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# Metatranscriptomes of California grassland soil microbial communities in response to rewetting

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**ABSTRACT** When very dry soil is rewet, rapid stimulation of microbial activity has important implications for ecosystem biogeochemistry, yet associated changes in microbial transcription are poorly known. Here, we present metatranscriptomes of California annual grassland soil microbial communities, collected over 1 week from soils rewet after a summer drought—providing a time series of short-term transcriptional response during rewetting.

**KEYWORDS** metatranscriptomics, biogeochemistry

In Mediterranean grasslands, the first rainfall after the dry season is a time of high activity (1) and stress (2) for soil microbial communities, when a large portion of previously fixed organic carbon is mineralized and lost as CO<sub>2</sub> (3). Despite its importance, we have minimal understanding of the genetic controls that dictate soil microbial response to rewetting. Here, we describe a total of 47 metatranscriptomes that illustrate the short-term rewetting response of soil microbial communities.

Samples were collected in an annual grassland at the Hopland Extension and Research Center, Hopland, California, USA (39.00092, -123.07962) from an established field experiment with reduced and controlled precipitation treatments. The site contained 16 3.25 m<sup>2</sup> plots covered by rainout shelters; half received 50% of annual precipitation, and the rest received 100% of annual precipitation. Soils were collected in September 2018, at the end of the Mediterranean climate dry season. The top 0–15 cm of a 0.5 m<sup>3</sup> area was collected from four plots per treatment and transported to Lawrence Livermore National Lab, Livermore, CA, USA.

Soils were homogenized *via* a 2-mm sieve and weighed into 44 incubation chambers per treatment, each containing 5 g. Samples were rewet to 22% gravimetric water content with either H<sub>2</sub><sup>18</sup>O (98 atom% <sup>18</sup>O) or natural abundance water and then 4 samples per treatment were destructively harvested at 3, 24, 48, 72, or 168 h post-rewetting, with four samples harvested per treatment just prior to rewetting. Harvested soils were immediately frozen in liquid N<sub>2</sub> and stored at –80°C.

Samples treated with H<sub>2</sub><sup>18</sup>O were used in a quantitative stable isotope probing study (4); however, this treatment was not predicted to influence transcription and RNA was therefore extracted from four randomly selected samples according to the time and precipitation treatments only. RNA was extracted using an RNEasy PowerSoil Total RNA Kit (Qiagen) using the standard protocol. RNA quality was assessed using a Nanodrop One Spectrophotometer (Thermo Fisher Scientific Inc) and gel electrophoresis, and quantity was determined using a Qubit Fluorometer (Invitrogen).

High-quality RNA samples were shipped to the Department of Energy Joint Genome Institute (JGI; Berkeley, CA, USA) for sequencing. From 100 ng of RNA, rRNA was depleted using QIAseq FastSelect kits (Qiagen): 5S/16S/23S, rRNA Plant, and rRNA Yeast.

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TABLE 1 Sample names and descriptions for metatranscriptomes included in data release<sup>a</sup>

Sample name	Collection time (h)	Precipitation treatment (%)	Plot #	IMG taxon OID	NCBI Bioproject accession no.	NCBI Biosample accession no.	SRA accession no.	Assembly size (bp)	% reads assembled	Gene count	GC %	Pfam %	KEGG %
0_100_14	0	100	14	3300052403	PRJNA1088043	SAMN40460437	SRR28364973	25,399,712	73.44	63,946	54.73	30.56	14.22
0_100_16	0	100	16	3300052404	PRJNA1088044	SAMN40460260	SRR28364976	23,831,343	71.12	57,445	54.31	27.61	12.11
0_100_3	0	100	3	3300052401	PRJNA1088041	SAMN40460257	SRR28364970	24,098,858	72.77	59,306	55.36	31.21	14.21
0_100_8	0	100	8	3300052402	PRJNA1088042	SAMN40460344	SRR28364972	34,770,642	75.95	85,127	53.37	33.76	15.52
0_50_10	0	50	10	3300052399	PRJNA1088039	SAMN40460687	SRR28364958	28,981,743	73.48	70,692	53.28	34.48	15.75
0_50_13	0	50	13	3300052400	PRJNA1088040	SAMN40460256	SRR28364965	52,172,601	72.55	125,523	55.98	40.11	18.66
0_50_9	0	50	9	3300052778	PRJNA1088038	SAMN40460644	SRR28364957	33,695,301	68.52	81,945	55.86	37.59	18.17
3_100_14	3	100	14	3300052779	PRJNA1088051	SAMN40460651	SRR28364975	105,728,744	61.32	274,876	62.87	58.34	35.29
3_100_16	3	100	16	3300052780	PRJNA1088052	SAMN40460443	SRR28364982	84,267,711	65.55	214,512	58.38	58.59	37.2
3_100_3	3	100	3	3300052409	PRJNA1088049	SAMN40460440	SRR28364979	123,576,902	66.53	310,871	61.19	61.6	38.27
3_100_8	3	100	8	3300052410	PRJNA1088050	SAMN40460385	SRR28364983	127,204,214	66.3	323,308	62.12	59.23	36.06
3_50_10	3	50	10	3300052407	PRJNA1088047	SAMN40460691	SRR28364977	95,579,820	58.45	251,086	60.18	0	37.13
3_50_13	3	50	13	3300052408	PRJNA1088048	SAMN40461255	SRR28364981	164,870,248	69.69	415,011	60.81	60.67	37.94
3_50_4	3	50	4	3300052405	PRJNA1088045	SAMN40460439	SRR28364971	128,475,800	60.55	329,338	61.27	60.7	37.34
3_50_9	3	50	9	3300052406	PRJNA1088046	SAMN40460441	SRR28364978	236,320,059	69.9	593,793	63.07	59.6	34.88
24_100_14	24	100	14	3300051593	PRJNA1088059	SAMN40460648	SRR28364931	122,400,676	52.75	329,589	60.27	52.42	30.5
24_100_16	24	100	16	3300051594	PRJNA1088064	SAMN40460354	SRR28364932	98,278,248	68.24	232,798	48.31	59.22	33.78
24_100_3	24	100	3	3300051591	PRJNA1088057	SAMN40460352	SRR28364987	161,064,172	58.27	408,717	56.78	57.7	33.31
24_100_8	24	100	8	3300051592	PRJNA1088058	SAMN40460696	SRR28364935	153,578,695	58.87	402,956	58.7	54.65	31.57
24_50_10	24	50	10	3300051589	PRJNA1088055	SAMN40460693	SRR28364990	116,710,461	55.71	310,045	58.38	53.23	30.93
24_50_13	24	50	13	3300051590	PRJNA1088056	SAMN40460264	SRR28364988	162,284,959	56.88	423,797	59.58	53.78	31.78
24_50_4	24	50	4	3300052411	PRJNA1088053	SAMN40460346	SRR28364980	216,338,638	62.75	536,872	56.68	59.01	34.26
24_50_9	24	50	9	3300051588	PRJNA1088054	SAMN40460388	SRR28364984	33,288,343	47.85	87,781	59.69	54.2	32.45
48_100_14	48	100	14	3300051600	PRJNA1087816	SAMN40461215	SRR28364940	54,246,179	50.91	146,807	57.85	47.48	26.5
48_100_16	48	100	16	3300051601	PRJNA1087817	SAMN40460268	SRR28364933	67,130,837	50.47	180,611	56.7	49.16	27.76
48_100_3	48	100	3	3300051598	PRJNA1088069	SAMN40460393	SRR28364937	196,494,752	56.1	499,735	56.13	50.18	28
48_100_8	48	100	8	3300051599	PRJNA1088070	SAMN40460394	SRR28364942	139,629,737	54.68	366,859	56.43	50.71	28.48
48_50_10	48	50	10	3300051596	PRJNA1088067	SAMN40460345	SRR28364936	49,414,151	45.36	134,654	57.18	48.18	26.99
48_50_13	48	50	13	3300051597	PRJNA1088068	SAMN40461249	SRR28364939	87,214,943	50.21	232,967	57.94	47.92	26.76
48_50_4	48	50	4	3300051595	PRJNA1088065	SAMN40460448	SRR28364930	42,519,384	42.41	116,035	59.37	50.04	28.54
48_50_9	48	50	9	3300051257	PRJNA1088066	SAMN40460697	SRR28364938	213,718,347	52.19	557,367	60.25	53.56	29.96
72_100_14	72	100	14	3300051608	PRJNA1087824	SAMN40461243	SRR28364946	79,072,549	54.63	212,098	60.08	45.34	24.11
72_100_16	72	100	16	3300051609	PRJNA1087825	SAMN40460645	SRR28364955	77,178,599	56.62	201,538	57.69	46.08	25.7
72_100_3	72	100	3	3300051606	PRJNA1087822	SAMN40460436	SRR28364961	229,619,457	57.34	599,881	59.05	50.46	27.76
72_100_8	72	100	8	3300051607	PRJNA1087823	SAMN40460686	SRR28364945	122,320,207	53.27	321,251	58.24	46.4	25.29
72_50_10	72	50	10	3300051604	PRJNA1087820	SAMN40460359	SRR28364941	62,140,121	49.78	164,797	57.25	44.48	24.68

(Continued on next page)

TABLE 1 Sample names and descriptions for metatranscriptomes included in data release<sup>a</sup> (Continued)

Sample name	Collection time (h)	Precipitation treatment (%) MAP)	Plot #	IMG taxon OID	NCBI Bioproject accession no.	NCBI Biosample accession no.	SRA accession no.	Assembly size (bp)	% reads assembled	Gene count	GC %	Pfam %	KEGG %
72_50_13	72	50	13	3300051605	PRJNA1087821	SAMN40460643	SRR28364944	140,421,172	62.51	357,387	56.28	46.07	25.25
72_50_4	72	50	4	3300051602	PRJNA1087818	SAMN40460382	SRR28364934	75,072,475	51.97	201,293	58.16	47.1	25.99
72_50_9	72	50	9	3300051603	PRJNA1087819	SAMN40460396	SRR28364943	28,879,462	49.84	78,031	56.73	41.64	23.4
168_100_14	168	100	14	3300051439	PRJNA1087832	SAMN40460255	SRR28364960	19,731,979	52.47	52,772	57.89	35.44	18.19
168_100_16	168	100	16	3300051258	PRJNA1087833	SAMN40460261	SRR28364956	39,560,723	57.06	105,123	57.19	38.95	19.93
168_100_3	168	100	3	3300051613	PRJNA1087830	SAMN40460647	SRR28364963	136,483,723	57.36	350,286	58.18	43.84	22.64
168_100_8	168	100	8	3300051614	PRJNA1087831	SAMN40460474	SRR28364968	140,143,059	57.67	364,092	58.3	42.61	21.35
168_50_10	168	50	10	3300051612	PRJNA1087828	SAMN40460432	SRR28364962	19,858,972	47.85	53,097	56.24	36.87	19.7
168_50_13	168	50	13	3300052781	PRJNA1087829	SAMN40460689	SRR28364969	143,895,565	62.7	372,889	57.53	41.92	20.97
168_50_4	168	50	4	3300051610	PRJNA1087826	SAMN40460646	SRR28364966	44,418,056	55.98	116,610	56.65	37.51	19.12
168_50_9	168	50	9	3300051611	PRJNA1087827	SAMN40460433	SRR28364967	56,071,808	55.81	149,367	58.49	41.11	21.13

<sup>a</sup>Precipitation treatment refers to field treatment prior to rewetting, consisting of either 100% or 50% mean annual precipitation. Collection time refers to hours after rewetting, with collection at 0 h being a dry pre-wet control.

The TruSeq Stranded mRNA kit (Illumina) was used with heat-fragmented 300–400 bp RNA to create the first strand of cDNA using random hexamers and SuperScript Reverse Transcriptase (Thermo Fisher Scientific) followed by the second strand. cDNA was A-tailed and ligated with JGI's dual index adapters. Prior to sequencing, one RNA sample was discarded because of low quality, leaving 47 samples. Prepared libraries were quantified with a KAPA Biosystem NGS library qPCR kit (Roche) and run on a LightCycler 480 real-time PCR instrument (Roche). Samples were sequenced on an Illumina NovaSeq sequencer using a NovaSeq XP V1.5 reagent kits on S4 flow cells, following the 2 × 151 read protocol. Raw reads were QC filtered using BBTools (v 38.86)—by trimming and removing contaminants (human, mouse, cat, dog), duplicate, ribosomal, and low-quality reads. QC-filtered reads were assembled into contiguous sequences using MEGAHIT v 1.2.9 (5) (--k-list 23, 43, 63, 83, 103, 123). The input reads were then mapped to assemblies using bbmap (v. 38.86; with option --ambiguous=random) (6). Assembled contiguous sequences were annotated using the IMG Annotation Pipeline v.5.1.5 (7).

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**DATA AVAILABILITY**

The metadata for this project is on the Genomes OnLine Database (GOLD) (8) under the study ID [Gs0156567](https://www.ncbi.nlm.nih.gov/). Raw reads are located on NCBI (<https://www.ncbi.nlm.nih.gov/>) under the SRA numbers in Table 1. Assemblies, annotations, and gene counts are in IMG/M under the IMG genome IDs listed in Table 1. We note that the samples described here and listed under "Soil microbial communities from Hopland Research and Extension Center, CA, USA" on IMG/M are a subset of the full number collected for this experiment (see Table 1 for samples included in this data release).

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