

Metatranscriptomes of two biological soil crust types from the Mojave desert in response to wetting

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ABSTRACT We present eight metatranscriptomic datasets of light algal and cyanolichen biological soil crusts from the Mojave Desert in response to wetting. These data will help us understand gene expression patterns in desert biocrust microbial communities after they have been reactivated by the addition of water.

KEYWORDS biocrust, RNA, transcriptome, wetting, desert, soil

Biological soil crusts comprise diverse microbial communities that carry out vital ecological functions in dryland ecosystems (1). Under dry conditions, biocrust microbes primarily persist in dormancy (2–4). When water becomes available, they quickly respond by exploiting moisture to repair cell damage and synthesize new biomass (5, 6). Nevertheless, the specific gene expression and metabolic processes underlying these responses remain poorly understood.

We sought to compare two kinds of biocrust commonly found in the Sheephole Valley Wilderness (Mojave Desert): light algal crust (LAC) and cyanolichen crust (CLC). In all, 10 biocrust samples, each measuring 5 cm², were collected at GPS location 34.1736 N, 115.3888 W. Each sample was placed in a 10 cm petri dish with 2 mL of sterile ultrapure water added on top, covered with a petri dish cover, and incubated at ambient laboratory conditions. After 0.5, 6, 18, 30, and 50 h time points, an entire biocrust sample was transferred and stored at –80°C for subsequent total RNA extraction using a NucleoBond RNA Soil Midi kit (740140.20, Macherey-Nagel, Nordrhein-Westfalen, Germany). We pursued rRNA depletion of 100 ng of total RNA using a QIAseq FastSelect 5S/16S/23S kit for bacteria and FastSelect rRNA yeast and plant depletion for eukaryotes (335921, 334219, and 334319, QIAGEN, Germantown, MD) following the manufacturer's instructions. The resulting RNA was reverse transcribed to create first-strand cDNA using a TruSeq Stranded mRNA Library prep kit (20020594, Illumina Inc., San Diego, CA). To synthesize second-strand cDNA, deoxyuridine triphosphate was incorporated in place of deoxythymidine triphosphate to quench the second strand during amplification and achieve strand specificity. Double-stranded cDNA fragments were A-tailed and ligated to JGI dual-indexed Y-adapters, followed by 10 cycles of PCR. The prepared libraries were quantified using KAPA Biosystems' next-generation sequencing library qPCR kit and run on a LightCycler 480 real-time PCR instrument (Roche Diagnostics Corporation, Indianapolis, IN). NovaSeq sequencing (Illumina Inc., San Diego, CA) was performed using NovaSeq XP V1 reagent kits and an S4 flowcell following a 2 × 151 bp indexed run recipe. BBDuk version 38.87 (<https://jgi.doe.gov/data-and-tools/bbtools/>) was used to remove contaminants, trim adapters from Illumina raw sequencing reads, remove any reads that contained "N" bases, and were shorter than 51 bp. Filtered reads were assembled with MEGAHIT version v1.2.9 (7) and mapped back to the final transcriptome assembly and coverage determined using BBMap version 38.86 (8).

Editor Frank J. Stewart, Montana State University, USA

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The authors declare no conflict of interest.

See the funding table on p. 4.

Received 16 November 2023

Accepted 13 December 2023

Published 8 January 2024

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TABLE 1 Accession numbers and characteristics of metatranscriptomes from two types of biological soil crusts, light algal crust (LAC) and cyanolichen crust (CLC), over the course of a re-wetting experiment (times shown indicate sample harvest time post-wetting; CLC samples at 0.5 and 30 h time points did not generate sufficient high-quality RNA yields for sequencing). All contigs are ≥ 0.1 kb

Meta-transcriptome	NCBI BioSample ID	NCBI BioProject ID	No. of raw reads	No. of filtered reads	Assembly BioSample ID	No. of Contigs	No. of assembled (150 bp) reads	Assembly length (bp)	Transcriptome coverage	N50 (bp)	Max contig length (KB)
LAC 0.5 h	SAMN17674635	PRJNA697426	378,329,084	15,399,682	GKPO000000000	58,795	12,494,595	31,311,788	59.9x	18,350	7.034
LAC 6 h	SAMN18245122	PRJNA710733	406,275,950	19,607,874	GKPP000000000	88,036	16,171,069	50,130,842	48.4x	25,380	20.259
LAC 18 h	SAMN17675269	PRJNA697427	437,433,136	20,442,408	GKPN000000000	72,020	16,932,941	38,371,519	66.2x	22,289	7.537
LAC 30 h	SAMN17675483	PRJNA697428	500,168,512	20,768,548	GKPP000000000	86,683	17,426,116	50,104,316	52.2x	24,532	14.942
LAC 50 h	SAMN17674330	PRJNA697429	670,916,034	38,911,978	GKPP000000000	109,448	32,668,699	61,798,533	79.3x	31,386	18.369
CLC 6 h	SAMN17674629	PRJNA697430	590,894,720	32,744,316	GKPS000000000	88,422	27,681,580	50,698,865	81.9x	24,701	23.151
CLC 18 h	SAMN18247024	PRJNA710734	528,673,374	28,175,474	GKPT000000000	60,086	23,018,914	35,379,485	97.6x	15,771	19.855
CLC 50 h	SAMN18245957	PRJNA710735	682,130,280	29,262,602	GKPU000000000	94,375	23,172,351	51,949,060	66.9x	27,333	27.808

Nearly 95% of reads aligned to ribosomal reference sequences in the SILVA database (9) using BBDuk (version 38.87, default settings), suggesting that experimental rRNA depletion was not effective. Nevertheless, these rRNA reads could be assembled and used to comprehensively survey the taxonomic diversity contained within these biocrusts (10). We obtained at least 25 million mRNA reads per sample, of which 80% could be assembled into contigs; this represents an average transcriptome coverage of ~69× and should be sufficient depth for functional analyses of wetting the reanimation process.

ACKNOWLEDGMENTS

We thank the BLM Needles CA office for their assistance with permitting at the Sheephole Valley Wilderness. This work was performed and supported in part by the Facilities Integrating Collaborations for User Science (FICUS) program (proposal: <https://doi.org/10.46936/fics.proj.2018.50356/60000035>) and used resources at the DOE Joint Genome Institute (JGI) (<https://ror.org/04xm1d337>) and the National Energy Research Scientific Computing Center (NERSC) (<https://ror.org/05v3mvq14>), which are DOE Office of Science User Facilities operated under Contract No. DE-AC02-05CH11231; Bureau of Land Management Cooperative Agreement L15AC00153 (NPI) and permit number 6850-CAD0000.06 (NPI and JES); the U.S. Department of Agriculture, National Institute of Food and Agriculture Hatch project CA-R-PPA-211-5062-H to NPo and JES; a Royal Thai Government Scholarship to NPo; and NSF GoLife grant DEB-1541538 and CAREER grant DEB-1846376 to EFYH. JES is a CIFAR fellow in the Fungal Kingdom: Threats and Opportunities program. This is UM's Center for Biodiversity and Conservation Research Publication No. 39.

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FUNDING

Funder	Grant(s)	Author(s)
National Science Foundation (NSF)	DEB-1541538, DEB-1846376	Erik F. Y. Hom
Joint Genome Institute (JGI)	FICUS 50356	Jason E. Stajich Erik F. Y. Hom Nicole Pietrasiak
U.S. Department of Energy (DOE)	DE-AC02-05CH11231	Marcel Huntemann Alicia Clum Brian Foster Bryce Foster Simon Roux Krishnaveni Palaniappan Neha Varghese Supratim Mukherjee Chris Daum Alex Copeland I-Min A. Chen Natalia N. Ivanova Nikos C. Kyrpides Miranda Harmon-Smith Emiley A. Eloë-Fadrosh T. B. K. Reddy
U.S. Department of Agriculture (USDA)	CA-R-PPA-211-5062-H	Nuttapon Pombubpa Jason E. Stajich
Royal Thai Government	Scholarship	Nuttapon Pombubpa

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DATA AVAILABILITY

Raw sequencing data and assemblies are accessible at the NCBI using the BioSample and BioProject IDs listed in Table 1. The data are also available from JGI's genome portal (<https://genome.jgi.doe.gov/portal/ProMicSoilCrusts/ProMicSoilCrusts.info.html>) or GOLD database (<https://gold.jgi.doe.gov/study?id=Gs0142145>).

REFERENCES

1. Bowker MA, Maestre FT, Eldridge D, Belnap J, Castillo-Monroy A, Escolar C, Soliveres S. 2014. Biological soil crusts (biocrusts) as a model system in community, landscape and ecosystem ecology. *Biodivers Conserv* 23:1619–1637. <https://doi.org/10.1007/s10531-014-0658-x>
2. Rodríguez-Caballero E, Aguilar MÁ, Castilla YC, Chamizo S, Aguilar FJ. 2015. Swelling of biocrusts upon wetting induces changes in surface micro-topography. *Soil Biol Biochem* 82:107–111. <https://doi.org/10.1016/j.soilbio.2014.12.010>
3. Leung PM, Bay SK, Meier DV, Chiri E, Cowan DA, Gillor O, Woebken D, Greening C, Stegen JC. 2020. Energetic basis of microbial growth and persistence in desert ecosystems. *mSyst* 5:e00495–19. <https://doi.org/10.1128/mSystems.00495-19>
4. Bay SK, Waite DW, Dong X, Gillor O, Chown SL, Hugenholtz P, Greening C. 2021. Chemosynthetic and photosynthetic bacteria contribute differentially to primary production across a steep desert aridity gradient. *ISME J* 15:3339–3356. <https://doi.org/10.1038/s41396-021-01001-0>
5. Karaoz U, Couradeau E, da Rocha UN, Lim H-C, Northen T, Garcia-Pichel F, Brodie EL. 2018. Large blooms of bacillales (firmicutes) underlie the response to wetting of cyanobacterial biocrusts at various stages of maturity. *mBio* 9:e01366–16. <https://doi.org/10.1128/mBio.01366-16>
6. Steven B, Belnap J, Kuske CR. 2018. Chronic physical disturbance substantially alters the response of biological soil crusts to a wetting pulse, as characterized by metatranscriptomic sequencing. *Front Microbiol* 9:2382. <https://doi.org/10.3389/fmicb.2018.02382>
7. Li D, Liu CM, Luo R, Sadakane K, Lam T-W. 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de bruijn graph. *Bioinformatics* 31:1674–1676. <https://doi.org/10.1093/bioinformatics/btv033>
8. Bushnell B. 2014. BBMap: a fast, accurate, splice-aware aligner. Lawrence Berkeley National Laboratory LBNL-7065E. Available from: <https://escholarship.org/uc/item/1h3515gn>
9. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41:D590–6. <https://doi.org/10.1093/nar/gks1219>
10. Nuccio EE, Nguyen NH, Nunes da Rocha U, Mayali X, Bougoure J, Weber PK, Brodie E, Firestone M, Pett-Ridge J. 2021. Community RNA-seq: multi-kingdom responses to living versus decaying roots in soil. *ISME Commun* 1:72. <https://doi.org/10.1038/s43705-021-00059-3>