






Metagenomes and Metatranscriptomes of a Glucose-Amended Agricultural Soil

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ABSTRACT The addition of glucose to soil has long been used to study the metabolic activity of microbes in soil; however, the response of the microbial ecophysiology remains poorly characterized. To address this, we sequenced the metagenomes and metatranscriptomes of glucose-amended soil microbial communities in a laboratory incubation.

Soil microbial communities are considered to be limited by carbon and energy (1, 2). The addition of glucose to soil has been used to study the maintenance energy demands of soil microbes (3), soil organic matter priming (4), and taxon-specific growth rates of soil bacteria (5, 6). However, a detailed description of the response of microbial community metabolism to a glucose addition is lacking. Here, we present metatranscriptomes and metagenomes of glucose-amended agricultural soil in a short-term laboratory incubation.

Soils from a long-term crop rotation experiment (7) at the West Virginia University Certified Organic Farm (Morgantown, WV, USA) (39.647502°N, 79.93691°W; 243.8 to 475.2 m above sea level) were sampled at 0- to 10-cm depths and shipped to Northern Arizona University (Flagstaff, AZ, USA). There, soils from separate cores were homogenized and separated into Mason jars to contain 30 g of soil each. Samples were then preincubated for 2 weeks at room temperature. After preincubation, each sample was amended with 1.6 ml of a 0.13 M glucose solution, which added 0.7 mg of glucose C per g of dry soil. Before and 8, 24, and 48 h after glucose addition, 4 replicates collected from different Mason jars were frozen using liquid N₂ and stored at -80°C. RNA was extracted using the RNeasy PowerSoil total RNA kit (Qiagen) and treated with RNase-free DNase (Qiagen). DNA was extracted using the RNeasy PowerSoil DNA elution kit (Qiagen). A Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) was used to determine the concentrations of extracted nucleic acids, and a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) was used to assess purity.

RNA and DNA were shipped to the Joint Genome Institute (JGI) for sequencing. An Illumina TruSeq stranded RNA LT kit was used to generate cDNA libraries. Prior to sequencing, heavily degraded samples were discarded, resulting in the elimination of 3 DNA samples. Plate-based DNA library preparation for Illumina sequencing was performed on the PerkinElmer Sciclone next-generation sequencing (NGS) robotic liquid-handling system using a Kapa Biosystems library preparation kit. Two hundred nanograms of sample DNA was sheared to 300 bp using a Covaris LE220 focused ultrasonicator. Sheared DNA fragments were size selected by double solid-phase reversible immobilization (SPRI), and

Citation Chuckran PF, Huntemann M, Clum A, Foster B, Foster B, Roux S, Palaniappan K, Varghese N, Mukherjee S, Reddy TBK, Daum C, Copeland A, Ivanova NN, Kyrpides NC, del Rio TG, Eloë-Fadrosh EA, Morrissey EM, Schwartz E, Fofanov V, Hungate B, Dijkstra P. 2020. Metagenomes and metatranscriptomes of a glucose-amended agricultural soil. *Microbiol Resour Announc* 9:e00895-20. <https://doi.org/10.1128/MRA.00895-20>.

Editor Frank J. Stewart, Georgia Institute of Technology

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Received 30 July 2020

Accepted 6 October 2020

Published 29 October 2020

TABLE 1 Sample numbers and summaries for metagenomes and metatranscriptomes of laboratory-incubated agricultural soil amended with glucose

IMG identification no.	Sample name	Time (h)	JGI analysis project type	NCBI BioProject accession no.	NCBI BioSample accession no.	SRA accession no.	No. of reads	Assembled genome size (bp)	No. of genes	No. of scaffolds	N ₅₀ (bp)	GC content (%)	% of genes with predicted function using:			
													COG database	Pfam database	KEGG database	
3300032003	C0D1	0	Metagenome	PRJNA539715	SAMN11528526	SRR9032300	122,905,108	1,355,893,536	3,466,757	2,855,912	851,672	63.9	53.7	51.5	24.9	
3300031892	C0D2	0	Metagenome	PRJNA539712	SAMN11533409	SRR9032202	111,310,488	1,152,269,571	2,964,388	2,455,617	739,392	64.1	53.9	51.6	25.0	
3300032000	C0D3	0	Metagenome	PRJNA539720	SAMN11533240	SRR9032615	126,283,392	1,651,468,217	4,265,445	3,536,653	1,067,790	64.4	53.9	51.7	25.3	
3300032122	C0D4	0	Metagenome	PRJNA539713	SAMN11532952	SRR9032258	128,790,659	1,467,471,286	3,759,875	3,098,631	927,590	64.1	53.8	51.6	25.0	
3300034668	C0R1	0	Metatranscriptome	PRJNA570403	SAMN12814391	SRR10849656	99,039,727	302,858,706	718,971	631,914	217,331	60.4	45.8	43.2	17.8	
3300034480	C0R2	0	Metatranscriptome	PRJNA570401	SAMN12814786	SRR10904363	70,227,109	64,495,153	148,170	137,255	47,055	57.4	37.6	35.3	14.8	
3300034674	C0R3	0	Metatranscriptome	PRJNA570409	SAMN12812743	SRR10849749	61,722,077	92,886,127	211,898	192,180	64,296	58.7	41.0	38.6	15.9	
3300036405	C0R4	0	Metatranscriptome	PRJNA647753	SAMN15601796	SRR12334071	94,316,601	394,204,764	925,493	806,549	272,244	59.2	46.7	43.6	18.0	
3300031908	C24D1	24	Metagenome	PRJNA539717	SAMN11533408	SRR9032509	205,837,446	3,525,197,514	8,695,255	7,014,884	1,984,116	64.0	52.6	51.5	24.4	
3300031940	C24D2	24	Metagenome	PRJNA539718	SAMN11532657	SRR9032510	111,794,949	1,202,955,554	3,148,390	2,627,975	810,700	64.3	54.4	51.8	25.6	
3300032012	C24D3	24	Metagenome	PRJNA539719	SAMN11532357	SRR9032617	170,867,131	2,561,938,476	6,450,661	5,267,248	1,531,865	63.9	52.9	51.4	24.7	
3300034671	C24R1	24	Metatranscriptome	PRJNA570406	SAMN12813766	SRR10849744	70,179,661	321,462,175	764,600	659,038	224,121	60.5	54.5	51.4	26.6	
3300034672	C24R2	24	Metatranscriptome	PRJNA570407	SAMN12815093	SRR10849745	77,871,015	277,611,432	656,390	568,464	191,550	59.2	53.5	50.7	25.8	
3300036404	C24R4	24	Metatranscriptome	PRJNA570408	SAMN12812652	SRR10849807	58,429,105	296,367,375	697,412	602,090	202,562	59.6	53.7	50.9	25.6	
3300031854	C48D1	48	Metagenome	PRJNA647752	SAMN15601797	SRR12334066	86,292,361	280,662,482	666,005	577,618	197,189	60.7	52.0	48.5	23.5	
3300032013	C48D3	48	Metagenome	PRJNA539722	SAMN11532950	SRR9032716	174,461,389	2,640,998,970	6,618,271	5,384,216	1,554,238	64.2	53.0	51.5	24.7	
3300031847	C48D4	48	Metagenome	PRJNA539723	SAMN11532599	SRR9032694	134,609,818	1,664,342,186	4,245,763	3,497,047	1,045,780	64.0	54.0	51.8	25.3	
3300034675	C48R1	48	Metatranscriptome	PRJNA570410	SAMN12814942	SRR10849803	65,457,552	150,659,653	353,892	318,387	110,009	58.2	46.6	44.2	20.8	
3300034676	C48R2	48	Metatranscriptome	PRJNA570411	SAMN12812625	SRR10849941	81,394,607	36,204,275	860,612	746,114	253,443	59.8	50.4	47.7	22.7	
3300034677	C48R3	48	Metatranscriptome	PRJNA570412	SAMN12812728	SRR10849938	68,921,951	88,305,274	207,453	187,559	64,424	59.1	45.4	42.5	20.2	
3300034678	C48R4	48	Metatranscriptome	PRJNA570413	SAMN12814182	SRR10849936	67,586,882	252,944,636	602,314	526,545	180,208	60.7	50.3	47.3	22.6	
3300032211	C8D1	8	Metagenome	PRJNA539714	SAMN11532583	SRR9032259	131,787,411	1,841,805,081	4,756,397	3,945,810	1,187,662	64.1	52.6	50.8	24.4	
3300031858	C8D2	8	Metagenome	PRJNA539711	SAMN11533337	SRR9032199	172,412,632	2,503,797,881	6,199,898	5,009,710	1,429,077	64.6	53.4	51.9	24.7	
3300032017	C8D4	8	Metagenome	PRJNA539716	SAMN11532864	SRR9032267	125,460,262	1,413,722,004	3,629,789	2,995,887	896,872	63.7	53.2	51.2	24.7	
3300034667	C8R1	8	Metatranscriptome	PRJNA570402	SAMN12815108	SRR10849655	82,347,388	440,040,760	1,041,764	882,412	293,847	60.1	56.8	53.5	30.4	
3300034666	C8R2	8	Metatranscriptome	PRJNA570400	SAMN12813333	SRR10849401	91,524,568	366,955,569	892,052	759,185	260,726	61.0	58.0	53.8	30.9	
3300034669	C8R3	8	Metatranscriptome	PRJNA570404	SAMN12814692	SRR10849649	59,135,875	307,696,835	741,250	629,624	215,105	62.3	58.1	54.1	31.2	
3300034670	C8R4	8	Metatranscriptome	PRJNA570405	SAMN12814921	SRR10849743	73,470,419	259,146,587	613,760	533,046	180,822	60.1	54.5	51.4	28.8	

selected fragments were end repaired, A tailed, and ligated with Illumina-compatible sequencing adaptors (IDT) containing a unique molecular index barcode for each sample library. The prepared libraries were quantified using the Kapa Biosystems NGS library quantitative PCR kit and run on a Roche LightCycler 480 real-time PCR instrument. Quantified libraries were multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina NovaSeq sequencer using NovaSeq XP v1 reagent kits and an S4 flow cell, following a 2×150 -bp indexed run protocol. Metatranscriptome reads were filtered using BBTools v38 (8) to remove duplicate, ribosomal, low-quality, and human reads. Filtered reads were assembled using MEGAHIT v1.1.2. (9) using a custom k-mer size list (--k-list 23,43,63,83,103,123). Reads for metagenomic samples were filtered for contaminants and adaptors and trimmed for quality using BBTools v38 (8), corrected using BFC vr181 (10) (with the options -s 10g -k 21), and assembled using SPAdes v3.13.0 (11) (with the options --only-assembler, --meta, and -k33,55,77,99,127). Reads were mapped against the assembled read set using BMap v38 (8) with the option ambiguous=random. Assembled contigs were annotated using the IMG Annotation Pipeline v5.0.1 (12, 13). In total, 13 metagenomes (minimum of 3 per time point) and 16 metatranscriptomes (4 per time point) were sequenced, assembled, and annotated (Table 1). Detailed information on the bioinformatic processing of each library is available via the JGI Genome Portal (<https://genome.jgi.doe.gov/portal/Strinailability/Strinailability.info.html>).

Data availability. Metadata curation and public repository registration for the metagenomes and metatranscriptomes were managed by the Genomes OnLine Database (GOLD) (14) under study number Gs0135756 (<https://gold.jgi.doe.gov/study?id=Gs0135756>). Annotations are located in the IMG database. IMG identification numbers, SRA accession numbers, and sample information can be found in Table 1.

ACKNOWLEDGMENTS

This work was supported by funding from the USDA National Institute of Food and Agriculture Foundational Program (award 2017-67019-26396); additional support for P.D. was provided by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research, Genomic Science Program at Lawrence Livermore National Laboratory, Microbes Persist Soil Microbiome Scientific Focus Area (award SCW1632). The work conducted by the U.S. DOE JGI, a DOE Office of Science User Facility, is supported under contract DE-AC02-05CH11231.

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