AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements



| Environmental Microbiology | Announcement

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

**Thuy M. Nguyen,[1] Nuttapon Pombubpa,[2] Marcel Huntemann,[3] Alicia Clum,[3] Brian Foster,[3] Bryce Foster,[3] Simon Roux,[3] Krishnaveni Palaniappan,[3] Neha Varghese,[3] Supratim Mukherjee,[3] T. B. K. Reddy,[3] Chris Daum,[3] Alex Copeland,[3] I-Min A. Chen,[3] Natalia N. Ivanova,[3] Nikos C. Kyrpides,[3] Miranda Harmon-Smith,[3] Emiley A. Eloe-Fadrosh,[3] Nicole Pietrasiak,[4] Jason E. Stajich,[2] Erik F. Y. Hom[1]**

**AUTHOR AFFILIATIONS** See affiliation list on p. 3.

**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 $cm^2$, 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

Address correspondence to Erik F. Y. Hom, erik@fyhom.com.

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

**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 | GKPO00000000 | 58,795 | 12,494,595 | 31,311,788 | 59.9× | 18,350 | 7.034 |
| **LAC 6** h | SAMN18245122 | PRJNA710733 | 406,275,950 | 19,607,874 | GKPP00000000 | 88,036 | 16,171,069 | 50,130,842 | 48.4× | 25,380 | 20.259 |
| **LAC 18** h | SAMN17675269 | PRJNA697427 | 437,433,136 | 20,442,408 | GKPN00000000 | 72,020 | 16,932,941 | 38,371,519 | 66.2× | 22,289 | 7.537 |
| **LAC 30** h | SAMN17675483 | PRJNA697428 | 500,168,512 | 20,768,548 | GKPQ00000000 | 86,683 | 17,426,116 | 50,104,316 | 52.2× | 24,532 | 14.942 |
| **LAC 50** h | SAMN17674330 | PRJNA697429 | 670,916,034 | 38,911,978 | GKPR00000000 | 109,448 | 32,668,699 | 61,798,533 | 79.3× | 31,386 | 18.369 |
| **CLC 6** h | SAMN17674629 | PRJNA697430 | 590,894,720 | 32,744,316 | GKPS00000000 | 88,422 | 27,681,580 | 50,698,865 | 81.9× | 24,701 | 23.151 |
| **CLC 18** h | SAMN18247024 | PRJNA710734 | 528,673,374 | 28,175,474 | GKPT00000000 | 60,086 | 23,018,914 | 35,379,485 | 97.6× | 15,771 | 19.855 |
| **CLC 50** h | SAMN18245957 | PRJNA710735 | 682,130,280 | 29,262,602 | GKPU00000000 | 94,375 | 23,172,351 | 51,949,060 | 66.9× | 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.

## AUTHOR AFFILIATIONS

[1]Department of Biology and Center for Biodiversity and Conservation Research, University, University of Mississippi, Mississippi, USA
[2]Department of Microbiology and Plant Pathology, University of California, Riverside, California, USA
[3]US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California, USA
[4]School of Life Sciences, University of Nevada-Las Vegas, Las Vegas, Nevada, USA

## PRESENT ADDRESS

Nuttapon Pombubpa, Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

## AUTHOR ORCIDs

Thuy M. Nguyen http://orcid.org/0000-0002-0164-5340
Nuttapon Pombubpa http://orcid.org/0000-0003-3385-5331
Marcel Huntemann http://orcid.org/0000-0002-1284-3748
Alicia Clum http://orcid.org/0000-0002-5004-3362
Simon Roux http://orcid.org/0000-0002-5831-5895
Neha Varghese http://orcid.org/0000-0003-0580-0675
Supratim Mukherjee http://orcid.org/0000-0002-6322-2271
T. B. K. Reddy http://orcid.org/0000-0002-0871-5567
Chris Daum http://orcid.org/0000-0003-3895-5892
Alex Copeland http://orcid.org/0000-0002-3971-5439
Natalia N. Ivanova http://orcid.org/0000-0002-5802-9485
Nikos C. Kyrpides http://orcid.org/0000-0002-6131-0462
Miranda Harmon-Smith http://orcid.org/0000-0002-3980-1820

Emiley A. Eloe-Fadrosh http://orcid.org/0000-0002-8162-1276
Nicole Pietrasiak http://orcid.org/0000-0003-4636-8006
Jason E. Stajich http://orcid.org/0000-0002-7591-0020
Erik F. Y. Hom http://orcid.org/0000-0003-0964-0031

## 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<br>Erik F. Y. Hom<br>Nicole Pietrasiak |
| U.S. Department of Energy (DOE) | DE-AC02-05CH11231 | Marcel Huntemann<br>Alicia Clum<br>Brian Foster<br>Bryce Foster<br>Simon Roux<br>Krishnaveni Palaniappan<br>Neha Varghese<br>Supratim Mukherjee<br>Chris Daum<br>Alex Copeland<br>I-Min A. Chen<br>Natalia N. Ivanova<br>Nikos C. Kyrpides<br>Miranda Harmon-Smith<br>Emiley A. Eloe-Fadrosh<br>T. B. K. Reddy |
| U.S. Department of Agriculture (USDA) | CA-R-PPA-211-5062-H | Nuttapon Pombubpa<br>Jason E. Stajich |
| Royal Thai Government | Scholarship | Nuttapon Pombubpa |

## AUTHOR CONTRIBUTIONS

Thuy M. Nguyen, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review and editing | Nuttapon Pombubpa, Investigation, Methodology | Marcel Huntemann, Formal analysis, Methodology | Alicia Clum, Methodology, Supervision | Brian Foster, Formal analysis, Methodology | Bryce Foster, Methodology | Simon Roux, Methodology | Krishnaveni Palaniappan, Methodology | Neha Varghese, Formal analysis, Methodology | Supratim Mukherjee, Data curation | T. B. K. Reddy, Data curation | Chris Daum, Supervision, Methodology | Alex Copeland, Supervision, Methodology | I-Min A. Chen, Methodology, Supervision | Natalia N. Ivanova, Supervision, Methodology | Nikos C. Kyrpides, Supervision, Methodology | Miranda Harmon-Smith, Project administration | Emiley A. Eloe-Fadrosh, Methodology, Supervision | Nicole Pietrasiak, Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review and editing | Jason E. Stajich, Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Writing – review and editing | Erik F. Y. Hom, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing

## 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