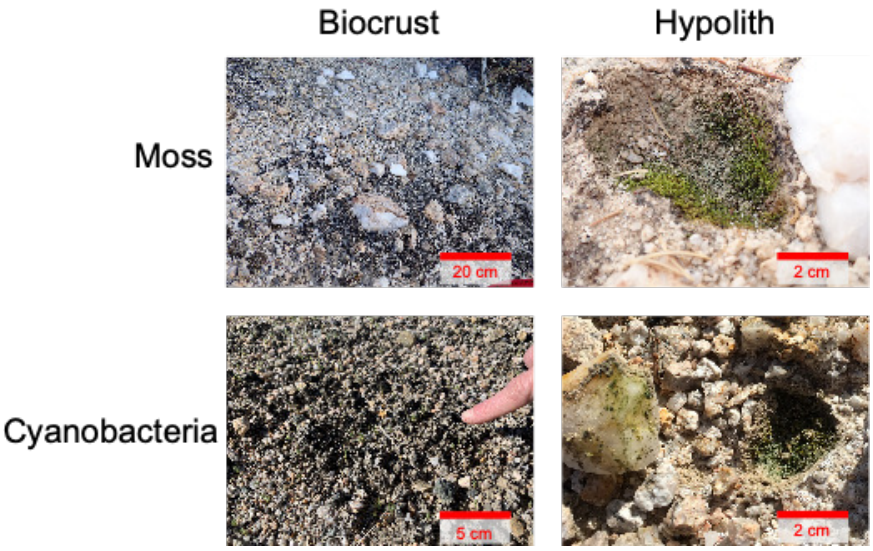

Figure 1. Examples of biocrust and hypolith environments with moss or cyanobacteria as the dominant photoautotroph anchor.

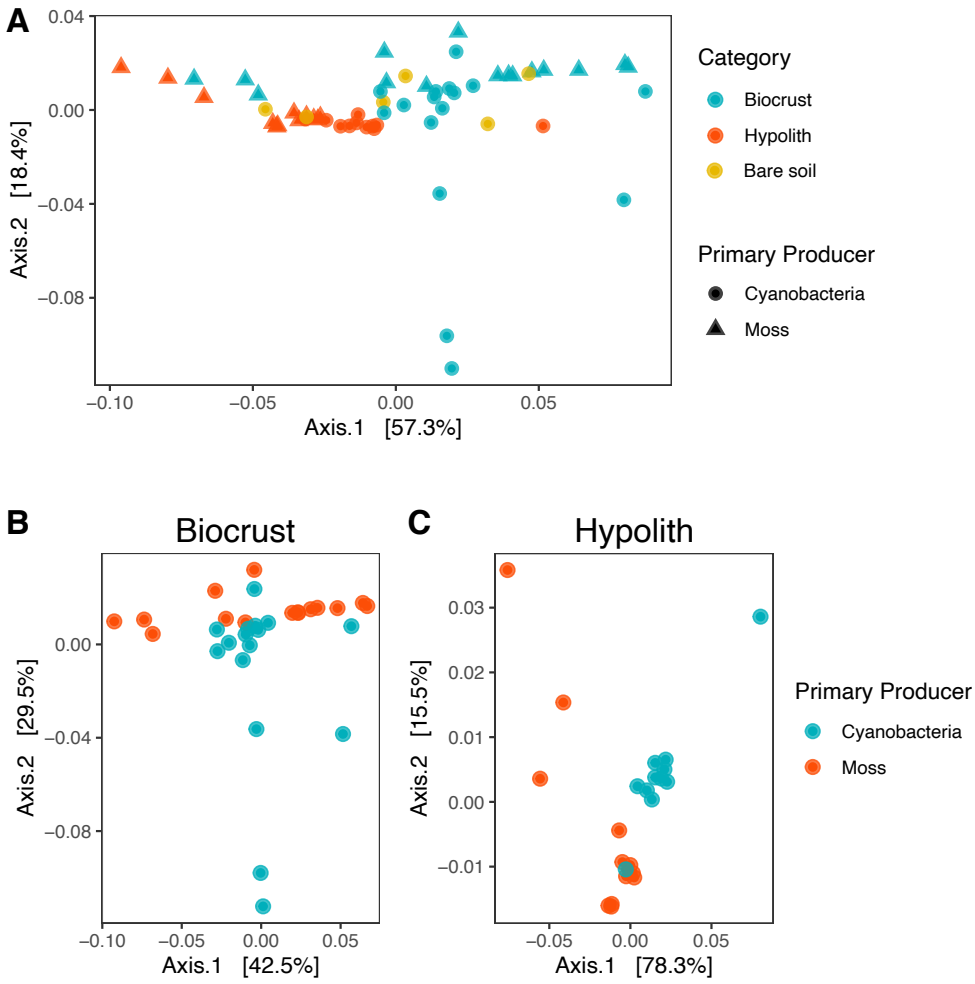

Figure 2. Principle Coordinate Analysis (PCoA) ordination plots of (A) all samples, (B) biocrust samples, and (C) hypolith samples. In panel A, samples are colored according to environmental niche (blue: biocrust, red: hypolith, yellow: bare soil) and shaped by dominant primary producer (circle: cyanobacteria, triangle: moss). Panel B shows biocrust samples, which are colored by primary producer (blue: cyanobacteria, red: moss). Panel C shows hypolith samples, which colored by the same scheme as in panel B.

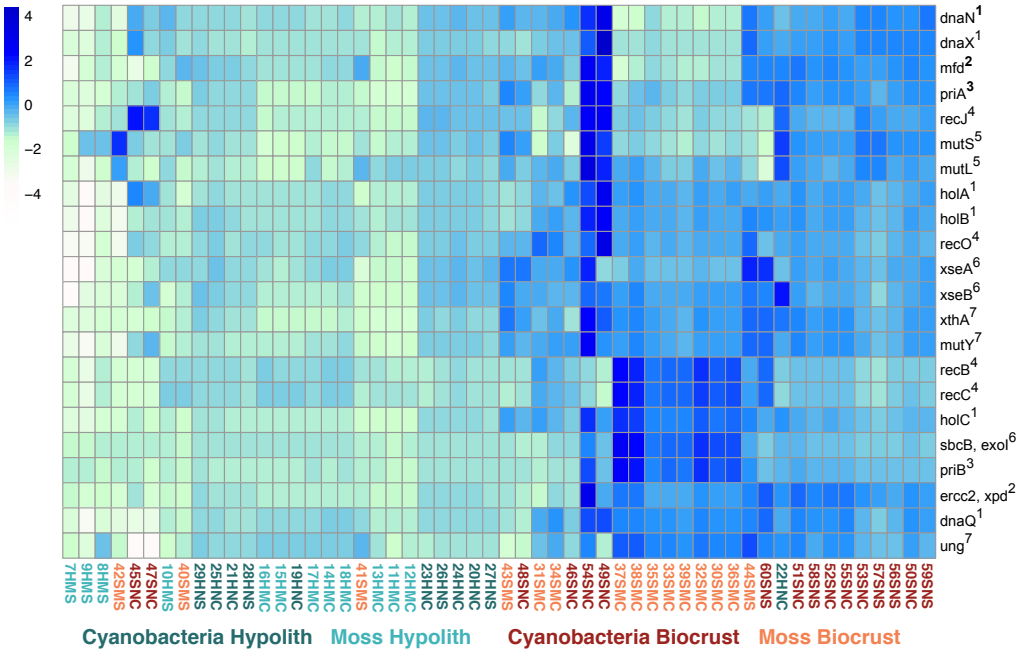

Figure 3. Heatmap of DNA repair genes significantly more abundant in biocrust communities versus hypolith communities. Genes are shown in rows and samples are shown in columns. Heat map colors show the relative abundances of genes scaled by row. Rows are labeled by gene names. Sample names are colored 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, 7: base excision repair. A list of genes with the corresponding KEGG orthology numbers can be found in Supplemental Table 6.

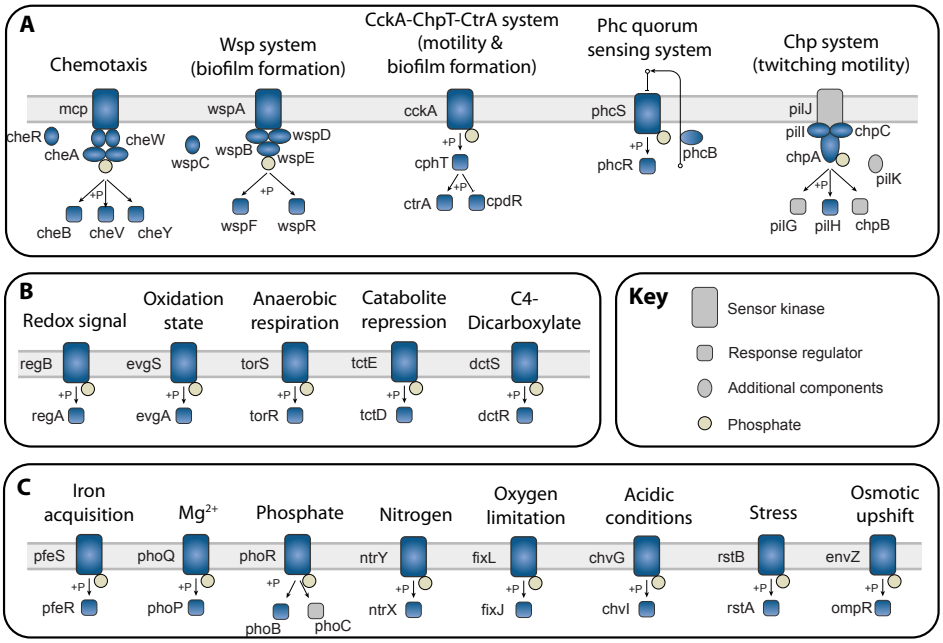

Figure 4. Selected two component systems enriched in biocrust crust communities. Two component systems canonically contain a sensor kinase (shown as rectangles within the membrane) and response regulator (shown as squares), which mediate downstream cellular responses when phosphorylated. Additional components 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 ($P < 0.01$). Grey shows genes with p-values > 0.01. Panel A contains lifestyle-related two component systems, B contains two component systems related to redox signals and catabolites, and C 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 Supplemental Table 7.

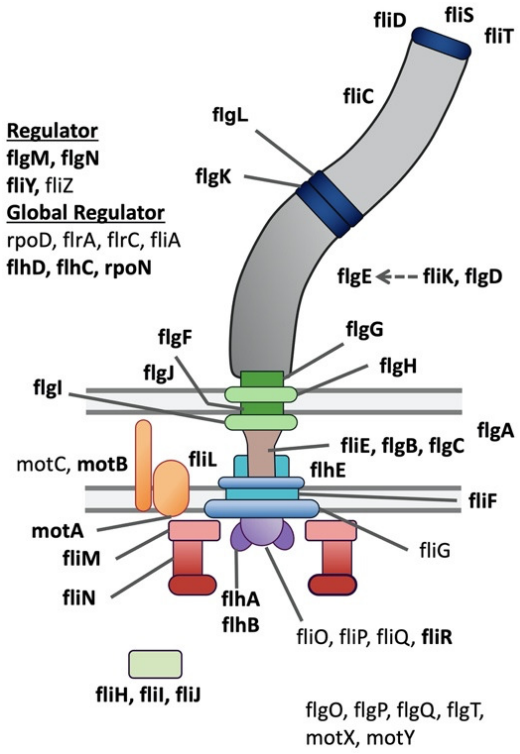

Figure 5. Diagram of the KEGG flagellar assembly pathway. Genes significantly more abundant in biocrusts versus hypoliths ($P<0.01$) are indicated in bold text. Genes where $P > 0.01$ are not bolded. KEGG orthology numbers corresponding to gene names are listed in Supplemental Table 8.

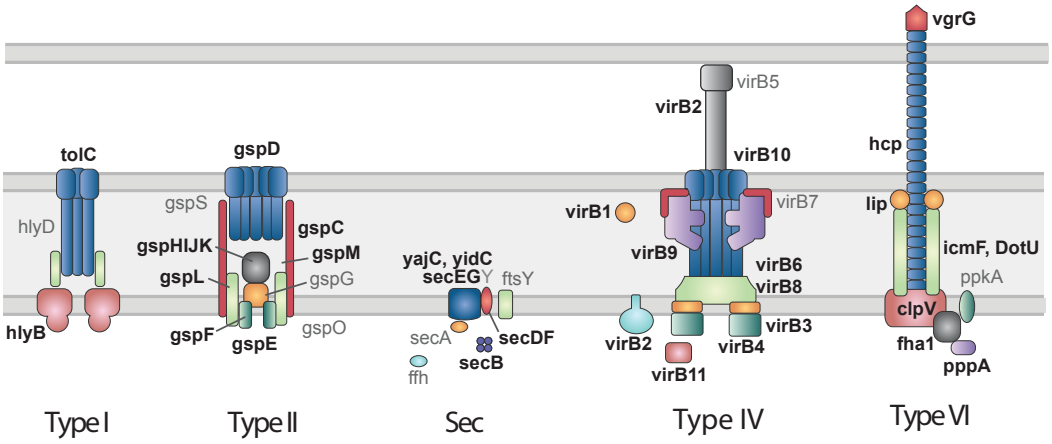

Figure 6. Schematic of bacterial secretion systems significantly more abundant in biocrust compared with hypolith communities. Each component is labeled with a gene name. Names in bold represent significant genes (P<0.01) and names in gray represent non-significant genes (P>0.01). A full list of bacterial secretion system genes significantly more abundant in biocrusts versus hypoliths can be found in Supplemental Table 11. The pathway schematic is based on the KEGG secretion system diagram, Costa et al. (2015), and (Mackelprang et al. 2017).

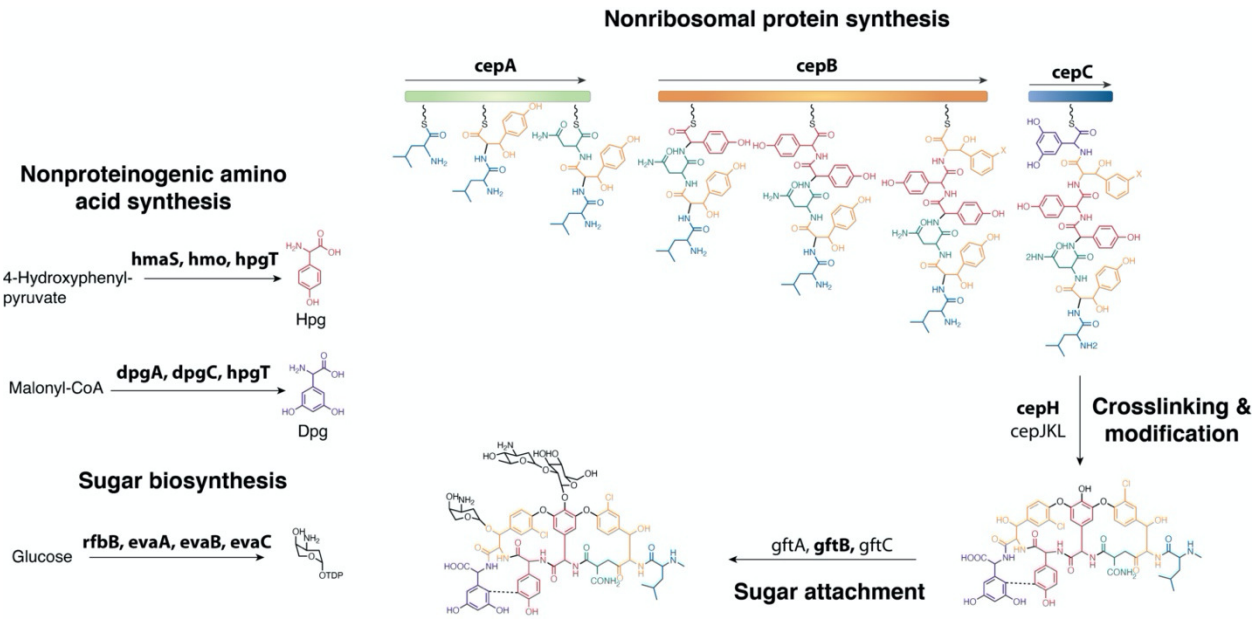

Figure 7: **Overview of vancomycin biosynthesis pathway labeled with genes significantly more abundant in hypolith compared with biocrust microbial communities.** Schematic represents all major steps in the pathway, including synthesis of nonproteinogenic amino acids 4-Hydroxyphenlglycine (Hpg) and 3,5-Dihydroxyphenylglycine (Dpg), sugar biosynthesis, assembly of the peptide backbone via nonribosomal proteins synthesis, crosslinking 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 Supplemental Table 12.

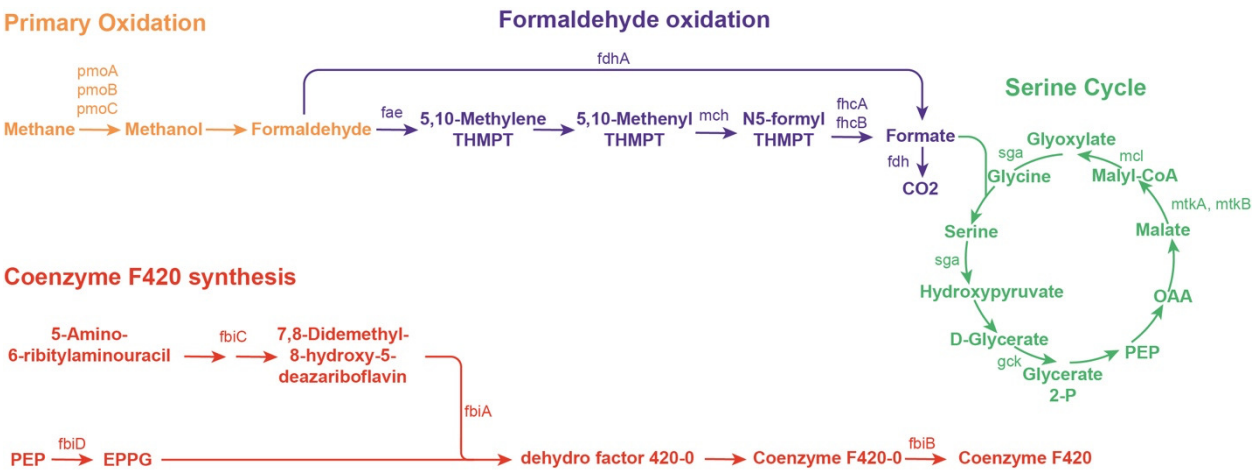

Figure 8. Overview of methanotrophy/methylotrophy pathways enriched in hypolith compared to biocrust communities. Significant genes (P<0.01) are indicated on the diagram. Genes with a p-value > 0.01 were omitted from the figure. Abbreviations are as follows. OAA: oxaloacetate, PEP: phosphenolpyruvate, EPPG: enolpyruvoyl-2-diphospho-5'-guanosine. A list of genes and the corresponding KEGG orthology numbers can be found in Supplemental Table 14.

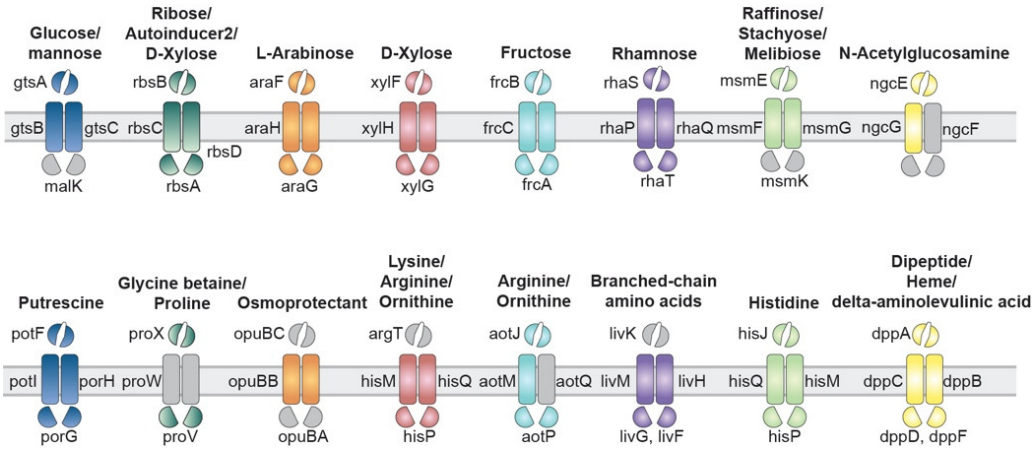

Figure 9: Selected ABC transporters significantly more abundant in moss-dominated samples compared to cyanobacteria-dominated samples. Each system is labeled by the substrate transported. Each component is labeled with the gene name. Colored proteins are significant (P<0.01). Greyed proteins represent genes with a p-value > than 0.01. A complete list of ABC transporter genes that are significantly more abundant in moss-dominated samples are found in Supplemental Table 17.

Tables

Table 1. KEGG pathways significantly enriched in biocrust versus hypolith communities

Pathway number	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*

*** P < 0.001
**P < 0.01
*P < 0.05

Table 2. KEGG pathways significantly enriched in hypolith versus biocrust communities

Pathway number	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*

*** P < 0.001
**P < 0.01
*P <0.05

Table 3. KEGG pathways significantly enriched cyanobacteria-anchored versus moss-anchored communities

Pathway number	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*

*** $P < 0.001$
**$P < 0.01$
*$P < 0.05$

Table 4. KEGG pathways significantly enriched moss-anchored versus cyanobacteria-anchored communities

Pathway number	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*

*** $P < 0.001$
**$P < 0.01$
*$P < 0.05$

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