Title

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Insights into drylands biocrust microbiome: geography, soil depth, and crust type affect biocrust microbial communities and networks in Mojave Desert, USA

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Abstract

Biocrusts are the living skin of drylands, comprising diverse microbial communities that are essential to desert ecosystems. Despite extensive knowledge on biocrust ecosystem functions and lichen and moss biodiversity, little is known about factors structuring diversity among their microbial communities. We used amplicon-based metabarcode sequencing to survey microbial communities from biocrust surface and subsurface soils at 4 sites located within the Mojave Desert. Five biocrust types were examined: Light-algal/Cyanobacteria, Cyano-lichen, Green-algal lichen, Smooth-moss, and Rough-moss crust types. Microbial diversity in biocrusts was structured by several characteristics 1) central versus southern Mojave sites displayed different community signatures, 2) indicator taxa of plant associated fungi (plant pathogens and wood saprotrophs) were identified at each site, 3) surface and subsurface microbial communities were distinct, and 4) crust types had distinct indicator taxa. Network analysis ranked bacteria-bacteria interactions as the most connected of all within-domain and cross-domain interaction networks in biocrust surface samples, with Actinobacteria, Proteobacteria, Cyanobacteria, and Ascomycota as hubs among all phyla. Specifically, the genera with highest node degree was *Pseudonocardia sp.* (Pseudonocardiales, Actinobacteria) in bacteria and *Alternaria sp.* (Pleosporales, Ascomycota) among fungal genera. Our findings provide crucial insights for dryland microbial community ecology, conservation, and sustainable management.

Keywords

Biological crusts; Fungi-bacteria networks; Bacteria; Desert Microbes; Cyanobacteria

1. Introduction

In vegetation-sparse drylands, plant interspaces are often covered by biological soil crusts (hereafter biocrusts) (Belnap et al. 2001). Microbial communities form biocrusts by interweaving soil particles as sticky biofilms and biofilaments establishing a living soil aggregate at the soil surface. Evolutionarily diverse organisms such as bryophytes, lichens, eukaryotic algae, cyanobacteria, bacteria, and fungi combine to form different types of biocrust distinguished by their dominant photoautotrophic community member as light (cyanobacterial/algal), dark (cyanobacterial/algal), lichen, and bryophyte crusts (Belnap et al. 2001; Bowker et al. 2006; Büdel et al. 2009; Pietrasiak et al. 2013; Maier et al. 2018; Weber et al. 2016). The complex combinations of microorganisms in biocrusts affect a range of ecosystem functions, such as: mediating soil nutrient cycles, preventing soil erosion and improving soil stabilization, assisting with regeneration of vegetation, as well as fertilizing and transforming subsurface soils (Belnap et al. 2001; Belnap et al. 2016; Maier et al. 2018; Weber et al. 2016; Belnap and Gardner 1993).

While there is an extensive body of literature on biocrust lichen and bryophyte diversity, as well as their roles in dryland ecosystems, studies have only recently begun exploring their less conspicuous community members. The earliest published work employed culture dependent approaches to survey biocrusts' microbial composition, which likely underestimated microbial diversity as was demonstrated by Amann et al. (1995) and Viaud et al. (2000). Modern DNA-based procedures greatly improved biodiversity assessment of microbial communities and environmental DNA sequencing approaches were recently introduced to biocrust diversity analysis (Garcia-Pichel et al. 2001; Garcia-Pichel et al. 2003; Gundlapally and Garcia-Pichel 2006; Steven et al. 2014; Steven et al. 2015; Maier et al. 2016; Maier et al. 2018; Couradeau et al. 2019; Moreira-Grez et al. 2019). These amplicon sequencing studies allow general profiles of biocrust bacterial communities to be drawn up. Abundant bacterial phyla in most biocrust systems included Acidobacteria, Actinobacteria, Proteobacteria, and Cyanobacteria. In particular, Cyanobacteria are fundamental for biocrust formation. For example, *Microcoleus* is one of the most well studied cyanobacterial genera in biocrust, functioning both as primary producer in the microbial community and as filament builder essential to the biocrust physical structure (Belnap and Gardner 1993; Büdel et al. 2016; Couradeau et al. 2019). In contrast, our knowledge of biocrust fungal diversity and function is extremely poor. Modern tools such as

microbial community network analysis will allow us to investigate how photoautotrophic diversity in biocrusts is associated with non-phototrophic archaea, bacteria, and fungi.

Cross-domain microbial network analysis such as SPIEC-EASI (SParse InverseE Covariance estimation for Ecological ASsociation Inference) can provide essential insights into relationships among microbial populations (Kurtz et al. 2015; Tipton et al. 2018), yielding a better understanding of connections between microorganisms as hubs and key connectors. A microbial hub is hereby defined as a microbe which has a high degree of connections to other microorganisms in a community while a key connector microbe is a bottleneck that serves as essential connection in microbial networks (Tipton et al. 2018). Cross-domain microbial network analysis has not yet been applied in biocrust systems, but could be crucial to discover biocrust microbial community linkages. Although network analysis presents correlation and not actual causation/interaction, joint surveying of fungal and bacterial/archaeal communities with amplicon sequencing data allows us to investigate the deeper complexity of biocrust microbiome diversity.

Equally underexplored are questions about regional patterning of biocrust diversity, or how diversity changes vertically when comparing the biocrust to the underlying soil. Denaturing gradient gel electrophoresis (DGGE) studies have reported similar major fungal phyla and bacterial phyla in biocrusts from different localities, albeit with different relative abundances (Gundlapally and Garcia-Pichel 2006; Steven et al. 2013; Liu et al. 2017). However, sampling was usually conducted over a small spatial scale, and the procedures employed often focused on a single group of organisms. To date, only a handful of studies have reported on geographical patterns of biocrust microbial communities across larger scales in North America (Nagy et al. 2005; Bates et al. 2009; Mogul et al. 2017; Fisher et al. 2019). Moreover, only a few surveys of biocrusts have included the underlying subsurface soil microbial community, to investigate the differences between surface and subsurface communities (Garcia-Pichel et al. 2003; Steven et al. 2013; Mueller et al. 2015). Vertical heterogeneity not only provides us with additional insights into the ecology of taxa found inside biocrusts, but may also create confounding effects when comparing alpha and beta diversity results from different surveys, especially if standard soil depths are sampled so as to combine surface biocrust material with subsurface soil.

These gaps in our understanding led us to investigate microbial community composition and structure at different levels of complexity: regionally, structurally among biocrust types, vertically by soil depth, as well as across multiple microbial phyla. We surveyed the microorganisms from three domains of life including Archaea,

Bacteria, and Fungi, using high-throughput amplicon sequencing targeting both the 16S rRNA and ITS1 markers. We collected biocrust samples from four different sites along a north-south axis within the Mojave and at the ecotone of the Mojave and Colorado Deserts, separately collecting surface and subsurface material from five different biocrust types at each site. We hypothesized that: 1) geographical locations do structure biocrust microbial communities: our 3 central Mojave sites will have similar microbial composition while the Joshua Tree National Park (JTNP) site at the ecotone of the Mojave and Colorado desert will have different microbial composition; 2) different geographical locations will harbor indicator species that are unique to each site; 3) soil depth affects fungal and bacterial diversity, light dependent microbes (Cyanobacteria) have higher abundances on the surface than subsurface soil. Both alpha and beta diversity will distinguish subsurface soil microbial community composition from surface communities; and 4) biocrust types relate to microbial diversity: more structurally complex assemblages such as lichen and moss crusts will have greater alpha diversity in both fungal and bacterial composition than structurally less complex types such as light algal crusts.

2. Materials and Method

2.1 Sampling sites and biocrust sampling

Biocrust samples were collected from four different sites in the Mojave Desert and at its southern edge. Our Joshua Tree National Park site (JTNP, GPS: 34.10N, -115.45W) was located at the ecotone of the Mojave Desert with the Colorado Desert, while sites at Granite Mountains (GMT, GPS: 34.78N, -115.63W), Kelso Dunes (KELSO, GPS: 34.89N, -115.69 W), and Cima volcanic field (CIMA, GPS: 35.20N, -115.87W) were located further north in the central Mojave Desert (Figure 1D). Using sterile sampling technique, five biocrust types were collected with a spatula. The underlying subsurface soil for each biocrust type was also collected (Figure 1G) by pushing a 5cm diameter brass core to a depth of 5cm (or less if subsurface rock was hit at a shallower depth). Light algal/cyanobacterial crust (LAC, Figure 1A), Cyanolichen crust (CLC, *Collema* spp., Figure 1C) and Green algal lichen crust (GLC, *Clavascidium lacinulatum,* Figure 1B & 1E) were collected at all four sites, while rough moss crust (RMC, *Syntrichia* spp., Figure 1F) and smooth moss crust (SMC, *Bryum* spp., Figure 1H) were collected at KELSO, GMT, and CIMA (neither type was sufficiently prevalent for collection at the JTNP sampling site). For each type of biocrust, surface versus subsurface soil samples were collected into separate sampling containers. In total, there were 10 soil samples per site collected from KELSO, GMT, and CIMA (1 surface and 1 subsurface

samples per crust type) while there were 18 samples from JTNP (3 replicates of 1 surface and 1 subsurface samples per crust type, no moss crusts present). Biocrust samples were stored on ice and transferred to a -80°C freezer at University of California, Riverside.

2.2 Amplicon sequencing data analysis

DNA extraction was performed with 0.15 g of biocrust using the QIAGEN DNeasy PowerSoil kit (Qiagen, Germantown, MD, USA) following the manufacturer's protocol. The ITS1F and ITS2 primer pair was used to amplify the ITS1 for the fungal communities according to Smith and Peay's Illumina MiSeq protocol (Smith and Peay 2014). The 515F and 806R primers were used to amplify the 16S rRNA V4 gene region for bacterial communities following Caporaso et al. (2011). PCR reactions were processed in 25 ul total volume in three replicates which included 1 ul of each primer (10 uM), 1 ul of genomic DNA, 12.5 ul of Taq 2X DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA), and 9.5 ul of nuclease-free water (Sigma-Aldrich, St.Louis, MO, USA). PCR conditions were: initial denaturation at 93°C for 3 min; 35 cycles of denaturation at 95°C for 45 sec, annealing at 50°C for 1 min, extension at 72°C for 90 sec, and a final extension at 72°C for 10 min using a C1000 thermal cycler (BioRad, Hercules, CA, USA). PCR products from three replicates were combined, purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Hoerdt, France) and pooled to produce equimolar mixture. Pooled libraries were quantified using Qubit dsDNA HS Assay (Life Technologies, Carlsbad, CA, USA) and analyzed using Agilent 2100 Bioanalyzer and Fragment Analyzer (Agilent Technologies, Santa Clara, CA, USA). Then pooled libraries were sequenced using Illumina MiSeq (San Diego, CA) with the V3 kit to generate paired-end reads in 2 x 300bp format, at the Institute for Integrative Genome Biology, Core Facilities, University of California, Riverside (http://iigb.ucr.edu). A total of 8,918,345 paired end sequence reads were produced and submitted to the Sequence Read Archive (SRA) databases associated with BioProject accession number PRJNA544067.

2.3 Bioinformatics

The fungal ITS1 amplicon sequences were analyzed with AMPtk: the Amplicon Toolkit for NGS data (formally UFITS) (Palmer et al. 2018) (https://github.com/nextgenusfs/amptk). The demultiplexed paired-end sequences were pre-processed by trimming forward and reverse reads to a maximum of 300 bp, trimming primer

sequences, and discarding reads less than 100 bp in length. The paired-end reads were merged to produce a single long read using USEARCH v9.1.13 (Edgar 2010) where they could be found to overlap. After pre-processing, a total of 3,040,944 valid paired sequence reads were produced. Sequence quality filtering was performed with the expected error parameter of 0.9 (Edgar and Flyvbjerg 2015), which produced 2,392,561 quality filtered reads. This cleaned sequenced dataset was clustered with UPARSE using a 97% percent identity parameter, which generated 2,569 Operational Taxonomic Units (OTUs) following the procedure of Palmer et al. 2018. Chimeric OTUs, sequences produced from PCR amplification of templates or parent sequences, were filtered using VSEARCH (v 2.3.2) (Rognes 2016) which removed 65 chimeras after comparison to the database. Finally, taxonomic assignment for 2,504 OTUs was performed with the AMPtk hybrid approach using names from UNITE v8.0 with 97% similarity (Nilsson et al. 2019) and functional guilds were assigned using FUNGuild v1.0 (Nguyen et al. 2016).

The 16S V4 amplicon sequences were analyzed using Quantitative Insights Into Microbial Ecology version 2 (QIIME2 v2019.1) (Bolyen et al. 2019) using bacterial 16S processing workflows. Demultiplexed sequence data (5,757,892 reads) were imported to QIIME2 then pre-processed by trimming primers from forward reads and quality control was performed using DADA2 (q2-dada2 plugin) (Callahan et al. 2016). The sequences were truncated to 250 bp based on the base call quality score during this step. After pre-processing steps, the resultant dataset contained 5,042,292 reads and amplicon sequences variant (ASV) tables with associated sequences were generated from DADA2. Taxonomy classification was performed using q2-feature-classifier (Bokulich et al. 2018) with extracted 515-806 SILVA database version 132 (Quast et al. 2013) based on ASV tables and associated sequences, which were well-developed for bacterial data processing through QIIME2 following published protocols (Bolyen et al. 2019, Callahan et al. 2016, Bokulich et al. 2018, Quast et al. 2013). Mitochondria and chloroplast sequences were removed from the dataset resulting in 18,564 ASVs. Functional Annotation of Prokaryotic Taxa (FAPROTAX) was used to assign ecological relevant functions to bacterial species (Louca et al. 2016).

2.4 Data analysis

Both fungal and bacterial (including archaeal) data were rarefied to 6,842 reads per sample in fungal data and 37,435 reads per samples in bacterial data, then analyzed using Phyloseq packages in R version 3.5.1 (R Core Team 2013) and Rstudio version 1.1.463 (RStudio Team 2015) for taxonomic composition, alpha diversity (observed OTUs/ASVs), and beta diversity (McMurdie and Holmes 2013). Differences in alpha diversity were

evaluated for homoscedasticity using Levene's test with the "leveneTest" function in the "car" package (Fox 2018). Homoscedastic data (location and crust type) were compared using ANOVA with the "Anova" function and pairwise multiple comparison (Tukey test) was performed with the "TukeyHSD" function in R. A type = "III" ANOVA was used to account for unbalanced design when comparing crust type. When data were heteroscedastic (soil depth), Welch correction was performed. Beta diversity was compared using PERMANOVA with the "adonis" function in the "vegan" package in R (Bray-curtis distance for fungi and Unifrac distance for bacteria, with permutation=999) (Oksanen et al. 2010). Network analysis was implemented with the Sparse InversE Covariance estimation of Ecological Association and Statistical Inference (SpiecEasi) package targeting network stability threshold of 0.05 (Kurtz et al. 2015) and followed the pipeline procedure for cross domain analysis using node degree to define hubs and betweenness centrality to examine connected networks (Tipton et al. 2018). Circular fungal-bacterial networks plots were generated using the "circlize" package in R to visualize cross-domain connections (Gu et al. 2014). We also performed indicator species analysis (function "indval" in "labdsv" package, Roberts 2016) in R to identify the significant OTUs/ASVs at p = 0.05 that are predicted to be part of the structured crust types and sites.

The analysis R script is available at (https://github.com/stajichlab/MojaveCrusts_2019).

3. Results

3.1 Does geographical location structure biocrust microbial communities?

No geographical differences of fungal richness of biocrust microbial communities were identified by analysis of alpha diversity of crust surface in our 4 sites (ANOVA, $F(3,20) = 1.64$, $p = 0.212$, Figure 2A). There were 38 fungal taxonomic classes observed across all samples (Figure S1). Although overall fungal alpha diversity analysis did not show significant difference among sites, fungal richness was significantly different among sites for 3 fungal classes (Leotiomycetes (ANOVA, $F(3,20) = 8.2575$, $p = 0.0009$), Blastocladiomycetes (ANOVA, $F(3,20) = 4.1667$, $p = 0.0191$), and Mucoromycetes (ANOVA, $F(3,20) = 3.1865$, $p = 0.046$) (Figure S1). GMT had the highest richness among the sites for Leotiomycetes and JTNP had the lowest richness. The chytrid lineages of Blastocladiomycete richness was greater in CIMA and GMT (central Mojave sites) than at JTNP, while Mucoromycetes richness was greatest at CIMA.

Alpha diversity analysis showed significant geographical differences for bacterial and archaeal richness (ANOVA, $F(3,20) = 4.745$, $p = 0.0117$, Figure 2B). At JTNP, bacterial species richness was significantly lower than

at GMT and KELSO, but not significantly different from the values at CIMA (Figure 2B). Bacterial species richness comparison (for each phylum) by site indicated a variable distribution of richness among 10 bacterial phyla (ANOVA, $p < 0.05$); including Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, Planctomycetes, Patescibacteria, Armatimonadetes, Gemmatimonadetes, and Verrucomicrobia (Figure S2A and Table S1). Across the bacteria phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, and Verrucomicrobia richness was lowest at JTNP, mirroring the pattern observed in the 3 dominant fungal classes. The Acidobacteria, Planctomycetes, Patescibacteria, and Gemmatimonadetes richness was greatest at GMT.

Beta diversity analysis of biocrust fungal communities differed significantly by site (PERMANOVA, $p = 0.001$, $R^2=0.178$) (Figure 3A). These differences in beta diversity were visualized in PCoA plots revealing a geographical pattern: JTNP biocrust fungal composition clustered separately from central Mojave fungal communities (KELSO, GMT, and CIMA). Evaluation of bacterial members of the samples found that communities were significantly different by site (PERMANOVA, $p = 0.001$, $R^2=0.129$) (Figure 3B). The distinct clustering of JTNP bacterial communities away from the three sites of the central Mojave resembled findings from the fungal communities.

3.2 Do biocrusts contain indicator microbial species at each site?

Overall, our analysis revealed fewer numbers of fungal indicator taxa for geographic location than bacteria across all surface samples. A total of 11 indicator fungal OTUs was revealed based on sampling site: 2 OTUs for CIMA (closest related taxon: *Catenulomyces convolutus* - with an unassigned functional guild and *Preussia terricola* - a dung saprotroph and/or plant saprotroph); 4 OTUs for JTNP (closest related taxon: *Allophoma labilis* - a plant pathogen; *Curvularia inaequalis* - a plant pathogen; *Entoloma halophilum* - an ectomycorrhizal, fungal parasite, and/or soil saprotroph; and *Preussia africana* - a dung saprotroph and/or plant saprotroph; and 5 OTUs for KELSO (closest related taxon: *Alternaria hungarica* - an animal pathogen, endophyte, plant pathogen, and/or wood saprotroph; *Cladosporium herbarum* - a plant pathogen and/or wood saprotroph; *Colletotrichum gloeosporioides* - an endophyte and/or plant pathogen; *Fusarium oxysporum* - a plant pathogen, soil saprotroph, and/or wood saprotroph; and *Ulocladium dauci* - a plant pathogen. No fungal indicator species were predicted by the analysis for the GMT site (Table S2).

Bacterial and archaeal indicator species analysis of biocrust surface samples found 67 ASVs when analyzed by site. Indicator ASVs include 29 indicator ASVs for CIMA (3 classifiable ASVs were most closely similar to bacteroidetes *Segetibacter aerophilus* - with an unassigned functional guild, cyanobacteria *Chroococcidiopsis sp. BB79.2* - with an unassigned functional guild, deinococcus-thermus *Deinococcus maricopensis DSM 21211* - a nitrate reducer), 15 species/ASVs for GMT (only 1 classifiable species, most closely similar to bacteroidetes *Parahymenobacter deserti* - with an unassigned functional guild), 12 species/ASVs for JTNP (1 classifiable species was bacteroidetes *Hymenobacter rigui* - a nitrate reducer), and 11 species/ASVs for KELSO (closest to proteobacteria *Roseomonas pecuniae*, proteobacteria *Sphingomonas kaistensis*) (Table S3).

3.3 Do biocrust microbial surface communities differ from those in the adjacent soil subsurface?

When comparing alpha diversity by soil depth, biocrust surface samples had significantly lower species richness than subsurface soil samples, both for fungal and bacterial richness (fungal Welch's t-test, $t(43.594) = 3.208$, $p = 0.0025$) (bacterial Welch's t-test, $t(31.587) = 9.84$, $p = 3.856e\text{-}11$) (Figure 2C and 2D). Ten fungal classes showed significantly higher richness in subsurface soils (Welch's t-test, $p < 0.05$): Ascomycete classes: Sordariomycetes, Schizosaccharomycetes, Saccharomycetes, Orbiliomycetes, Pneumocystidomycetes; Basidiomycota class Agaricomycetes; Mucoromycota classes: Mucoromycetes, Mortierellomycetes; and Zoopagomycota classes: Basidiobolomycetes and Entomophthoromycetes (Table S4, Figure 4B, 4C, and 4D). Nevertheless, most fungal OTUs (514 OTUs) were shared between surface biocrust and subsurface samples (Figure S3B). For bacterial and archaeal communities, distinct differences can already be seen in the relative abundance values in the taxonomic composition bar plot among the 30 prokaryotic phyla (Figure 5 and S2). Nineteen phyla showed significant differences between surface vs. subsurface samples (Welch's t-test, $p < 0.05$); Proteobacteria, Firmicute, Actinobacteria, Euryarchaeota, Nanoarchaeota, Thaumarchaeota, Acidobacteria, Planctomycetes, Patescibacteria, Elusimicrobia, Armatimonadetes, Chloroflexi, Gemmatimonadetes, Entotheonellaeota, Cyanobacteria, Nitrospirae, FBP, Fibrobacteres, and Verrucomicrobia (Figure 5 and Table S5). Nearly all of these phyla showed greater species richness in subsurface soil than in biocrust samples. Cyanobacteria were the only bacterial phylum with significantly greater richness in biocrust surface samples than in subsurface soil. Similar to the fungal community the majority of bacterial surface ASVs (2883 ASVs) were shared between surface biocrust and subsurface samples (Figure S3A).

Beta diversity analysis of biocrust fungal communities showed significant difference by soil depth (PERMANOVA, p = 0.001, R^2=0.071) (Figure 3A). These differences in beta diversity were visualized in PCoA plots revealing that JTNP showed the strongest surface-subsurface clustering while central Mojave showed some surface-subsurface clustering, but not as clearly distinct as we observed in JTNP. Evaluation of bacterial members of the samples found that communities were significantly different by soil depth (PERMANOVA, p = 0.001, R^2=0.113) (Figure 3B). Bacterial communities displayed surface-subsurface patterning: surface samples clustered closer together in the PCoA plots and the majority of subsurface samples were clustered near each other (noting that two subsurface samples clustered with surface samples).

Bacterial networks were the most connected in biocrust surface samples (~54% were bacterial-bacterial connections) among all microbial communities both within (bacteria-bacteria, archaea-archaea, and fungi-fungi) and across (fungi-bacteria, bacteria-archaea, and fungi-archaea) domain networks for surface community (network stability = 0.047) (Figure 6). The network inferred from the ASV abundances in surface samples indicated that these communities are mostly structured within a single large connected network (in the center of Figure 6) instead of several distinct clusters of networks. Betweenness centrality and node degree analysis showed that microbial hubs of biocrust surface community included Actinobacteria, Proteobacteria, Cyanobacteria, and Ascomycota: high node degrees were observed in these four phyla, indicating high numbers of network connections. *Pseudonocardia sp.* was found to have the highest node degree (most connections) while *Methylobacterium sp.*, *Microvirga sp.*, *Microcoleus sp.*, and *Belnapia sp.* also had high node degrees (high connections) representing microbial hubs for biocrust surface network. However, many other microbial hubs were uncultured bacteria and/or unknowns. *Alternaria sp.* had the highest node degree and betweenness centrality in the fungal community (Figure 7). For biocrust surface samples, overall cross domain (fungal-bacteria connections, within domain networks removed) links included; 1) Agaricomycetes and Dothideomycetes linked to Actinobacteria, 2) Agaricomycetes, Dothideomycetes, Eurotiomycetes, Orbilliomycetes, and Sordariomycetes linked to Cyanobacteria, 3) Dothideomycetes, Eurotiomycetes, and Pezizomycetes linked to Alphaproteobacteria, and 4) Dothideomycetes, Lecanoromycetes, and Sordariomycetes were linked to Blastocatellia (Figure 8). The complete network of microbial connections within and across domains is depicted in Figure S4.

Subsurface soil samples showed similar patterns to surface biocrust where bacterial networks were more connected than other microbial community networks (network stability = 0.048) (Figure S5). Betweenness centrality

and node degree analysis showed that microbial hubs of the biocrust subsurface community included Actinobacteria, Proteobacteria, Thaumarchaeota, and Ascomycota. *Microvirga sp.* was found to have the highest node degree (most connections) while *Modestobacter sp.* and *Candidatus Nitrososphaera* also had high node degrees and represented microbial hubs for biocrust subsurface network. Similar to the biocrust surface microbial network, many other microbial hubs were uncultured bacteria and/or unknowns. Identical to surface samples, *Alternaria sp.* had the highest node degree and betweenness centrality in subsurface fungal networks (Figure S6). Although large connected networks were observed as well, a major backbone of multiple fungal-bacterial networks in subsurface communities revealed features different from surface microbial communities. Fungal-bacterial networks in subsurface samples included; 1) Agaricomycetes, Dothideomycetes, Eurotiomycetes, and Lecanoromycetes were linked to Actinobacteria, 2) Agaricomycetes, Basidiobolomycetes, Dothideomycetes, Leotiomycetes, Orbilliomycetes, and Sordariomycetes were linked to Alphaproteobacteria, 3) Dothideomycetes were linked to Bacteroidia, 4) Dothideomycetes and Mortierellomycetes were linked to Blastocatellia, 5) Dothideomycetes were linked to Chloroflexia, 6) Dothideomycetes were also linked to Gammaproteobacteria, and 7) Eurotiomycetes were linked to Rubrobacteria (Figure S7).

3.4 Do biocrust types each have their own unique assemblages of microbes and do characteristic differences in richness exist between them?

Fungal and bacterial richness of biocrust surface samples differed significantly by crust type (fungal ANOVA, $F(4,13) = 5.5869$, $p = 0.007668$ (Figure 2E), bacterial ANOVA, $F(4,12) = 3.9425$, $p = 0.02869$ (Figure 2F). The GLC crusts had the lowest fungal species richness among crust types. The LAC samples had bacterial alpha diversity that was significantly lower than CLC, but not significantly lower than in other crust types (Figure 2F). Comparing fungal richness across crust types identified 9 fungal classes that differed significantly by crust type (ANOVA, $p < 0.05$), including most of the major classes in Ascomycota: Sordariomycetes, Eurotiomycetes, Lecanoromycetes, Dothideomycetes, Leotiomycetes, Schizosaccharomycetes, Pezizomycetes, and Basidiomycota classes: Agaricomycetes and Tremellomycetes (Figure S1B and Table S6). GLC generally had lower fungal richness than the other crust types when comparing class-specific richness. Moss crusts (RMC and SMC) had greater richness within the fungal classes Leotiomycetes, Peziozomycetes, and Tremellomycetes. For bacterial communities, ten phyla were significantly different by crust type (ANOVA, $p < 0.05$); Proteobacteria,

Acidobacteria, Planctomycetes, Patescibacteria, Armatimonadetes, Deinococcus-Thermus, Chloroflexi, Cyanobacteria, FBP, and Verrucomicrobia (Figure S2B and Table S7). In Proteobacteria, Acidobacteria, Planctomycetes, Patescibacteria, and Verrucomicrobia, species richness was greater in moss crusts (RMC and SMC) than in other crust types. Richness of Armatimonadetes was lowest in LAC while Chloroflexi richness was highest in CLC. Lastly, Cyanobacteria richness was lower in moss crusts than in other crust types, versus highest in CLC.

Beta diversity analysis of biocrust fungal communities showed significant differences by crust type (PERMANOVA, $p = 0.001$, $R^2=0.174$) (Figure S8). These differences in beta diversity were visualized in PCoA plots. GLC fungal community was distinctly different from other crust types, while the other biocrust types had overlaps. Evaluation of bacterial members of the samples found that communities were also significantly different by crust type (PERMANOVA, $p = 0.001$, $R^2=0.181$) (Figure S9).

Indicator analysis revealed that 2 fungal OTUs were detected by crust type at all locations. Specifically, for RMC there was 1 indicator OTU (closest related taxon: *Sporormia subticinensis,* dung saprotroph) and likewise for SMC 1 OTU (closest related taxon: *Acrophialophora levis,* plant pathogen). No detectable fungal indicator species occurred in CLC, GLC, and LAC (Table S8). Bacterial indicator analysis found 36 indicator ASVs within crust types. There were 9 indicator ASVs for CLC (most closely similar to Proteobacterium *Azospirillum soli* - nitrate respiration), 4 indicator ASVs for LAC (closest to Proteobacterium *Belnapia moabensis*), 6 indicator ASVs for RMC (closest to Proteobacterium *Salinarimonas sp. BN140002*) and 17 indicator species/ASVs for SMC (closest relatives were Deinococcus-Thermus *Deinococcus pimensis DSM 21231* - nitrate reduction and Cyanobacterium *Calothrix sp. HA4186-MV5* - nitrogen fixer). No bacterial indicator species/ASVs were observed in GLC (Table S9).

4. Discussion

In our study, we identified several distinct patterns structuring biocrust microbial communities in the Mojave Desert. These patterns included 1) a distinct geographical signal between our three central Mojave sites versus the southern Mojave site, 2) a soil depth pattern which clearly differentiated biocrust surface diversity from subsurface microbial communities, and 3) a biocrust type pattern which showed differences between algal, lichen, and moss crusts.

4.1 Geographical pattern: Does geographical location structure biocrust microbial communities and reveal unique microbial species?

Many studies have demonstrated biogeographical patterning of bryophyte and lichen biocrusts based on climatic, edaphic, topographic and biotic factors at various spatial scales (Büdel 2001; Bowker et al. 2016). Less is known about biogeographical patterns of the microbial taxa that make up biocrusts. We hypothesized that geography would structure biocrust microbial communities and detected distinct geographical patterns within the Mojave Desert in which both alpha and beta diversity differentiated microbial communities in central Mojave (GMT, KELSO, and CIMA sites) from southern Mojave (JTNP site). Our results support the findings of other studies in which microbial communities were more similar when collecting sites were in close proximity compared to further away (Nagy et al. 2005; Bates et al. 2012; Mogul et al. 2017). In addition to spatial autocorrelation relationships these major differences could likely point to environmental gradients based on changes in elevation, temperature, and rainfall stretching from central Mojave to the Mojave-Colorado desert ecotone at JTNP, which could contribute to the separation of southern Mojave microbial communities from central Mojave diversity. In previous research, boundaries between biomes such as the Mojave and Colorado Deserts have been identified using vascular plant community composition. The key species *Prosopis glandulosa* var. *torreyana* and *Fouquieria splendens* are indicative for the Colorado Desert which appear in lower elevation and warmer climate while *Yucca brevifolia* is characteristic for higher elevation with winter rain in the Mojave Desert (Holmgren et al. 2010). Microbial communities may similarly respond to the same drivers which can be detected as species turnover when surveying sites along environmental gradients that stretch from the Mojave to Colorado Desert. However, additional studies are needed to specifically identify these drivers.

To our knowledge, this is the first report of geographical fungal indicator taxa obtained from biocrust samples. We hypothesized that different geographical locations will harbor key species that are unique to each site. We also obtained first predictions of putative functional roles of the detected fungal taxa. Indicator taxa were mostly assigned to plant associated fungi belonging to pathogenic, endophytic and saprotrophic functional guilds. At JTNP all classifiable fungal indicator taxa were plant pathogens while an indicator taxon at CIMA was plant saprotroph. According to these results, certain functional guilds were more confined to colonize at particular sites which is likely because of more living plant availability at JTNP than CIMA versus more plant debris at CIMA volcanic fields than JTNP. The fungal loop hypothesis states that fungi metabolically link plants and biocrusts in drylands (Collins et al.

2008), yet our results suggest other important plant-biocrust interactions may be mediated through the fungal community found in biocrust. For example, biocrusts' ability to capture seeds (Zhang et al. 2016) could also mean that plant associated fungal spores trapped by biocrusts could establish and could interact with the local plant communities in terms of symbiotic or pathogenic relationships. In contrast to the fungal data, most bacterial indicator taxa functions could not be classified in our study and therefore functional patterns cannot be identified. Further study is needed to explore bacterial functional guilds.

4.2 Soil depth pattern: Do biocrust microbial surface communities differ from those in the underlying soil subsurface?

Because biocrusts are localized to the surface of soils, they are considered a living skin of drylands (Belnap et al. 2016) housing unique microbial communities. One pioneering study comparing microbial communities at the biocrust surface with lower soil layers was conducted on the Colorado Plateau, using a culture-based quantification of viable aerobic copiotrophs and microscopic counts. Bacterial populations were found to be higher in the biocrusts on the soil surface compared to its associated subsurface soil or to soil without crust (Garcia-Pichel et al. 2003). Two additional studies sampling the Colorado Plateau and central Mojave demonstrated that bacterial alpha and beta diversity separated samples by soil depth when amplicon sequencing was used, which provided a more comprehensive microbial diversity survey (Steven et al. 2013; Mogul et al. 2017). Yet, only one single study to date has surveyed both the fungal and bacterial communities in biocrusts as well as the soil below, finding that in southern Nevada biocrusts there was lower diversity for both domains compared to the subsurface soil (Mueller et al. 2015).

Based on these studies we hypothesized that structuring of soil microbial communities is driven by higher richness of light dependent microbes (Cyanobacteria) on the biocrust surface. Similar to Mueller et al. (2015) we found lower overall species richness within biocrust samples as compared to their soil underneath. We observed that cyanobacteria richness was higher in the biocrust consistent with their dependence on light availability for photoautotrophic metabolism (Garcia-Pichel et al. 2003; Steven et al. 2013). In previous studies, the bacterial phyla Acidobacteria, Actinobacteria, Chloroflexi, and Proteobacteria were found to be more diverse in subsurface soil (Steven et al. 2013; Mogul et al. 2017) and we observed a similar pattern. In addition, in our work we found 12 additional bacterial and 3 archaeal phyla with significantly greater richness in surface samples than subsurface soil

(Table S4). Our observations found that bacterial communities showed a distinct depth dependent organization in Mojave Desert biocrust.

Examination of the fungal community revealed a similar pattern and fungal species richness in biocrust subsurface samples was found to be greater than in surface soil. These findings indicated that Sordariomycetes were skewed in a similar vertical distribution as was found in Nevada biocrusts (Mueller et al. 2015), but we also identified nine additional fungal classes with significant soil depth association at our collection sites. Overall, the majority of fungal OTUs were found in both California and Nevada but most did not display significant differences in soil depth in Nevada.

We used cross domain (fungal-bacterial) networks to further explore the soil depth patterns. Incorporating both fungi and bacteria communities in microbial network analysis improved network stability compared to single-domain microbial networks (Tipton et al. 2018). To better understand the entire microbial network in biocrust systems, bacteria and fungi were jointly analyzed in a single cross-domains network analysis, identifying key, or also known as hub, microorganisms in both domains. Our network analysis showed that Cyanobacteria were key to fungal-bacterial connections for biocrusts (surface), which supports previous hypotheses of their importance (Belnap and Gardner 1993; Büdel et al. 2016). Cyanobacteria have been inferred to be key taxa in biocrusts due to the high photoautotrophic biomass they contribute to the surface of biocrusts (García-Pichel et al. 2003; Steven et al. 2013; Mueller et al. 2015). We also identified Pleosporales (Dothideomycetes) as key to fungal-bacterial connections. Their dominance among fungal taxa in biocrust and semiarid and arid areas in general correspond to their substantial reference database and diverse lifestyle (Bates et al. 2012; Porras-Alfaro et al. 2011). We also found that Agaricomycetes were another major group of fungal connectors, which fits the abundance of the group as reported in the southern Nevada study (Mueller et al. 2015). Top OTUs in this group are ectomycorrhizal, mushroom, fungal parasite, and/or soil saprotroph which could be essential in plant-microbe and microbe-microbe interaction. Thus, our data suggested two groups of fungi that could potentially be key microorganisms for biocrusts in both southern California and Nevada.

Particular key microbes identified as microbial hub taxa included *Pseudonocardia* sp., *Methylobacterium* sp., *Microvirga* sp., *Microcoleus* sp., and *Belnapia* sp. Specific traits of these microbes could be essential for the functioning and community dynamics, such as structuring biocrust with polysaccharide by *Microcoleus* spp. (Belnap and Gardner 1993), producing antibiotics against microfungal parasites by *Pseudonocardia* sp. (Cafaro et al. 2011;

Carr et al. 2012), *Microvirga* sp. forming root nodules in plants (Ardley et al. 2012; Radl et al. 2014), and *Methylobacterium* sp. facilitating seed germination and plant development (Lidstorm and Ludmila 2002).

In contrast to surface microbial networks, Cyanobacteria were not present as hubs in our analyses of subsurface soil samples. However, Pleosporales (Dothideomycetes) were still a major connector for fungal-bacterial networks in subsurface soil while Agaricomycetes were also found as a minor key connector. The fungal networks appear to be similar between surface and subsurface, while bacterial networks were different primarily due to the lack of photoautotrophs. Furthermore, identifiable hubs for subsurface microbial networks seem to drive different functions than networks of the surface community. Only *Microvirga sp.* was found to be similar microbial hubs to the surface network. *Modestobacter sp.*, and *Candidatus Nitrososphaera* were the other two major hubs for subsurface soil which are known to inhabit extreme environments (temperature) (Busarakam et al. 2016; Pitcher et al. 2010). *Candidatus Nitrososphaera* is an ammonia oxidizing bacteria which plays a role in the nitrogen cycle while one of the key functions of *Modestobacter sp.* is melanin production (Busarakam et al. 2016; Pitcher et al. 2010). However, the functional roles of these microbial hubs will need to be explored further to better understand these hubs in Mojave Desert soils. Overall, we noted a strong soil depth pattern in our Mojave biocrusts, with greater numbers of bacterial phyla and fungal classes contributing to these patterns than previously reported (Garcia-Pichel et al. 2003; Steven et al. 2013; Mueller et al. 2015). Nonetheless, functional guilds could not as yet be identified for both bacteria and fungi that contributed mainly to soil depth patterning.

4.3 Biocrust type pattern: Are biocrust types linked with microbial diversity?

The classification of biocrusts have been based on a combination of their morphology, aggregation strength, overall functional role, and by their dominant photoautotrophs (Bowker et al. 2006, Büdel et al. 2009, Pietrasiak et al. 2013, Belnap et al. 2016, Maier et al. 2018). Environmental factors such as temperature, moisture, salinity, soil texture, dust deposition, geomorphology etc. influence the occurrence and abundance crust types at a local to regional scale (Williams et al. 2013, Pietrasiak et al. 2014, Belnap et al. 2016). Crusts found in different localities are classified as common types such as “cyanobacterial” or “lichen crusts” with visibly similar morphologies, but it remains unknown how much the constituent microbial communities vary among the same crust type found in different locations. We examined and compared the fungal and bacterial communities of five different biocrust types including LAC, CLC, GLC, SMC, and RMC. We hypothesized that microbial diversity will be

strongly associated with crust type, and structurally complex assemblages such as lichen and moss crusts will have greater alpha diversity in both fungal and bacterial composition than structurally less complex types such as light algal/cyanobacterial crusts. The bacterial species richness was indeed lower in LAC than in the more highly structured lichen and moss crusts, matching previous findings (Maier et al. 2018; Chilton et al. 2018). However, such differences were not mirrored in fungal communities. These observations raise new questions. If fungal communities are more similar to each other based on geographic location but do not differ among crust types, is there substantial crust to crust exchange of fungi with minimal dispersal limitations? Is there a core of fungal taxa required to promote crust establishment that is universal to all types? Alternatively, the patterns of fungal diversity could be explained by other abiotic and biotic factors or just be randomly assembled across biocrust types. Further sampling to test new hypotheses about geographic structure will need to be undertaken to more fully explore these ideas.

Cyanobacteria, which were inferred to be major microbial hubs in our network analysis, were more abundant in LAC, CLC, and GLC than in SMC and RMC. The dominance of Cyanobacteria in LAC and lichen crusts, but not in moss crusts, is indicative of their central role as primary autotrophic community members versus their less prominent role where mosses are dominant. Alpha diversity analysis also differentiated cyanobacterial (LAC) and lichen (CLC, GLC) crusts from moss crusts (SMC and RMC) in their richness of Proteobacteria, Actinobacteria, and Acidobacteria with greater alpha diversity in moss crusts than in cyanobacterial and lichen crusts. Moss crusts have been shown to retain more moisture than light cyanobacterial crust (Kidron and Benenson 2014) as well as fix carbon at higher rates (Pietrasiak et al. 2013). Greater microhabitat moisture availability and fertility may increase microbial diversity (Bao et al. 2019).

In addition, our indicator species analysis also showed that both types of our Central Mojave moss crusts contained fungal as well as bacterial indicator species (Table S6 and S8) suggesting that moss crusts may have a very defined core microbiome. However, due to limited sample numbers in this study, this possibility will need validation through geographically extensive sampling efforts in future studies. We were not able to match identical fungal species from sequences with lichen biocrust types as we identified from external morphology, but several OTUs matched Peltigerales (with high abundance in our CLC samples) and could possibly be the fungal symbionts in *Collema sp.* while many OTUs matched Verrucariales (with high abundance in our GLC samples) that might be the symbionts in *Clavascidium sp*. (Figure S1B) This issue clearly shows that better molecular markers are needed

for these lichens. Lastly, due to heterogeneous soil microbial communities and small sample size, increased crust types sampling and replicates are needed in future studies to be able to better understand biogeographical biocrust type patterns and investigate within and between variabilities in community composition and structure.

Lastly, we wanted to compare the microbial communities within the same functional crust type but sampled from different locations. Despite sampling the same functional biocrust types in all 4 localities, beta diversity and indicator species analysis indicated that central Mojave Desert localities had unique microbial communities in the surveyed crust types dissimilar from the same crust types sampled in JTNP. Although external morphology was not visibly different, microbial communities differed and indicator taxa were detected for specific locations. If this pattern persists in other desert ecosystems or even other locations within the Mojave Deserts this would mean that we cannot readily assume similarities in microbial community composition when classifying biocrusts by functional groups or morphological community types. Future investigations could focus on a broader more extensive sampling of crust types and exploring the questions of how local some of the microbial communities may be or if communities are rather stochastically assembled.

4.4 Implication to conservation and restoration management

Current efforts to restore biocrusts in heavily disturbed landscapes often yield limited success in the field (Doherty et al. 2015; Antoninka et al. 2016; Chiquoine et al. 2016; Ayuso et al. 2017). We think that such challenges arise from our lack of a comprehensive knowledge of local and regional community dynamics, dispersal modes, physiological constraints, taxonomic identities, biotic interactions, and functional roles of the microscopic community members. Our findings stimulate several new thoughts towards biocrust conservation and restoration management. In our dataset, although limited to five biocrust types from four sampled sites which were 10-50 km apart, microbial communities from the same biocrust type in different locations were not identical. Our results suggest that community heterogeneity could be related to biogeography and ecological processes such as dispersal limitations, competitive exclusion, local-scale microhabitat specializations, etc that could influence biocrust microbial community assembly (Warren et al. 2019) and can represent an additional drivers of biocrust community composition that has yet to be considered in biocrust research. Followup studies are needed to comprehensively investigate what environmental factors impact alpha, beta, and gamma diversity of microbial communities. Such knowledge can be highly informative when considering a source of biocrust inoculum for restoration especially

between sites that are far apart. More sites and crust types within the Mojave Desert need to be studied to identify the differences in microbial profiles among five crust types in the northern, eastern, and western parts of Mojave Desert compared to central and southern locations in our study. Moreover, efforts to implement restoration methods by inoculation should be preceded by combined domain amplicon sequencing surveys like the present work. This is especially true in drylands which lack baseline studies of microbial diversity that can be used to make observations about foundational species important for crust colonization of new soils.

Efforts to establish biocrust restoration using an inoculum based on intact crusts from other sites primarily focus on promoting biomass growth of photoautotrophs, while much less attention is given to other biocrust associated microorganisms, even though some taxa could nevertheless be important components too. Microbes which are hubs in microbial networks may regulate microbial community functioning and are thus potentially necessary components for growth and sustained health of newly seeded biocrust. We found complex linkages within and between the two surveyed microbial domains. We also demonstrated that hub taxa and indicator species occur in biocrust and are discoverable by the applied methods. However, we have limited information about particular functional guilds and community regulation of these species identified from amplicon sequencing alone. As a result, more research is needed on the functional roles of the vast majority of microbes including desert soil fungi and how they may affect biocrust microbial communities, to inform effective inoculation experiment designs.

Another aspect still overlooked in biocrust restoration is the risk of potentially distributing pathogens or other types of antagonists. Our results indicated by molecular sequence that several fungi which are typically plant pathogens occur in Mojave biocrusts and could be dispersed as part of restoration procedures such as biocrust transplantation, wetting events, and recurring fertilizations. Second, many of the predicted hubs in microbial networks lack much identification beyond a sequence OTU and their functional guild is unknown. The OTUs of these microorganisms were detected in the soil beneath the crust or in both the biocrusts and the subsurface, suggesting that source material for biocrust production or inoculation should incorporate more than simply the surface. This strategy is consistent with previous restoration experiments which observed small shifts in the cyanobacterial community when using local soil/biocrust inoculum (Ayuso et al. 2017), in contrast to significant changes in cyanobacterial composition when extraneous inocula was used. In addition, the temporal variability of biocrust microbial communities remains unknown. A better understanding of how temporal changes and seasonality

impact the hub species among microbes of resident biocrust communities will be important to know to inform restoration managers which communities are more or less suited for active microbial inoculation.

5. Conclusion

In summary, our findings provide the most extensive characterization of local biocrust microbiota to date from the central and southern Mojave Desert. It is to our knowledge the first comprehensive biocrust microbial community investigation to reveal geographical, soil depth, and crust type diversity patterns when considering both fungi and bacteria microbes. Although identification of biocrust types by their external structural morphology is practical for preliminary observation in the field, we have shown that microbial components within each type can be distinct geographically. Biocrust surface and subsurface communities also have distinct microbial compositions. Our results supported the hypothesis that Cyanobacteria are key microorganisms in biocrust types, with network analysis demonstrating that they are major hubs for cross-domain microbial community connectivity. We also identified Pleosporales fungi as a major hub for fungal-bacteria networks. Our key findings imply that microbial species composition and community dynamics need to be taken into account in future biocrust conservation and management efforts. It is imperative that we improve our understanding of spatial variation in the microbial composition and functioning of biocrusts and improve the taxonomic identification of potentially essential species. Neglecting these differences could possibly lead to counter-effective consequences to both biocrust microbial communities and desert ecosystems, such as risk of pathogen/antagonist spread, potential loss of microbial diversity, altered functioning, introduction of invasive microbial species, and conceivably even destruction of any remaining biocrusts.

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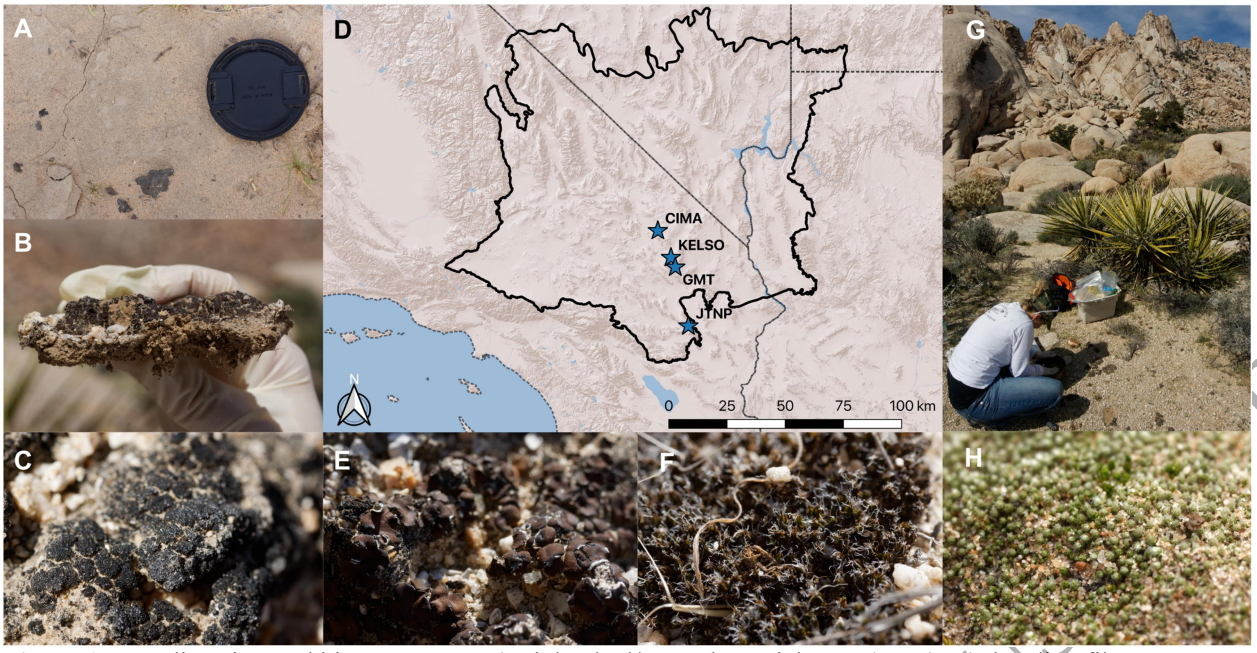

Figure 1. Sampling sites and biocrust types. A) Light algal/Cyanobacterial crust (LAC), B) dangling filaments underneath GLC, C) Cyanobacteria lichen crust (CLC), D) our 4 sampling sites including Cima Volcanic Flows (CIMA), Kelso Sand Dunes (KELSO), Granite Mountains Research Center (GMT) within the Mojave Desert (black outlined area), and Joshua Tree National Park (JTNP) at the edge of the Mojave and Colorado Deserts, E) Green algal lichen crust (GLC), F) Rough moss crust (RMC), G) crust sampling in Mojave Desert, H) Smooth moss crust (SMC)

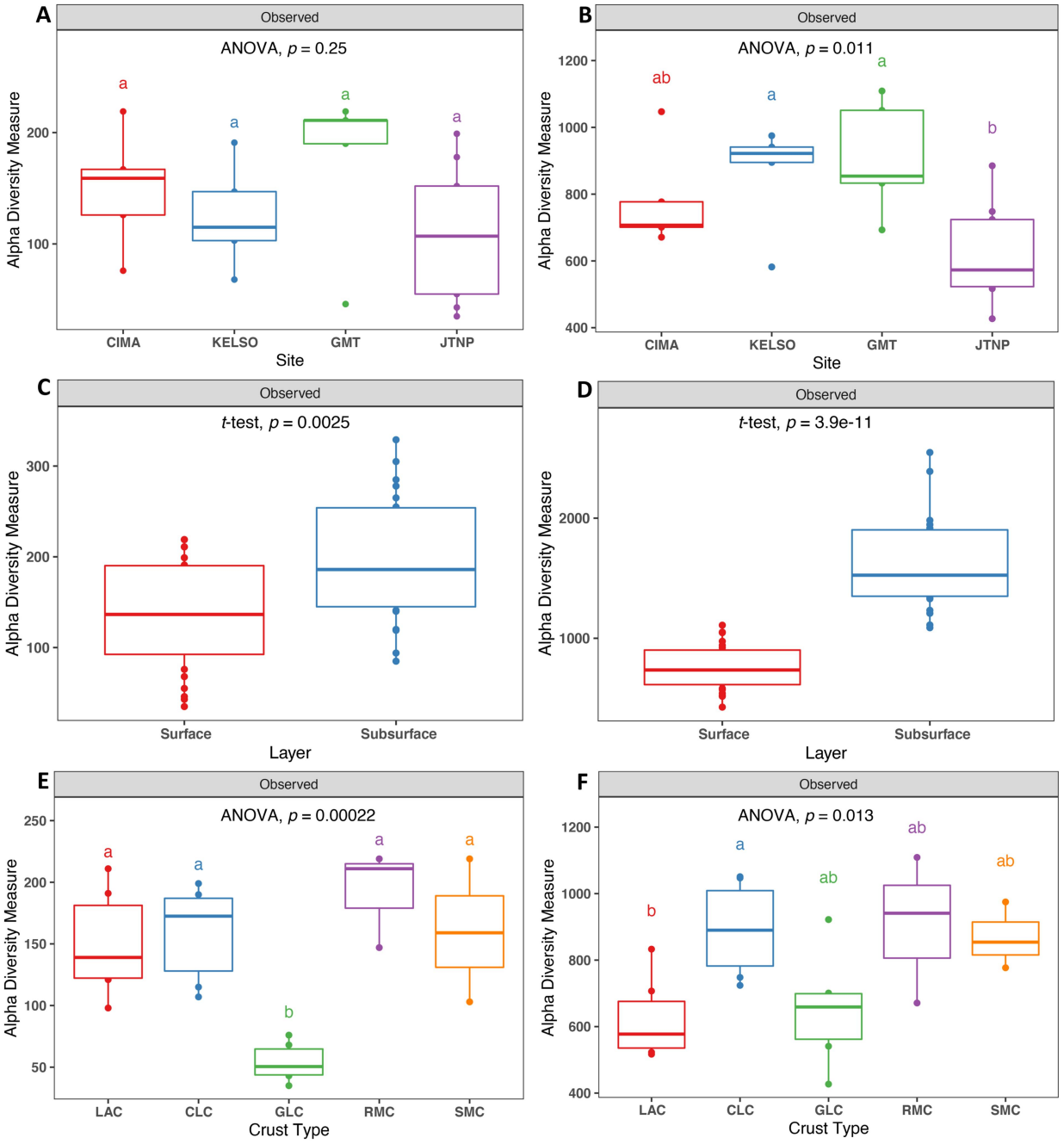

Figure 2. Boxplots showing alpha diversity as OTU richness in different site, soil depth, and crust type. A) Mojave biocrust fungal alpha diversity by site with rarefaction of 6842 reads per sample, B) Mojave bacterial alpha diversity by site with rarefaction of 37435 reads per samples, C) fungal alpha diversity by soil depth, D) bacterial alpha diversity by soil depth, E) fungal alpha diversity by crust types, and F) bacterial alpha diversity by crust type. Boxplots show 25th and 75th percentile while median was shown as lines inside boxes. Error bars show 1st and 99th percentile. Tukey HSD significant differences (P<0.05) are indicated by different letters.

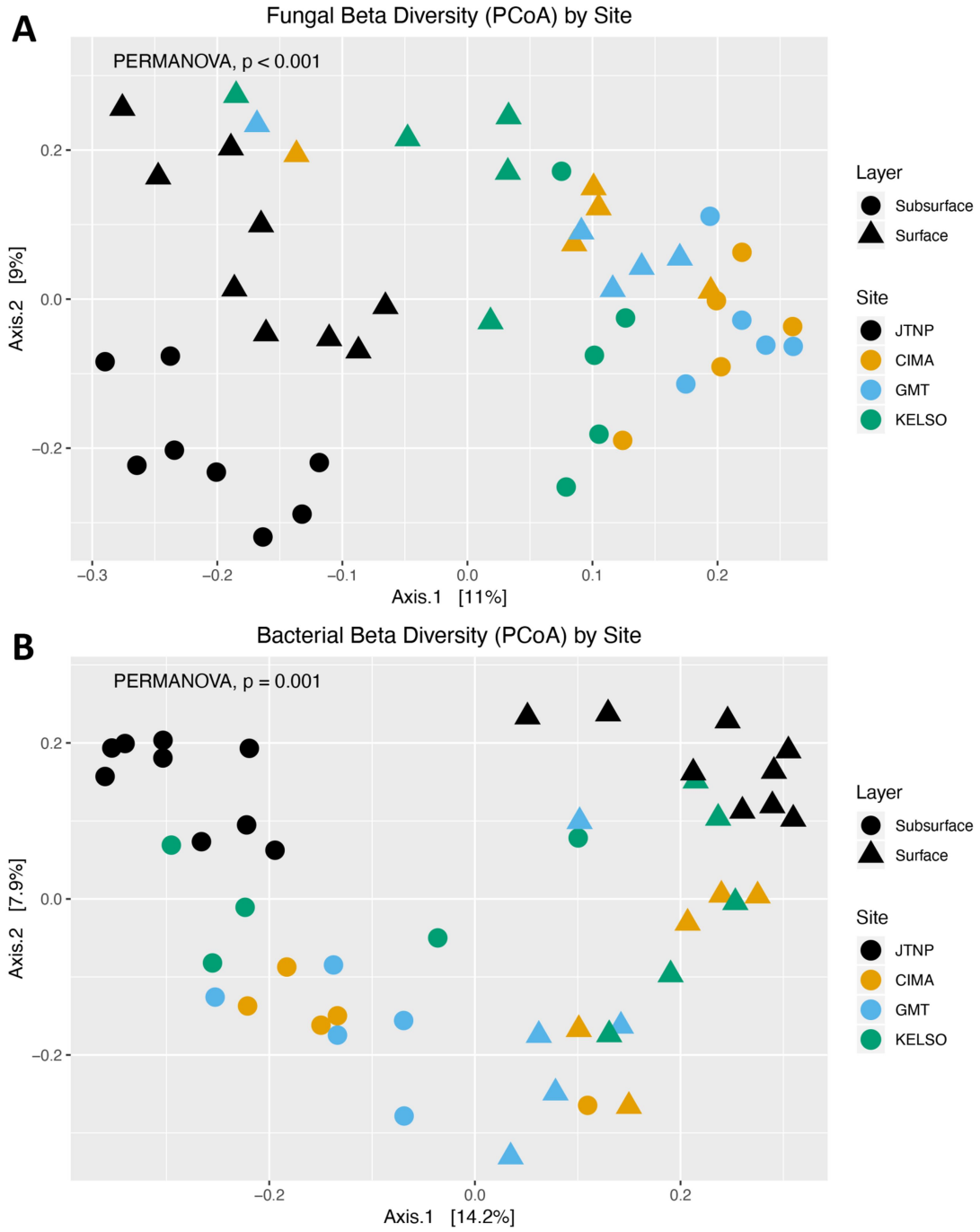

Figure 3. Beta diversity analysis of biocrust microbial communities. Dissimilarity of A) fungal and B) bacterial community composition in the comparison between site and soil depth (layer) using Principal Coordinate Analysis (PCoA). Different colors indicated four sampling sites including black color for JTNP, yellow color for CIMA, blue color for GMT, and green color for KELSO. Circle points showed subsurface samples while triangle points indicated surface samples. Significant differences (PERMANOVA, $P < 0.05$) were shown on PCoA plots.

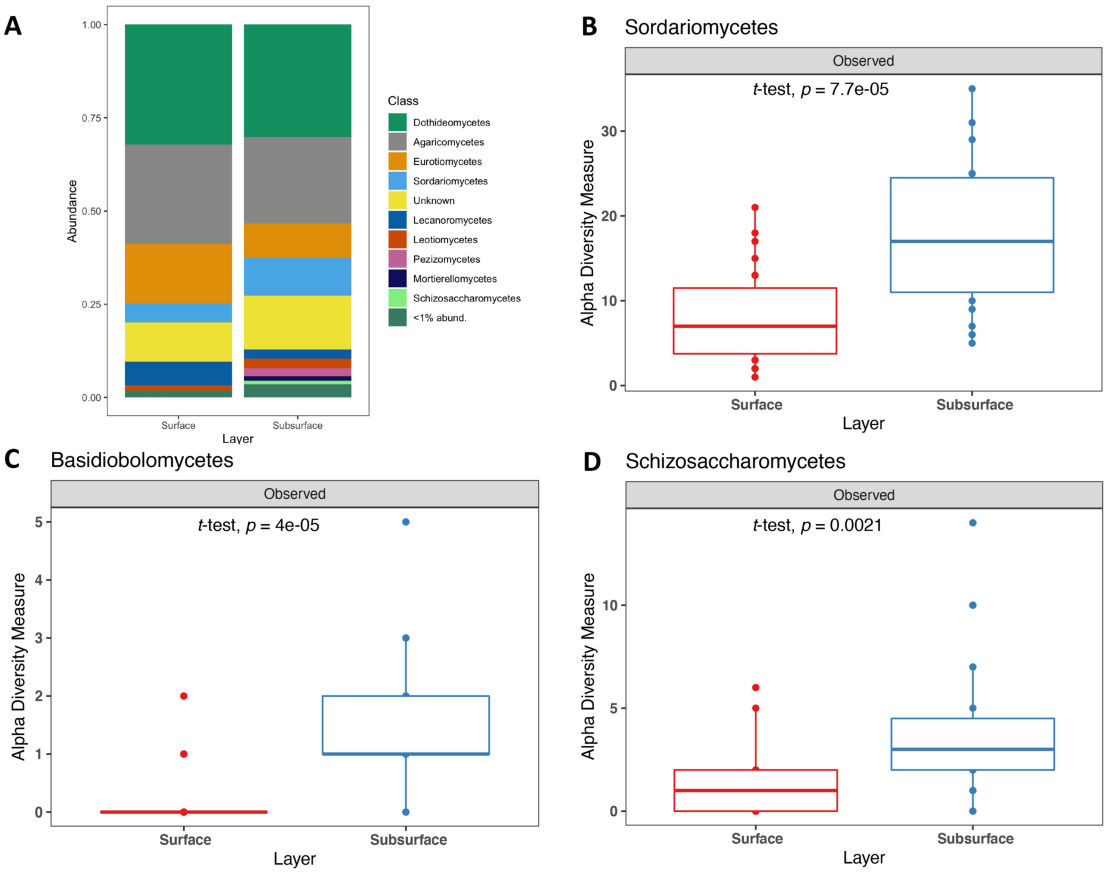

Figure 4. A) Fungal taxonomic composition bar plots at class level by layer ((<1% abund. = less than 1% relative abundance), Top three fungal classes with significantly different alpha diversity by layer, including B) Sodariomycetes, C) Basidiobolomycetes, and D) Schizosaccharomycetes. Subsurface soil also had greater species richness than surface biocrust for seven other fungal taxonomic classes (Agaricomycetes, Mucoromycetes, Saccharomycetes, Orbiliomycetes, Entomophthoromycetes, Mortierellomycetes, and Pneumocystidomycetes). Boxplots show 25th and 75th percentile while median was shown as lines inside boxes. Error bars show 1st and 99th percentile. Tukey HSD significant differences ($P<0.05$) are indicated by different letters.

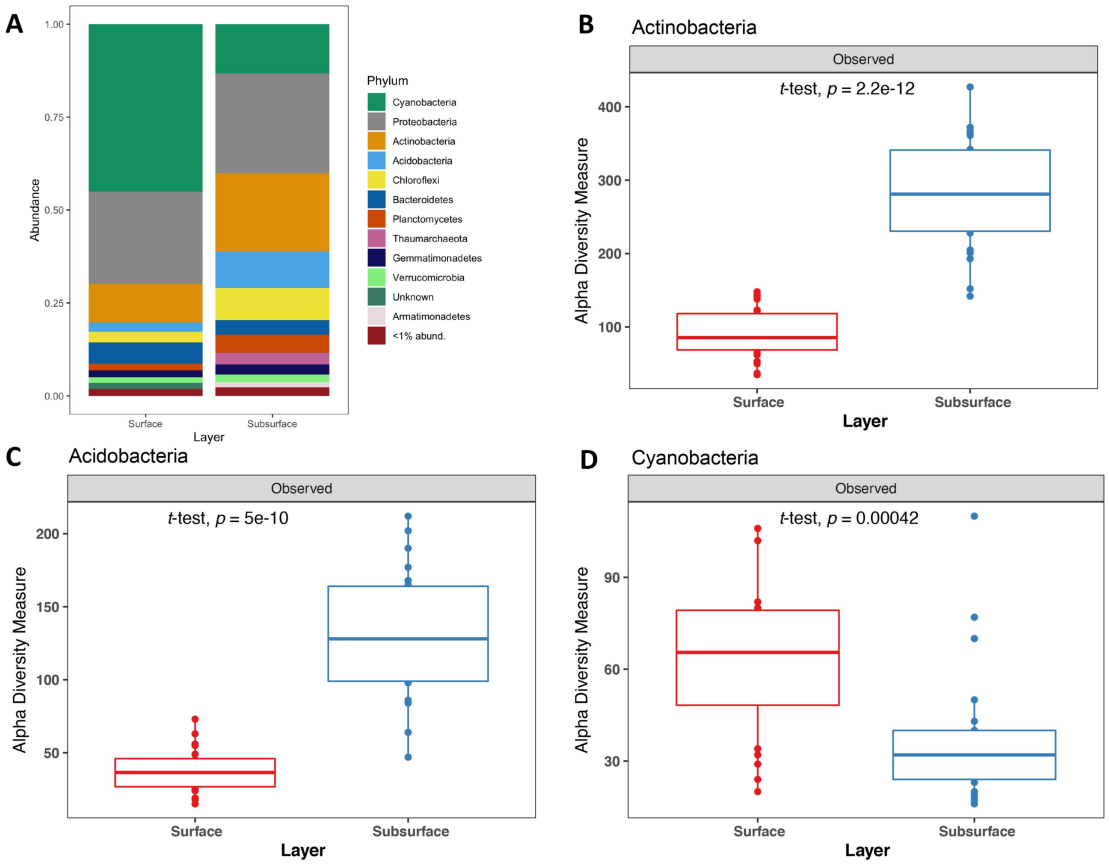

Figure 5. A) Bacterial taxonomic composition bar plot at phylum level by layer (<1% abund. = less than 1% relative abundance), Top two bacterial phyla in which alpha diversity by layer were significantly different including B) Actinobacteria and C) Acidobacteria. Same pattern was found in other 16 bacterial phyla in which subsurface soil had greater species richness than surface soil. D) Cyanobacteria bacterial richness on the soil surface was greater than in subsurface soil. Boxplots show 25th and 75th percentile while median was shown as lines inside boxes. Error bars show 1st and 99th percentile. Tukey HSD significant differences (P<0.05) are indicated by different letters.

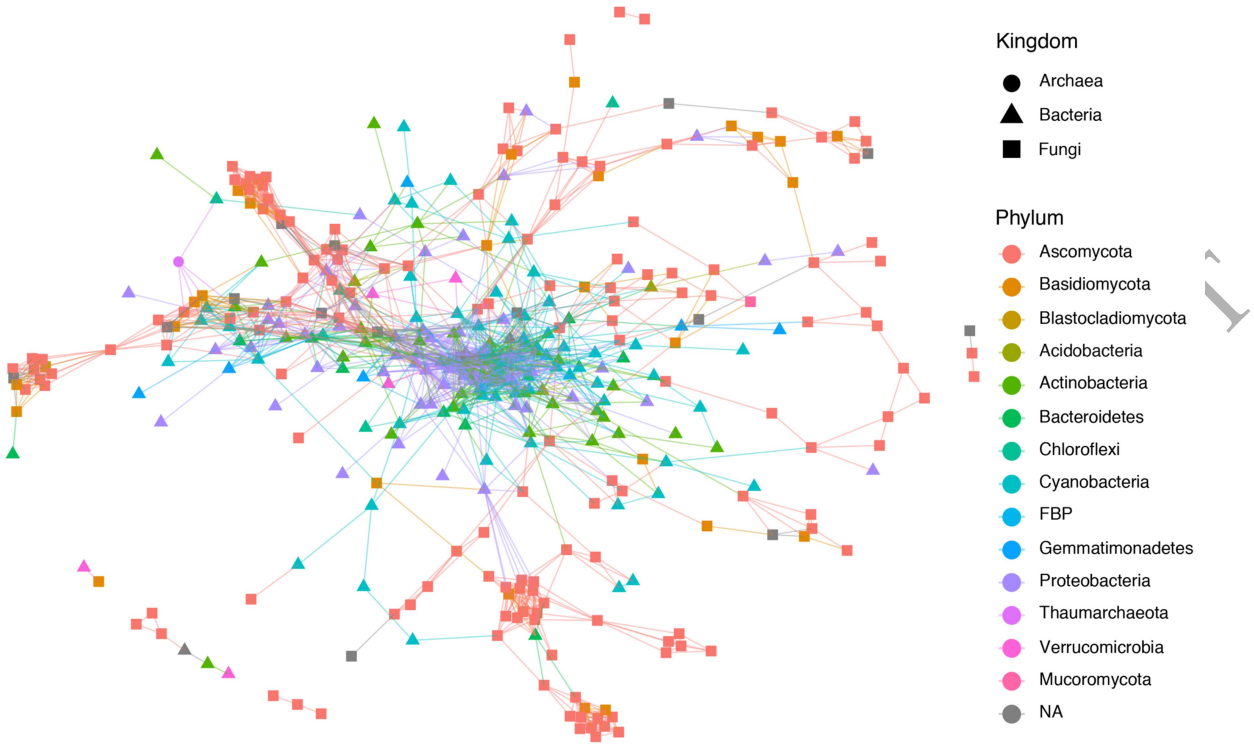

Figure 6. Microbial network analysis for biocrust surface samples. Each symbol/point on microbial network plot presents a single OTU. Microbial domains are indicated by different point shapes; archaea by circles, bacteria by triangles, and fungi by squares. Microbial networks are shown by line connection between points. Different colors indicate phylum for each point. Major network hubs concentrate at the center of microbial networks.

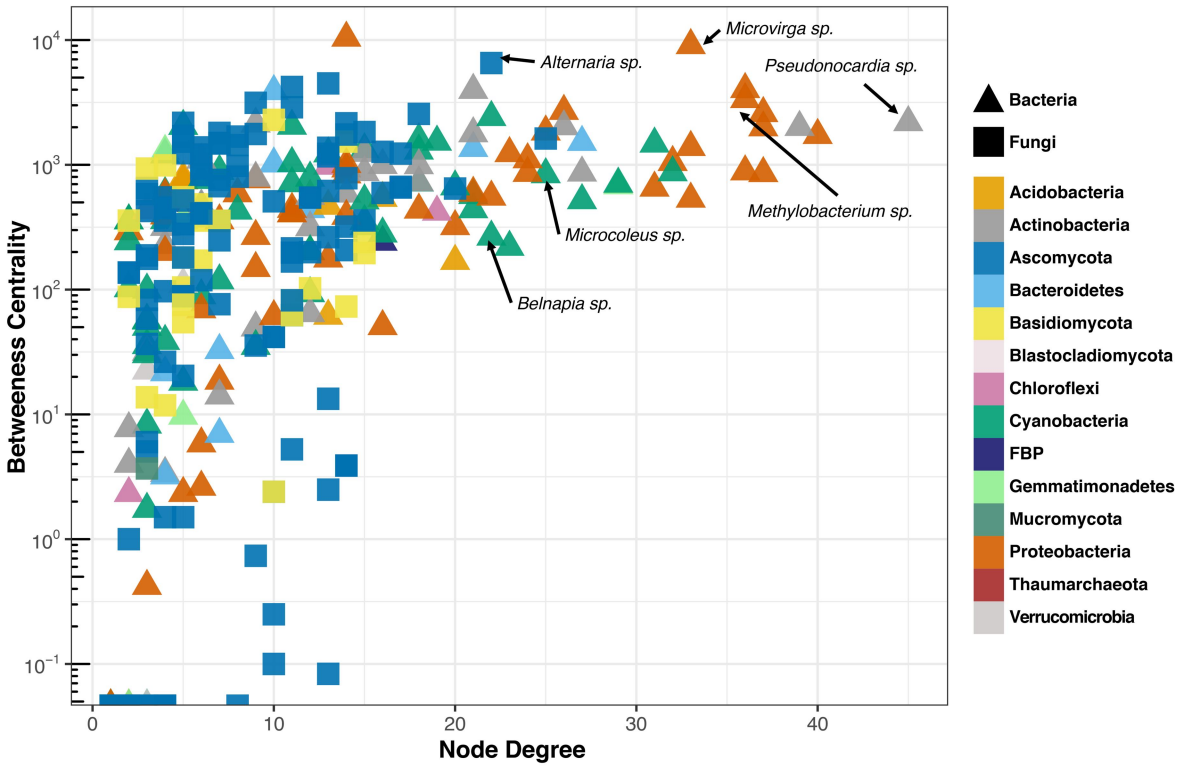

Figure 7. Betweenness centrality and node degree analysis of biocrust surface samples. Each symbol/point on the plot represents a single OTU. Microbial domains are indicated by different point shapes; archaea by circles, bacteria by triangles, and fungi by squares. High node degree indicates high network connections which represents microbial hubs. High betweenness centrality specify key connector for microbial network. When both high betweenness centrality and high node degree are observed, the species/nodes are the major hubs for the networks. Biocrust surface microbial network major hubs are clustered at the top right corner of the plots.

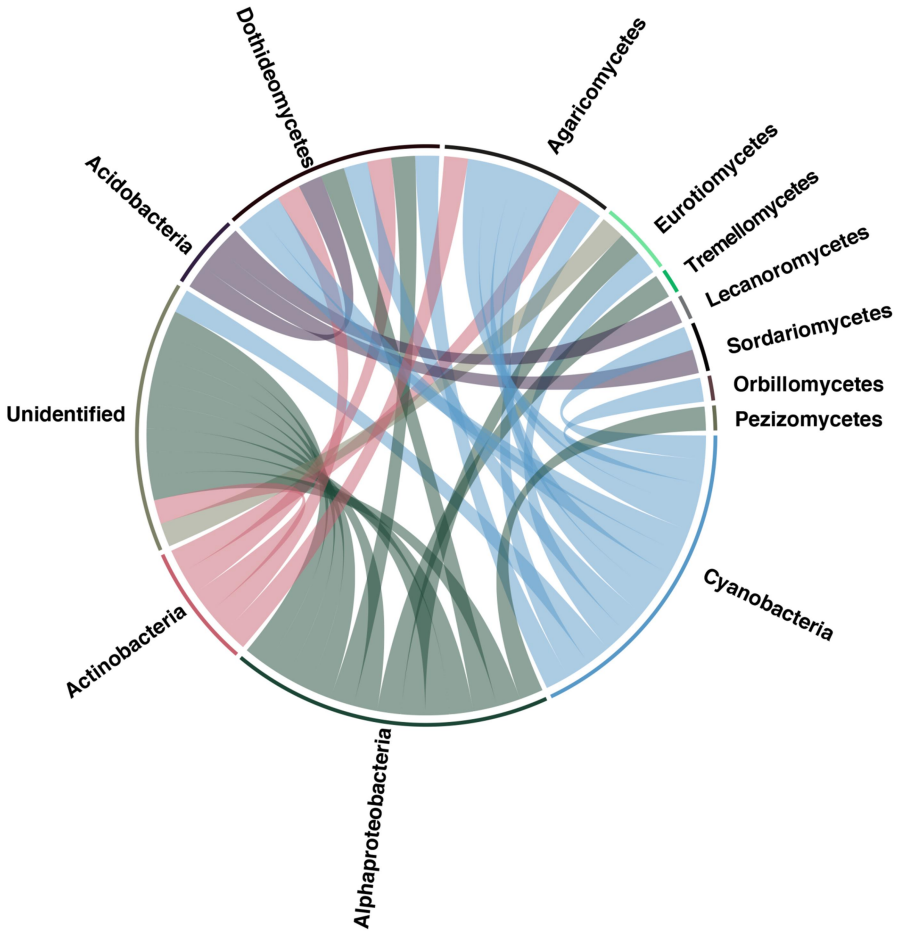

Figure 8. Cross-domain fungal-bacterial network analysis for biocrust surface samples. Cross-domain networks are a subset of total microbial networks showing in Figure 6. Each line represents the connection of a fungal OTU to a bacterial ASV. Different colors indicate different bacterial phyla. Cyanobacteria (in blue) had the highest number of connections to fungi. Cross-domain fungal-bacterial network analysis for biocrust subsurface samples are shown in Figure S7.