

Unveiling Microbial Carbon Cycling Processes in Key U.S. Soils using “Omics”

Our overarching goal for this research was to harness the power of multiple meta-omics tools to gain greater understanding of the functioning of whole-soil microbial communities and their role in C cycling (Myrold *et al.*, 2014). This entailed three objectives with multiple tasks.

Objective 1. Further develop and optimize a combination of meta-omics approaches to understand how climate shifts (precipitation timing/amount) impact microbially-mediated C cycling functions at different levels of expression and regulation.

Task 1.1-Generate a metagenome for the Kansas prairie soil. More than 500 Gb of metagenome sequence was generated for Kansas native prairie soil through the JGI Great Prairie Grand Challenge project. The data were assembled and annotated and used as a scaffold for metatranscriptome and metaproteome data generated in the project. One complication with sequencing of bulk soil DNA is that the DNA originates from populations that are dormant or even dead, in addition to actively-growing members of the community. To overcome this challenge and to reduce the diversity of the metagenome, we focused on the actively growing members of the soil community by specifically extracting DNA that had incorporated a thymine analog, bromodeoxyuridine (BrdU), during DNA replication in native bulk soils. The BrdU-labeled DNA was extracted using magnetic beads coated with goat anti-mouse IgG targeted to BrdU. By focusing on the active metagenome, the metagenome assembly was significantly improved based on a larger number and greater length of contigs (Fig.1; 6.8×10^6 contigs of at least 200 bp), which in turn resulted in better annotation. For example, a comparison of the active taxa based on extracting the reads annotated as *rrs* gene (16S) in the BrdU-labeled metagenomic shotgun data and the total DNA allowed us to identify taxa present and actively

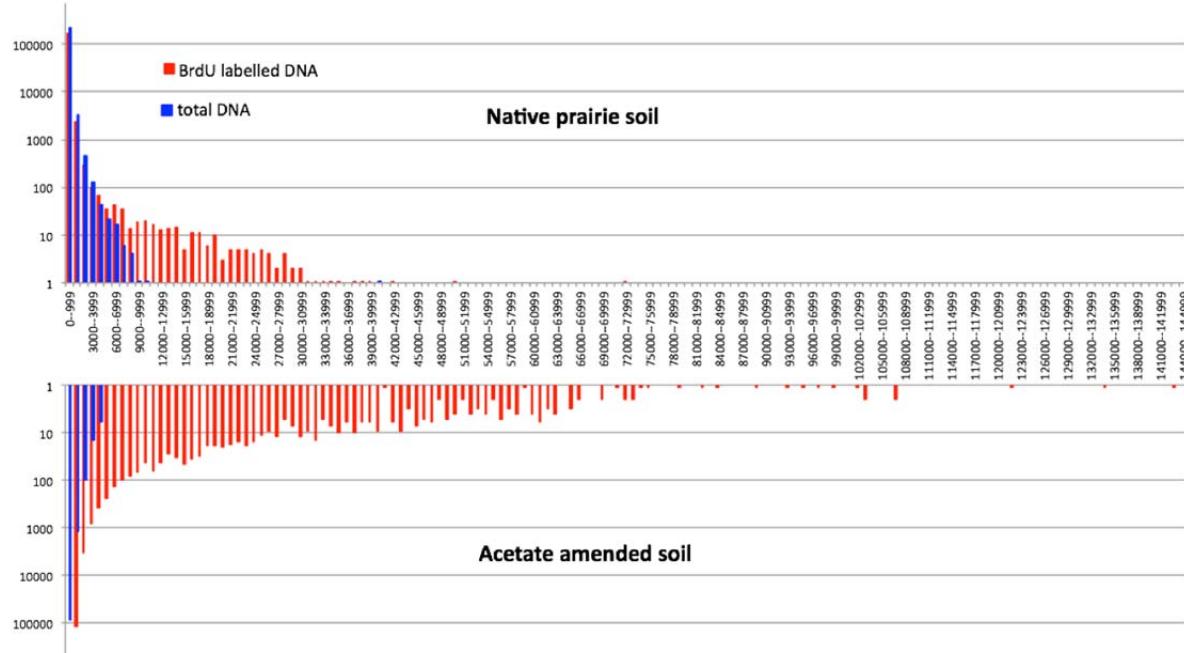


Figure 1. Comparison of metagenome assembly distribution between total and BrdU-labeled DNA extracted from Kanza native and acetate-amended prairie soil. The assembly has been performed using clc workbench, after quality trimming on galaxy (JGI platform). The figure shows longer contigs after BrdU-labeled metagenome showing (in red) than the total DNA (in blue).

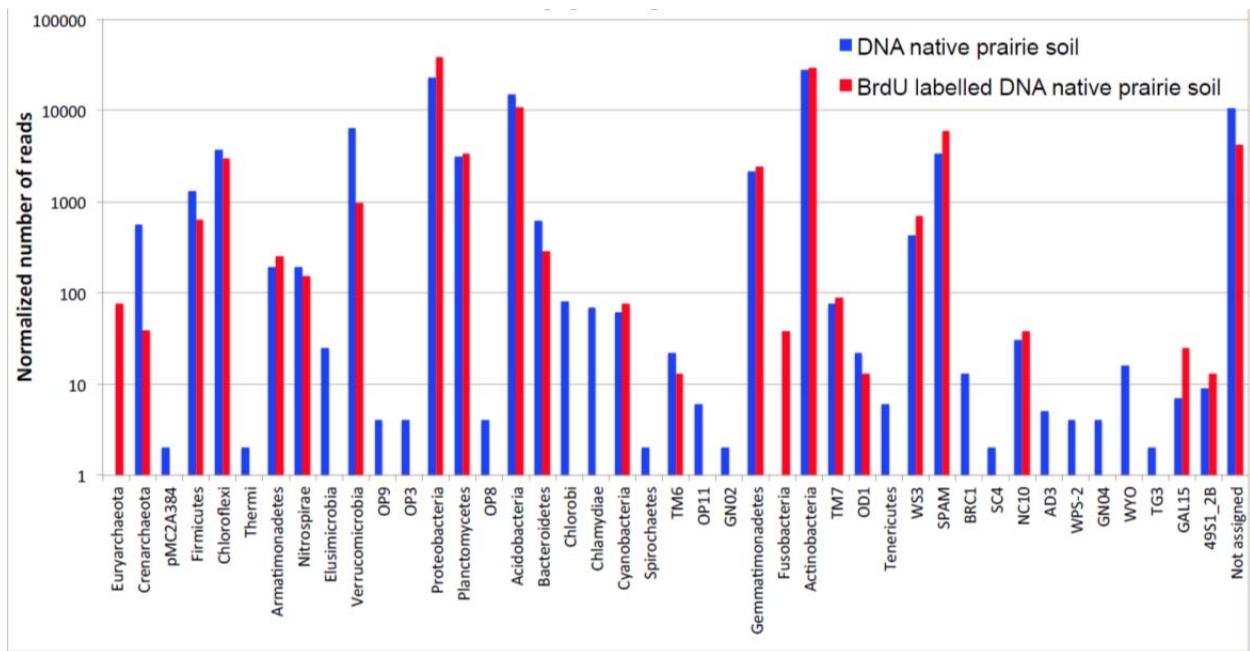


Figure 2. Comparison of total DNA and BrdU-labeled DNA from 16S annotated reads at phylum level. The results presented here provided from the metagenomic shotgun sequencing, extracting the reads annotated as 16S.

metabolizing, such as proteobacteria and to find phyla that were not even detectable in the total DNA, such as euryarchaeota (Fig. 2). In addition we performed co-occurrence network analysis of the bacterial community. An example, Fig. 3 shows the network for proteobacteria present in both the BrdU and non-labeled metagenome.

In addition, the use of the active metagenome as a new database on the same metaproteomics shotgun data (via 2d-LC-MS/MS on an LTQ Velos mass spectrometer) allowed us to identify 10 times more proteins per sample, reaching 1,235 protein identified—one of the highest protein yields for soil to date. We screened the metagenome and metaproteome data for specific functions involved in C cycling. These data show, for example, that when the soil was amended with acetate, specific cell transporters for acetate can be detected and identified in the metaproteome data.

In order to improve functional screening of the metagenome data, we built a comprehensive functional database manually curated into categories called FOAM (Functional Ontology Assignments for Metagenomes), where smallest chosen unit of the database was KOs (KEGG Orthology groups). KOs were retrieved to fit within the corresponding hierarchical organization for a specific function (such as denitrification, methanogenesis, etc.). In addition, to improve upon the speed and sensitivity of conventional BLAST searches versus FOAM, we turned each KOs set of sequences into Hidden Markov Models by fetching their corresponding *pfam* profiles and exploring the diversity of each KOs by retrieving the sequences of all entire genome data available in IMG for each orthology (Fig. 4). The resulting product is the first soil-specific HMM database of 35,781 HMMs for 2,870 unique KOs, allowing us to screen increasingly large “omics” datasets such as metagenomes, metatranscriptomes, and metaproteomes from soil samples with greater accuracy and speed. The details of this database have been published (Prestat *et al.*, in press). All the annotation methods developed in this project will be made available for the scientific community at <http://portal.nersc.gov/project/m1317/FOAM/>.

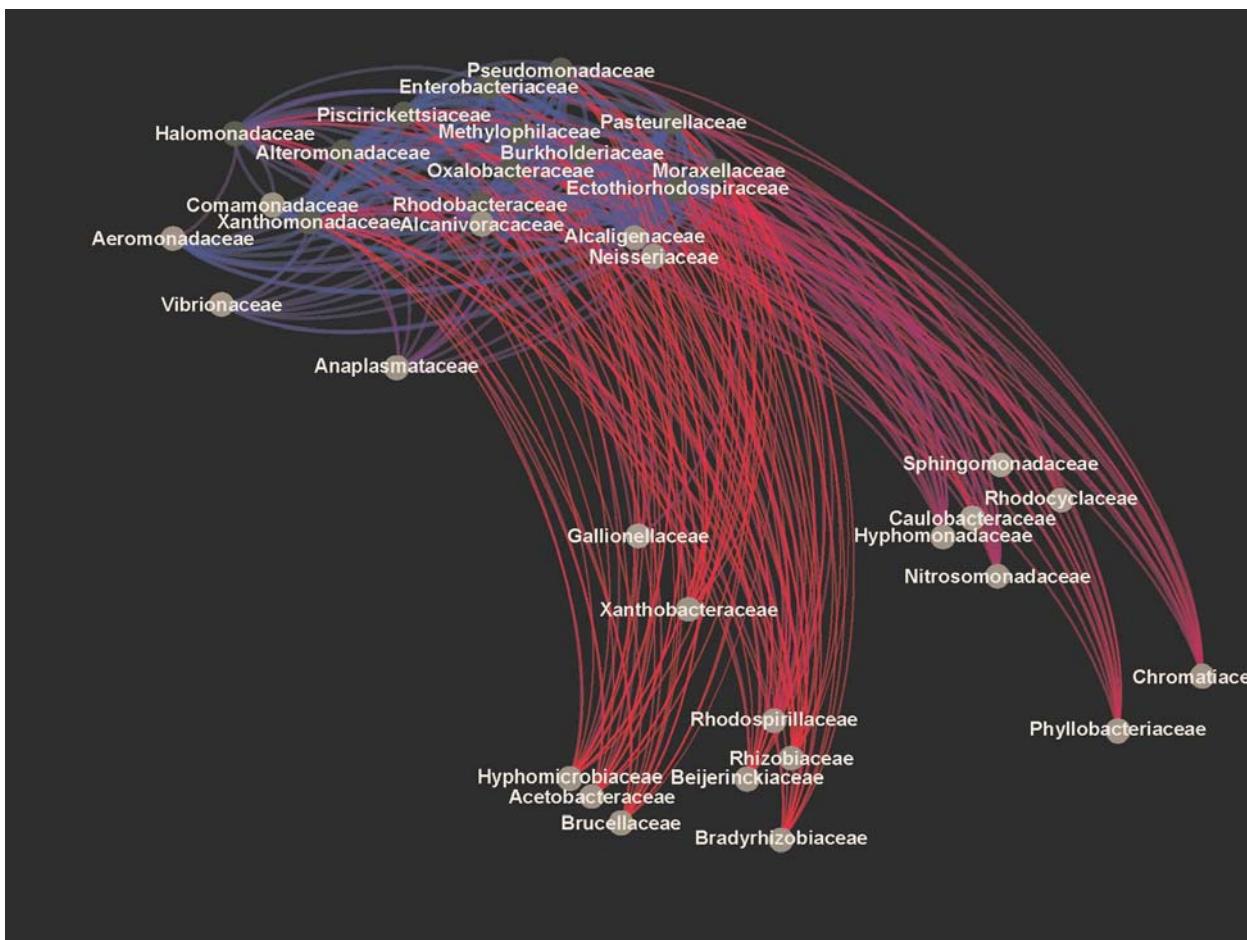


Figure 3. Co-occurrence network of proteobacteria based on a linear-linear model (attraction and repulsion, proportional to the distance between the nodes). The model was chosen as an intermediate position between Noack's LinLog and the algorithm of Fruchterman and Rheingold.

Task 1.2-Optimize metatranscriptomics protocol. Soil metatranscriptomics can inform mechanisms that drive microbially-mediated biogeochemical processes, by identifying the functional gene transcripts, or mRNAs, present under defined variations in environmental conditions. However, the pool of total RNA molecules in direct extraction preparations is dominated by ribosomal RNA (rRNA), which is not functionally informative. Therefore, a number of approaches exist to remove ribosomes from the total RNA pool to enrich the amount of mRNA sequence before preparation for sequencing. Although mRNA enrichment creates transcriptomic datasets with more functionally informative information, the manipulation of molecular composition may cause bias in the distribution of mRNAs sequenced, which could lead to inaccurate interpretations of experimental results. Therefore a simple experiment was used to evaluate the efficacy and bias in soil transcriptomes prepared using two straightforward mRNA enrichment protocols (rRNA removal via reverse hybridization (MICROBExpress Bacterial mRNA enrichment kit, Ambion/Life Technologies, Carlsbad, CA, USA) and physical removal of rRNA from total RNA via gel extraction) compared to total RNA preparation of metatranscriptomic libraries. To identify whether mRNA enrichment method affects the relative abundance of transcripts detected, we added log-phase *Pseudomonas putida* KT2440 cells (reference genome) to sterile Konza prairie soil, allowed the cells to metabolize soil-derived organic matter for 6 h, harvested total RNA from

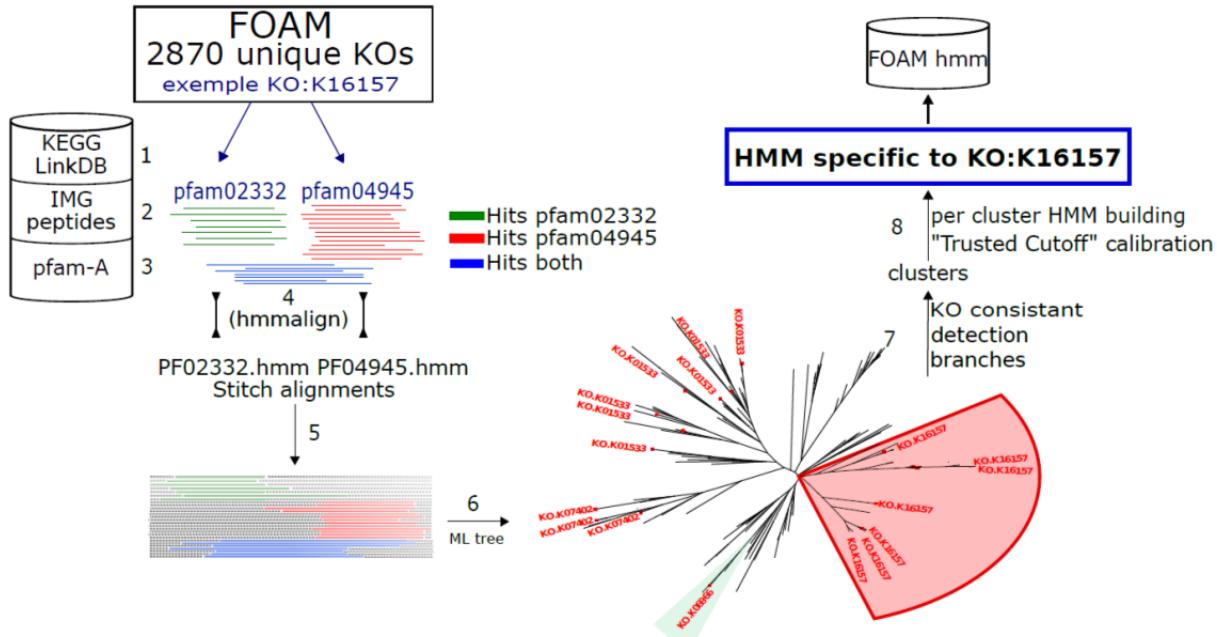


Figure 4. HMM building pipeline: example with KO:K16157 (methane monooxygenase). Step 1- find Pfam(s) combination assigned to the KO of interest (a) and (b) check for redundancy. Step 2- fetch IMG peptide sequences which hit the retrieved Pfam(s). Step 3- fetch from Pfam-A database the HMM of interest. Step 4-alignment (hmmalign) and filter each Pfam from extra sequences obtained in IMG. Step 5- stitch filtered alignments. Step 6- draw a Maximum Likelihood tree (fasttree). Step 7- find clusters in tree with same KO. Step 8- split alignment (step 5 output) by cluster (step 7 output) and build HMM for each, and process the “Trusted Cutoff” computation.

soil, then compared transcriptomes prepared with different ribosome removal (mRNA enrichment) methods. Results show that the relative abundance of recruitment of mRNA transcripts to the *P. putida* KT2440 genome was positively correlated between the total RNA and gel-enriched mRNA libraries, but not correlated between the total RNA and reverse-hybridized mRNA libraries (Fig. 5). Thus, soil transcriptome library preparation via gel-enrichment was determined to provide a more accurate snapshot of mRNA relative abundance within the total RNA pool than reverse hybridization library preparation.

A second laboratory incubation was set up to refine our methods for extracting RNA and protein from soil. We used a model bacterium that has been genome sequenced, *Arthrobacter chlorophenolicus*, as an inoculum. The model strain was inoculated into sterile and non-sterile Kansas prairie soil, and acetate and 4-chlorophenol were added as general and specific C substrates, respectively. The same substrates were added to the soil without inoculum to assess the response of the indigenous microbes in the soil. We extracted RNA using a phenol:chloroform extraction and PEG precipitation protocol followed by a Qiagen kit DNA/RNA separation and DNase cleanup. Key target genes were quantified by quantitative PCR and RT-QPCR. A first set of genes, 16S rRNA and gfp (encoding the green fluorescence protein that was stably inserted in the chromosome of the *A. chlorophenolicus* strain used) were chosen to estimate the *A. chlorophenolicus* cell number. Two other genes, *ICL* (isocitrate lyase, part of the 2-C bypass of the TCA cycle), and *sucAB* (succinyl CoA synthetase, an enzyme of the main TCA cycle) were used to track the pathways used by the microorganisms with different substrates. The gfp transcript could be detected in all soil samples and was thus a good estimator of the cell activity and abundance over the incubation course: gfp expression was highest in acetate incubations, which also showed the

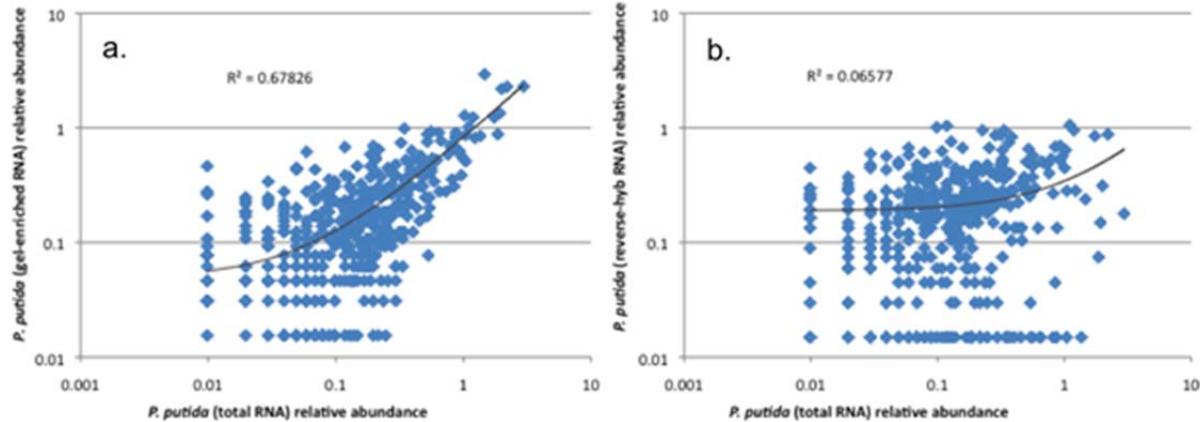


Figure 5. Correlation between relative abundance of recruitment of transcripts to model bacterium genome: (a) total RNA vs. gel-enriched mRNA, (b) total RNA vs. reverse-hybridization enriched mRNA.

highest level of substrate respiration and total RNA and DNA extracted from the soil. Sequencing of RNA libraries from soil with *A. chlorophenolicus* added was performed using the Illumina HiSeq 2000. These transcriptomes showed that different metabolic pathways were more predominantly expressed depending on the substrates and incubation conditions used (for example, a suite of genes that move acetate through the *A. chlorophenolicus* pyruvate/citric acid cycle for cellular energy generation were expressed in the acetate treatment), and these results mirrored the proteomic results, showing that the most expressed genes were also translated into active proteins. These data and observations highlight the cohesion and complementarity of the different meta-omics tools that we employed to answer our primary research questions.

RNA library data from the complex soil community showed that: (1) The relative abundance of transcripts from functional subsystems differed significantly between soil incubated with and without acetate. Soil with acetate added had a higher abundance of transcripts of genes involved in DNA, RNA, protein, sulfur, and phosphorus metabolism and in cell division and the cell cycle, consistent with growth. Soil with no acetate added had a higher abundance of transcripts of genes involved in basic nitrogen and carbohydrate metabolism, and in dormancy and sporulation. (2) The ribosomal abundance of certain microorganisms was significantly higher than the abundance of rRNA genes in the metagenomic library, implying that these groups of microbes were active, not dormant, in the soil habitat. In the soil with no acetate added, these “active” microbial groups included the Acidobacteria, Cytophaga, Fibrobacteres, Verrucomicrobia and various unclassified Bacteria, and the Agaricomycetes, Dothideomycetes, Eurotiomycetes and additional unclassified Ascomycota and Fungi. These are taxonomic groups typically associated with slow growth and complex organic matter decomposition, making them likely to be active in a soil habitat but difficult to isolate or grow in a lab culture. Overall, transcriptomic data generated from a complex soil matrix have successfully identified both active microbial metabolic and taxonomic differences between soils with different available C sources.

Task 1.3-Optimize metaproteomics protocol. One of the major hurdles to shotgun proteomics in soil is the effective extraction of protein molecules from the soil matrix. This is greatly aggravated by the presence of humic acids in high organic soils, such as the Konza prairie. We first applied protocols previously established in our consortium for low organic soils. These include a direct extraction with SDS/TCA and an indirect extraction based on differential centrifugation to first extract bacterial cells

prior to lysis. With the Konza soils, the humic acids overwhelmed the peptide signal after extraction using either approach. We partially solved this problem by modifying the SDS/TCA method with an additional acidification step followed by a 10-kilodalton filtration step to remove the humic acids. We used a GFP (green fluorescent protein)-tagged *A. chlorophenolicus* as a control soil microbe for microcosm experiments in order to test the effects of different C substrates on its proteome when incubated in Konza prairie soil. In addition, we used this experiment to demonstrate how one specific soil bacterium responds to differences in incubation conditions in soil (the rhizosphere was also added as an additional treatment). The *gfp* gene was used as an internal standard for RNA and protein quantification. The model strain was inoculated into sterile and non-sterile Kansas prairie soil, amended or not with acetate. Total proteins were extracted from the same samples to obtain metaproteomes by shotgun metaproteomics via 2d-LC-MS/MS on an LTQ Velos mass spectrometer. Differential protein expression patterns were observed (Fig. 6). For example, several proteins involved in response to stress (thioredoxin, chaperonin, cold-shock proteins, etc.) were expressed. We also detected high levels of flagellin proteins (FliC and FlgE) in the soil simultaneously with 24 genes responsible for flagella assembly in the transcriptome. *A. chlorophenolicus* proteins expressed in the rhizosphere showed several similarities to the proteome found in the soil amended with acetate. In addition, the expression of 114 genes involved in plant hormone responses were upregulated in the rhizosphere. We also confirmed that for the different conditions the proteome and the transcriptome matched well. Finally, a comparison of *A. chlorophenolicus* protein yields from sterile and non-sterile soil showed the impact of high background soil diversity on complicating the proteomic results, with more than 632 IDs identified in the sterile soil.

Objective 2. Determine the impacts of long-term climatic changes (precipitation) on soil C cycling using an existing long-term field manipulation.

Two field campaigns were completed at the Rainfall Manipulation Plots (RaMPs) at the Konza Prairie Long-Term Ecological Research site in north-eastern Kansas, USA. RaMPs is a replicated field manipulation of the timing and magnitude of natural precipitation that was established in 1998. This experiment does not modify the total amount of growing season rainfall; it imposes extended dry periods and larger, less frequent rainfall events. We collected soil before, during and after rainfall events in both Ambient and Altered precipitation interval (more “droughty”) treatments and measured

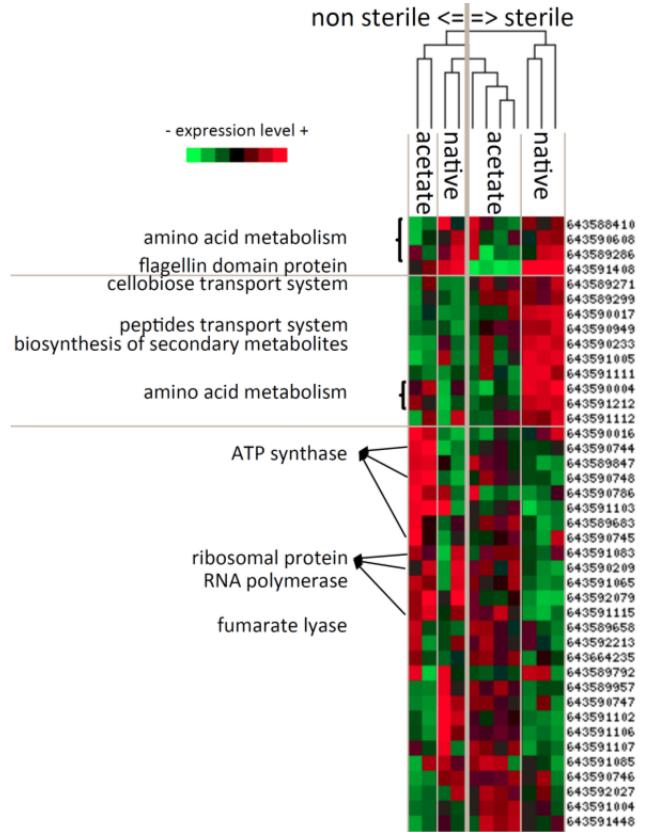


Figure 6. Heatmap of the proteins detected in all four incubations.

microbial growth, respiration and potential organic matter degradation responses. We sampled rainfall events in June and September 2011. At each sampling we collected soil immediately prior to a 1" rainfall event and at one and five days following the rainfall. Soil samples were divided and sent to the various research laboratories for analyses of soil microbial activities, microbial community composition, metatranscriptomes, and metaproteomes.

All activity measurements have been completed and published in Zeglin *et al.* (2013). A short summary of notable findings include: (1) Equivalent rainfall events caused equivalent microbial respiration responses (+1.77 in moist conditions and +0.95 mg CO₂-C kg⁻¹ dry soil h⁻¹ in dry conditions) in Ambient and Altered treatment soils, but biomass increased significantly after the rainfall in Altered treatment soils only (+171 and 147 mg C kg⁻¹ dry soil). (2) Microbial biomass pools were also larger in Altered than Ambient treatment soils (911 > 814 mg C kg⁻¹ dry soil, respectively). This implies that microbial C use efficiency (CUE) was higher in Altered than Ambient treatments (0.70 ± 0.03 vs. 0.46 ± 0.10). CUE was also higher in dry (September) soils. (3) Carbon-acquiring enzyme activities (β -glucosidase, cellobiohydrolase, and phenol oxidase) increased after rainfall in moist (June), but not dry (September) soils. (4) Both microbial biomass C:N ratios and fungal:bacterial ratios were higher at lower soil water contents, suggesting a functional and/or population-level shift in the microbiota at low soil water contents, and microbial community composition also differed following wet-up and between seasons and treatments. In summary, microbial activity may directly (C respiration) and indirectly (enzyme potential) reduce soil organic matter pools less in drier soils, and soil C sequestration potential (CUE) may be higher in soils with a history of extended dry periods between rainfall events. The implications include that soil C loss may be reduced or compensated for via different mechanisms at varying time scales, and that microbial taxa with better stress tolerance or growth efficiency may be associated with these functional shifts.

These results lead to hypotheses regarding microbial physiological adaptation to drought stress in prairie soils. Molecular data (454 sequencing and QPCR of bacterial 16S rRNA and fungal ribosomal genes and transcripts, full transcriptomes, and proteomes) were collected to test these hypotheses: (H1a) Microbial taxa that respond quickly to increased water availability after drought are more active in soil with an altered precipitation regime history. (H1b) Transcripts and proteins from COGs indicative of growth, not maintenance, will be more abundant after rainfall in the "droughty" plots. (H2a) In soils with low water contents, transcripts and proteins driving trehalose (or other compatible solute) production will be more abundant. (H2b) In soils with low water contents, fungal cells will be more abundant. (H3) Expression of extracellular (soil organic matter degrading) enzymes will be highest in moist soils after rainfall events. The following summarizes our findings to date with respect to gene and protein expression.

Although, bacterial and fungal metabolic activities (as inferred from indigenous exo-enzyme activities and soil respiration) and their biomass (PLFA and qPCR assays) were quick to respond to the precipitation pulse, their richness community composition were stable through the pulse and did not differ strongly among the Ambient or Altered precipitation interval treatments. However, we were able to decipher clear functional responses. Transcriptome libraries reflected a dynamic pool of genes expressed in the context of soil wetting and drying via both seasonal drought and individual rainfall events (Fig. 7). Despite this variability, there was no clear relationship between the taxonomic or functional gene expression in soils subject to long-term alteration of precipitation timing and soils

responding to short-term responses to soil water content, so there was no support for hypothesis H1a. However, finer-scale annotation and analysis of transcript data may identify patterns that did not emerge with high-level (i.e. phylum-level taxonomic or coarsest functional categorization) evaluation of gene expression in response to water availability at different time scales.

Hypothesis H1b was supported in that the relative abundance of oxidative phosphorylation (R) transcripts increased and decreased concurrent with the pulse of total microbial respiration following individual rainfall events, while the combined relative abundance of gluconeogenesis and saccharide synthesis (G) transcripts increased and remained elevated following rainfall (Fig. 8). In addition, soils in the Altered had significantly higher ratios of G:R transcripts across all timepoints than ambient soils ($0.75 \pm 0.33 > 0.20 \pm 0.07$). The expression of trehalose or compatible solute production genes was not correlated with soil water content, in contrast to expectations (hypothesis H2b); however, the sum of transcripts categorized as “cellular responses to stress-tolerance” (including oxidative stress, osmotic stress and general stress response, KO annotated) declined with soil water content (Fig. 9). Finally, hypothesis H3 could not be evaluated directly, because the expression rates of extracellular enzyme genes were relatively low; this could mean that either transcript libraries were not sequenced deeply enough to detect expression of certain genes (including extracellular enzymes), or that new enzyme production was minimal and changes in bulk soil enzyme potential activity was primarily driven by stabilized

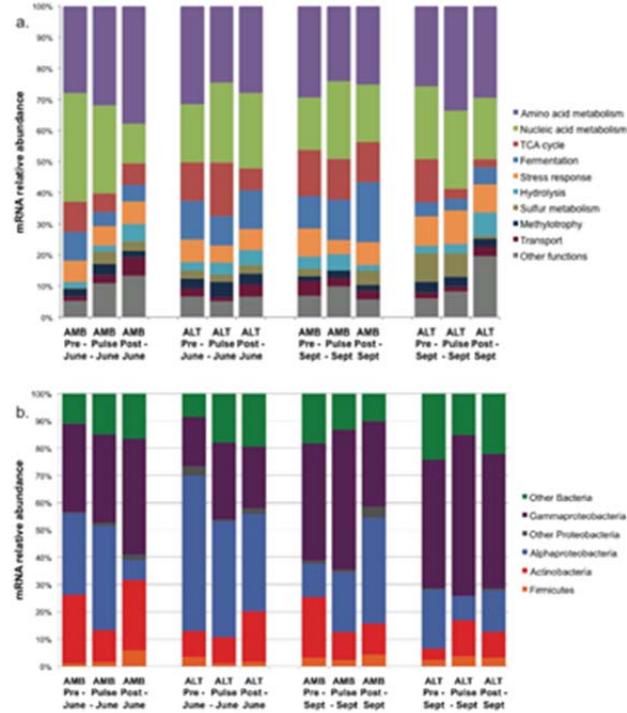


Figure 7. Functional (a; KO via FOAM categorization) and taxonomic (b; MG-RAST M5NR) annotation of mRNA libraries from field soil water experiments.

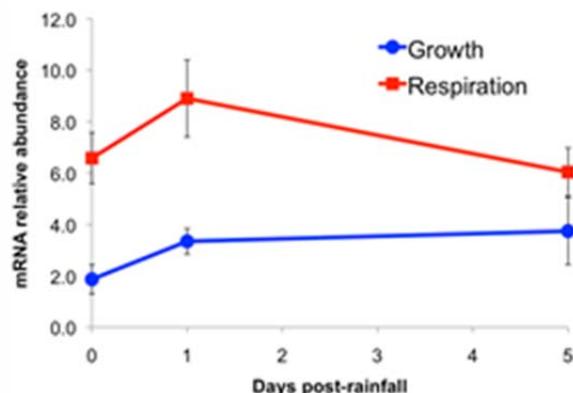


Figure 8. Mean relative abundance of growth (in blue) and respiration (in red) transcripts in field soils before and after rainfall events.

enzymes. A related link between soil water variability, soil organic matter (SOM) decomposition and microbial C utilization was apparent, however, in the concurrent increase in β -glucosidase potential activity and cellobiose transport following the June rainfall event, both indicating an increase in cellobiose (the dimer that comprises cellulose, a major component of SOM) availability for microbial utilization (Fig. 10). In summary, mRNA library data provided mechanistic insights into soil microbial carbon dynamics under changing soil water conditions in this field experiment. Still,

improvements in the volume of data acquired as well as the resolution and accuracy of annotation might allow more specific hypotheses to be addressed.

We prepared 24 soil samples for metaproteomics measurements using two different methods; the first approach was an indirect method in which the microbial biomass was physically separated from the soil matrix by centrifugation, and the second approach was a direct method involving the optimized SDS-TCA approach from Task 1.3. The samples were then digested with trypsin and analyzed with a high performance LTQ-Orbitrap-Elite mass spectrometer at ORNL. Of the total 24 samples, 16 of them had dense total ion chromatograms (TIC) and base-peak chromatograms, suggesting that these had sufficient high quality peptide mass spectra for extensive proteome coverage. We suspect that the remaining 8 poor quality runs might be due to either insufficient microbial biomass or the presence of an extracted, interfering component from the soil samples. The raw mass spectra were searched against predicted protein database constructed from various metagenomic assemblies of field samples, as well as a database constructed from 160 representative reference microbial species. For the Konza prairie soils, the metagenomes ranged in size from 700,000 to 7.8 million protein sequences, which provided computational challenges in the proteome searches. Searches were initiated on three representative samples that allowed evaluation of the performance of the various metagenome builds against raw MS data. The results yielded modest proteomic metrics (~200-300 unique proteins per sample), but also revealed a large number of high quality unassigned spectra (Fig. 11). A more detailed inspection of the metagenomes revealed a high percentage of very short sequence assemblies that likely confound the peptide/protein identification process (i.e. difficulty in assigning unique peptide sequences, presence of "fragmented proteins" in the database, etc.). This prompted the need for a revised metagenome construction to better assemble and/or remove the numerous short sequences. Alternatively, we are evaluating the application of a pseudo-metagenome (constructed from an expanded list of relevant reference isolates), even though this might be more

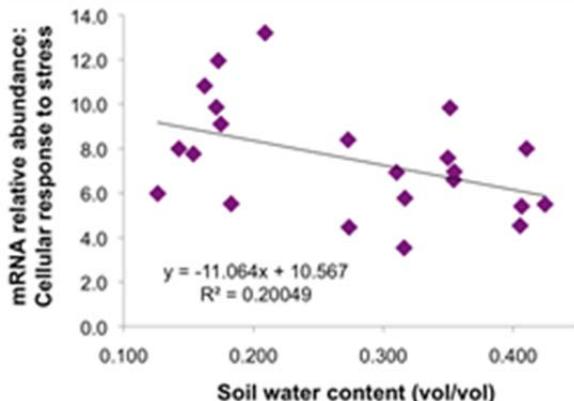


Figure 9. Correlation between soil water content and expression of microbial stress response genes.

Figure 10. Mean (a) β -glucosidase enzyme activity and (b) relative abundance of cellobiohydrolase transcripts before and after rainfall events in June (in green) and September (in orange).

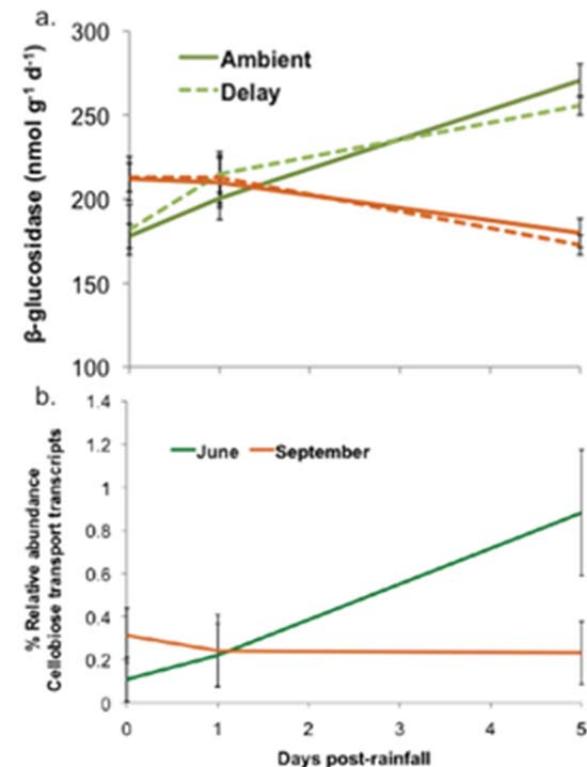


Figure 10. Mean (a) β -glucosidase enzyme activity and (b) relative abundance of cellobiohydrolase transcripts before and after rainfall events in June (in green) and September (in orange).

distantly related to the actual microbial species/strains in the environmental sample. We believe that such an iterative process of integrating metagenome with metaproteome data will greatly aid in the evaluation and improved assemblies/identification metrics. Work is underway to more fully execute this proposed work.

As a means of integrating the meta-omics and functional data of the field experiment, we are applying correlation network, using the approach described in Task 1.1. This network analysis shows an association between phenol oxidase and peroxidase, which are involved in primary lignin degradation (Fig. 12). The dissolved

organic C and phenol/peroxidase activities make sense, as small organics are needed as part of the lignin degradation activity. In addition, lignin degradation seems to be inversely related to N availability. The positive association of the two fungal taxa and biomass C, but negative to biomass N, might fit with fungi having a higher C:N ratio. The fungi presented here, Ascomycota, include numerous taxa that are known as secondary decomposers, colonizing dead plant material already colonized by other microfungi, and

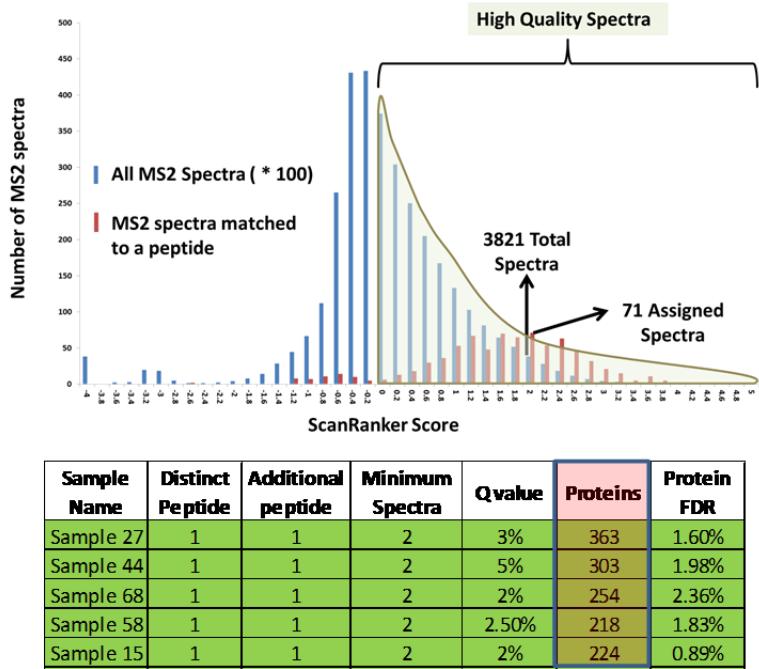


Figure 11. Proteome characterization success with modified method.

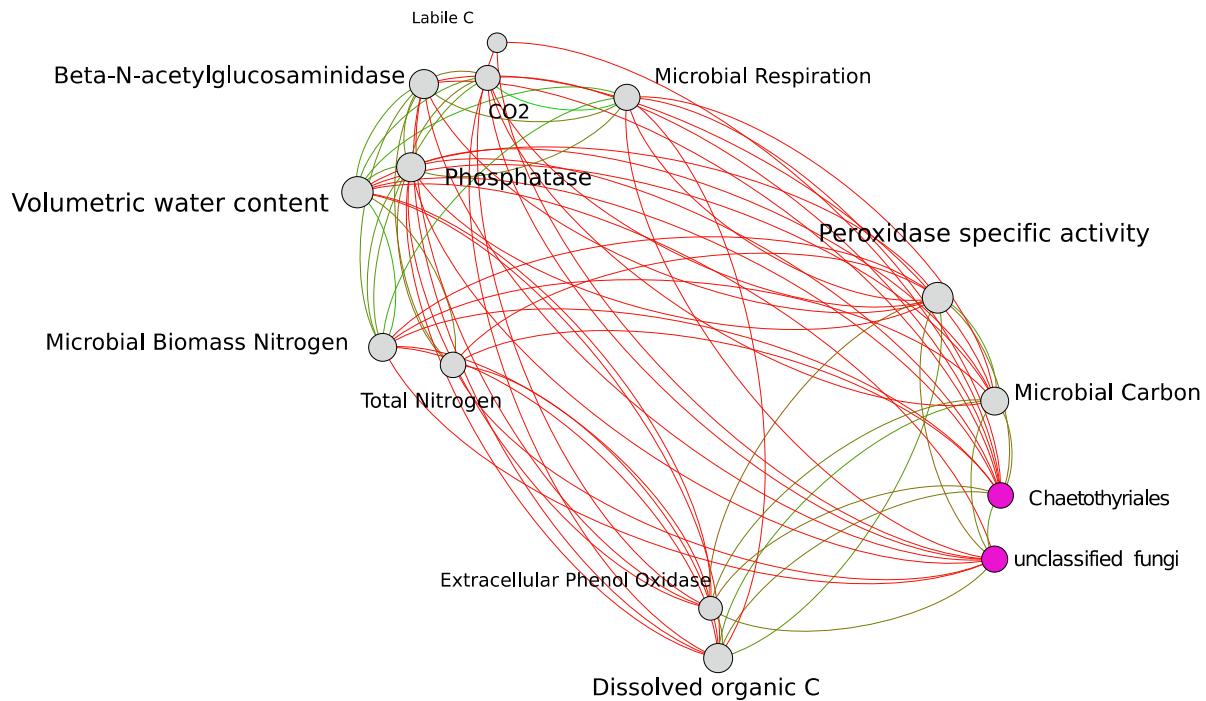


Figure 12. Association network d

soil.

scavenging on small carbohydrates, which could explain its association with dissolved organic C. In addition, it makes biological sense that fungi would be associated with phenol oxidase and peroxidases, as they are primarily involved in lignin degradation. Finally, the idea that lignin degradation is inversely related to N availability is represented in this network. The positive association of the two fungal taxa and biomass C, but negative to biomass N, might fit with fungi having a higher C:N ratio as suggested in Zeglin *et al.* (2013).

Objective 3. Conduct laboratory experiments of specific environmental variables (moisture, C inputs) to confirm field observations of the linkages between microbial communities and C cycling processes.

A laboratory experiment is underway to follow-up on the observation of greater CUE in drier soils in the field experiment. Two soils of different textures were adjusted to different water stress by varying water content (affects water potential and substrate transport) and additions of polyethylene glycol (affects water potential but not substrate transport). Work is underway to determine CUE using ¹³C-labeled substrates that mimic root exudates and/or plant litter.

Products Delivered

Referred journal articles (lead author in bold; postdocs, graduate students, or undergraduate students underlined)

Published

Jansson, J.K. 2011. Towards "Tera'Terra": Terabase sequencing of terrestrial metagenomes. Feature article. *Microbe* 6:309-315.

Bottomley, P.J., A.E. Taylor, and D.D. Myrold. 2012. A consideration of the relative contributions of different microbial subpopulations to the soil N cycle. *Front. Microbiol.* 3:373. (doi: 10.3389/fmicb.2012.00373)

Zeglin, L.H., P.J. Bottomley, A. Jumpponen, C.W. Rice, M. Arango, A. Lindsley, A. McGowan, P. Mfompeb, and D.D. Myrold. 2013. Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. *Ecology* 94:2334-2345. (doi: org/10.1890/12-2018.1)

Myrold, D.D., L.H. Zeglin, and J.K Jansson. 2014. The potential of metagenomic, and other omic, approaches for understanding soil microbial processes. *Soil Sci. Soc. Am. J.* 78:3-10. (doi:10.2136/sssaj2013.07.0287dgs)

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In preparation

A combination of proteomics and transcriptomics reveals specific functions expressed in soil and rhizosphere by *Arthrobacter chlorophenolicus*. Maude M. David, Lydia Zeglin, Emmanuel Prestat, Jill Dvornik, Robert Hettich, Kristen Corrier, Konstantinos Mavromatis, Renee

Koutsoukis, Steve Lindow, Manesh Shah, Nathan VerBerkmoes, David Myrold, and Janet K. Jansson

Sequencing the active metagenome facilitates omics of a complex native prairie soil. Maude M. David, Emmanuel Prestat, Lydia Zeglin, Robert Hettich, Ari Jumpponen, Charles Rice, Manesh Shah, Susannah Tringe, Nathan VerBerkmoes, David Myrold, and Janet K. Jansson

Non-refereed articles or abstracts

Myrold, D.D., L.H. Zeglin, M.M. David, E. Prestat, P.J. Bottomley, R. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, and N.C. VerBerkmoes. 2014. The Potential of Metagenomic Approaches for understanding nutrient cycling in soils. p. 38. *In* Proceedings of the 11th Dahlia Greidinger Memorial Symposium, 4-7 March 2013, Haifa, Israel.

Jumpponen, A., L. Zeglin, M. David, E. Prestat, S. Brown, J. Dvornik, K. Lothamer, R. Hettich, J. Jansson, C. W. Rice, S. Tringe, and D. Myrold. 2013. Fungal community responses to discrete precipitation pulses under altered rainfall intervals. *Phytopathology* 103:182-183.

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Myrold, D.D., P.J. Bottomley, M.M. David, R.L. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, N.C. VerBerkmoes, S.A. Yarwood, and L.H. Zeglin. 2011. Meta-“omics” analysis of microbial carbon cycling responses to altered rainfall inputs in native prairie soils. DOE Genomic Sciences Meeting, 10-13 April 2011. Arlington, VA.

David, M.M., L. Zeglin, M. Shah, S. Yarwood, K. Mavromatis, S. Tringe, R. Hettich, N. VerBerkmoes, D. Myrold, and J.K. Jansson. 2011. Assessing the microbial basis of carbon cycling in prairie soils with an integrated omics approach. DOE Genomic Sciences Meeting, 10-13 April 2011. Arlington, VA.

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David, M.M., L. Zeglin, R. Koutsouri, M. Shah, S. Yarwood, K. Mavromatis, S. Lindow, S. Tringe, R. Hettich, N. VerBerkmoes, D. Myrold, and J.K. Jansson. 2011. Development of an integrated omics approach for studying carbon cycling in prairie soils. 11th Conference on Bacterial Genetics and Ecology (BAGECO11), 29 May-2 June 2011. Corfu, Greece.

Myrold, D.D., P.J. Bottomley, M.M. David, R.L. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, N.C. VerBerkmoes, S.A. Yarwood, and L.H. Zeglin. 2011. Omics for soil: choose one or integrate all? 2011 International Conference on Soil Omics-Nanjing. 19-23 November 2011, Nanjing, China. (invited)

Zeglin, L.H., M. David, P. Bottomley, R.L. Hettich, J. Jansson, A. Jumpponen, C.W. Rice, S. Tringe, N. C. VerBerkmoes, and D. Myrold. 2011. Microbial response to modified precipitation patterns in

tallgrass prairie soil: molecular mechanisms, activity rates and organic matter dynamics. AGU Fall Meeting, 5-9 December 2011, San Francisco, California, USA.

Myrold, D.D., P.J. Bottomley, M.M. David, R.L. Hettich, J.K. Jansson, A. Jumpponen, E. Prestat, C.W. Rice, N.L. Tisdell, S.G. Tringe, N.C. VerBerkmoes, and L.H. Zeglin. 2012. Meta-omics analysis of microbial carbon cycling responses to altered rainfall inputs in native prairie soils. DOE Genomic Sciences Meeting, 26-29 February 2012. Bethesda, MD. (**invited**)

David, M.M., L. Zeglin, P. Bottomley, R. Hettich, A. Jumpponen, K. Mavromatis, D. Myrold, C. Rice, M. Shah, S. Tringe, N. VerBerkmoes, and J.K. Jansson. 2012. Development of integrated “omics” approach for assessing microbial cycling of carbon in prairie soil using a model soil bacterium: *Arthrobacter chlorophenolicus*. DOE Genomic Sciences Meeting, 26-29 February 2012. Bethesda, MD.

Zeglin, L.H., P.J. Bottomley, M.M. David, R.L. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, N.C. VerBerkmoes, and D.D. Myrold. 2012. Meta-omics Microbial response to modified precipitation patterns in tallgrass prairie soil: molecular mechanisms, activity rates and organic matter dynamics. DOE Genomic Sciences Meeting, 26-29 February 2012. Bethesda, MD.

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Zeglin, L.H., M. David, E. Prestat, A. Lindsley, M. Arango, P.J. Bottomley, R. Hettich, J. Jansson, A. Jumpponen, C. Rice, S. Tringe, N. VerBerkmoes, and D.D. Myrold. 2012. Microbial functional response to altered precipitation timing and duration – implications for the soil carbon cycle. Abstr. Ann. Meet. Ecol. Soc. Am., p. xxx.

David, M.M., L. Zeglin, E. Prestat, J. Dvornik, P. Bottomley, R. Hettich, K. Corrier, A. Jumpponen, K. Mavromatis, C. Rice, R. Koutsouri, S. Lindow, M. Shah, S. Tringe, N. VerBerkmoes, D. Myrold, and J.K. Jansson. 2012. Impact of soil, rhizosphere and growth conditions on the *Arthrobacter chlorophenolicus* transcriptome and proteome. 14th International Society for Microbial Ecology Meeting, 19-24 August 2012, Copenhagen, Denmark.

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proteome, and activity to rainfall pulses in a prairie soil. 14th International Society for Microbial Ecology Meeting, 19-24 August 2012, Copenhagen, Denmark.

Zeglin, L.H., P. Bottomley, A. Jumpponen, C. Rice, M. Arango, A. Lindsley, A. McGowan, and D.D. Myrold. 2012. Microbial functional response to altered precipitation timing and duration: implications for the soil carbon cycle. 2012 LTER All Scientists Meeting, 10-13 September 2006, Estes Park, CO.

Zeglin, L.H., M. David, E. Prestat, A. Lindsley, M. Arango, P.J. Bottomley, R. Hettich, J. Jansson, A. Jumpponen, C. Rice, S. Tringe, N. VerBerkmoes, and D.D. Myrold. 2012. Microbial functional response to altered precipitation timing and duration – implications for the soil carbon cycle. 4th Annual Argonne Soils Workshop. 3-5 October 2012. Argonne, IL.

Rice, C., L. Zeglin, P. Bottomley, N.L. Tisdell, A. Jumpponen, M.M. David, E. Prestat, J.K. Jansson, S.G. Tringe, N. VerBerkmoes, R.L. Hettich, A.W. McGowan, P. Mfombep, M. Arrango, and D. Myrold. 2012. Response of microbial transcriptome, proteome, and activity to rainfall pulses in a prairie soil. Abstr. American Society of Agronomy, Madison, WI. p. xxx.

Prestat, E., M.M. David, S.G. Tringe, E.A. Holman, A. Jumpponen, D.D. Myrold, K. Mavromatis, and J.K. Jansson. 2013. A custom database for functional annotation of soil omics datasets using Hidden Markov Models. DOE Genomic Sciences Meeting, 24-27 February 2013. Bethesda, MD.

David, M.M., E. Prestat, L.H. Zeglin, S.G. Tringe, N.C. VerBerkmoes, R.L. Hettich, D.D. Myrold, and J.K. Jansson 2013. Multi-omics assessment of active microbial communities and their C cycling function in native prairie soil. DOE Genomic Sciences Meeting, 24-27 February 2013. Bethesda, MD.

Zeglin, L.H., M.M. David, E. Prestat, S. Brown, J. Dvornik, P.J. Bottomley, R.L. Hettich, A. Jumpponen, J.K. Jansson, C.W. Rice, S.G. Tringe, N. VerBerkmoes, and D.D. Myrold. 2013. Multi-omics of RaMPs field study reveals impacts of altered rainfall patterns on carbon cycling in native prairie. DOE Genomic Sciences Meeting, 24-27 February 2013. Bethesda, MD.

Myrold, D.D., L.H. Zeglin, M. David, E. Prestat, P.J. Bottomley, R. Hettich, J. Jansson, A. Jumpponen, C. Rice, S. Tringe, N. VerBerkmoes. 2013. The potential of metagenomic approaches for understanding nutrient cycling in soils. The 11th Dahlia Greidinger Memorial Symposium, 4-7 March 2013. Haifa, Israel. **(Invited keynote)**

Myrold, D.D. 2013. Facilitating the integration of metaproteomics with soil metagenomic studies. Metaproteomics of the Soil: The Challenges and Possibilities satellite meeting of the 2013 Joint Genome Institute User Meeting, 25 March 2013, Walnut Creek, CA. **(Invited speaker)**

David, M.M., E. Prestat, L.H. Zeglin, S.G. Tringe, N.C. VerBerkmoes, R.L. Hettich, D.D. Myrold, and J.K. Jansson. 2013. Multi-omics assessment of active microbial populations and carbon cycling functions in native prairie soil. Gordon Research Conference, 7-12 July 2013, South Hadley, MA.

Jumpponen, A., L.H. Zeglin, M.M. David, E. Prestat, S.P. Brown, J. Dvornik, K. Lothamer, R.L. Hettich, J.K. Jansson, C.W. Rice, S.G. Tringe, and D.D. Myrold. 2013. Fungal community responses to discrete precipitation pulses under altered rainfall intervals. Mycological Society of American Meeting, 10-14 August 2013, Austin, TX.

Hettich, R.L., M.M. David, E. Prestat, L.H. Zeglin, P.J. Bottomley, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, N.C. VerBerkmoes, and D.D. Myrold. Integrating omics to understand soil C cycling

responses to precipitation variation. 5th Annual Argonne Soils Workshop. 2-4 October 2013. Argonne, IL.

Myrold, D.D., M.M. David, E. Prestat, L.H. Zeglin, P.J. Bottomley, R.L. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, and N.C. VerBerkmoes. 2013. Integrating omics to understand soil C cycling responses to precipitation variation. 2nd Thünen Symposium on Soil Metagenomics. 11-13 December 2013, Braunschweig, Germany. **(Invited speaker)**

Jumpponen, A., L.H. Zeglin, P.J. Bottomley, C.W. Rice, M.M. David, E. Prestat, J.K. Jansson, S.G. Tringe, R.L. Hettich, N.C. VerBerkmoes, and D.D. Myrold. 2014. Microbial community and functional responses to rainfall manipulations in a prairie soil. DOE Genomic Sciences Meeting, 24-27 February 2014. Bethesda, MD.

Tisdell, N.L., P.J. Bottomley, and D.D. Myrold. 2014. Desiccation effects on soil microbe carbon use efficiency: Is water or carbon more limiting? A preliminary experiment. Oregon Society of Soil Scientists, 27 February 2014, Bend, OR.

Myrold, D.D. 2014. Impact of altered precipitation patterns on carbon cycling dynamics in the Great Prairie. 114th General Meeting, American Society for Microbiology, 17-10 May 2014, Boston, MA. **(Invited speaker)**

Myrold, D.D., M.M. David, E. Prestat, L.H. Zeglin, P.J. Bottomley, R.L. Hettich, J.K. Jansson, A. Jumpponen, C.W. Rice, S.G. Tringe, and N.C. VerBerkmoes. 2014. Integrating omics to understand soil C cycling responses to precipitation variation. 20th World Congress of Soil Science, 8-13 June 2014, Jeju, Korea.

David, M.M., L.H. Zeglin, R. Sharma, E. Prestat, P.J. Bottomley, A. Jumpponen, C. W. Rice, S.G. Tringe, N.C. VerBerkmoes, R.L. Hettich, J.K. Jansson, and D.D. Myrold. 2014. Microbial community and functional responses to rainfall manipulations in a prairie soil. 15th International Society for Microbial Ecology Meeting, 24-29 August 2014, Seoul, Korea.

Zeglin, L.H., M.M. David, R. Sharma, E. Prestat, P.J. Bottomley, A. Jumpponen, C. W. Rice, S.G. Tringe, N.C. VerBerkmoes, R.L. Hettich, J.K. Jansson, and D.D. Myrold. 2014. Microbial community and functional responses to rainfall manipulations in a prairie soil. Abstr. American Society of Agronomy, Madison, WI. p. xxx.