U.S. Department of Energy: Research Grant Progress Report

Title of Project: Microbial drivers of global change at the aggregate scale: linking genomic function to carbon metabolism and warming

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Project Results:

Overview

Understanding and accurately predicting the microbial cycling of carbon in soil environments has been challenged by our ability to associate microbial community dynamics into ecosystem-scale biogeochemical models. Soil fractionation techniques provide an opportunity to examine intact microbial communities in a context that is relevant to both microbial community metabolism and ecosystem processes. We developed approaches that targeted metabolically active microorganisms and functions that drive carbon cycling in soils from bioenergy cropping systems. We used metatranscriptomics, genome sequencing, cell sorting, and cell isolation methods to access the key organisms involved in soil carbon cycling (e.g. cellulose decomposition) in soil aggregate fractions.

The objectives of our research were to:
1. Determine if the active carbon cycling microbial communities differ among soil aggregate fractions and bioenergy cropping systems
2. Identify the key organisms and functions involved in the metabolism of cellulose within soil communities
3. Evaluate the extent of functional redundancy among cellulose metabolizing assemblages
4. Investigate microbial functional responses to soil warming.
5. Incorporate our data and computational approaches as services within the DOE Systems Biology Knowledgebase or KBase to be used with community datasets and with scale-out capability.

Project Description

We aimed to identify the foundational organisms and metabolic pathways that drive microbial carbon cycling in soils from sustainable bioenergy cropping systems as well as provide an analytical framework for combining integrative approaches to inform intermediate and field scale models. We investigated microbial carbon metabolism within soil aggregates. Through our research we identified the main microbial membership and functions responding to carbon, created gene catalogs of high quality references to interpret field-scale soil sequencing datasets, and developed and evaluated novel approaches and analyses targeting microbial responses to metabolites. This foundational knowledge underlying microbial carbon metabolism is critical for harnessing the carbon sequestration potential of terrestrial ecosystems and for informing sustainable land management policies for our future. The scientific advances emanating from this research are broadly applicable to the study of other environments and are transferable to a broad range of applications, from biofuel production to managing systems to reduce greenhouse gas emissions.

Objective 1: Determine if the carbon cycling microbial communities differ among soil aggregate fractions and bioenergy crop treatments.

This objective was addressed at the community (enrichment cultures), mesocosm (laboratory incubations) and ecosystem (field experiments) scales with the intention of ultimately integrating microbial functions across scales. At the community scale, we developed an analytical framework for evaluating functional redundancy and microbial community relationships (Williams et al. 2014), and subsequently implemented this analysis to identify community relationships from field samples (Choi et al. 2018). The statistical code used to generate this analysis is already publicly available (https://github.com/chnops/bayezymes). This methodology has been further developed to be more statistically robust (https://github.com/ryanjw/bayezymes_2.0) in order to use it to link heterogeneous datasets (16S rRNA, metagenomes, metatranscriptomes, biogeochemical data; https://github.com/ryanjw/network_sim). Application of this methodology to combine 16S rRNA and biogeochemical data are available at (https://github.com/ryanjw/16S_bayes_co-occurrence).

We have published several papers demonstrating differences in microbial community composition and enzymatic potential among aggregate fractions and agricultural management systems. Importantly, our studies reveal that ecological interactions between soil microorganisms and organic matter vary spatially, among soil aggregate fractions, and temporally, throughout the growing season, influencing the cycling and storage of C and nutrients, including N (Upton et al. 2019, Upton et al. 2018, Bach et al. 2018, Bach et al. 2016).

Objective 2: Identify the key organisms and functions involved in the metabolism of cellulose within soil communities.

We conducted lab incubations to address our goal of identifying the key organisms and functions involved in the metabolism of cellulose within soil communities. We successfully enriched soil microbial communities from soil aggregates using fluorescently labeled cellulose nanocrystals as the sole C source. This work proved to be challenging because of the floccing nature of the cellulose degraders, making single cell sorting impossible. Instead we used metatranscriptomics to identify the key cellulose degrading communities, enzymes and metabolic pathways regulating cellulose cycling in soil aggregate fractions. This work was presented at national scientific meetings and invited talks at universities and a manuscript is in preparation to report our findings.

Objective 3: Evaluate the extent of functional redundancy among cellulose metabolizing assemblages.

We used soil metagenomics to identify "core" sequences widely present in multiple local soil samples from a fertilized prairie (FP) biofuel cropping system, specifically targeting sequences associated with C cycling (Howe et al. 2016). Our goal was to determine what core sequences are redundant among multiple soils, as a means of gaining insight into the drivers of soil function. In other words, is there a core set of sequences, genes, or functions that are present in *all* soils? Our research represents the first effort to identify a minimally functional core within soil metagenomes, with a focus on C-cycling proteins (Howe et al. 2016).

Of 226,887 sequences associated with known enzymes involved in the synthesis, metabolism, and transport of carbohydrates (the CAZy database), 843 were identified to be consistently prevalent across four replicate soil metagenomes from our field experiment. This core metagenome was functionally and taxonomically diverse, representing five major enzyme classes and 99 enzyme families within the CAZy database. Though it only comprised 0.4% of all CAZy-associated genes identified in FP metagenomes, the core was found to be functionally representative of the whole soil metagenome. These results suggest that these sequences may represent key C cycling genes in this soil. Comparing the FP CAZy-associated core sequences to other global soil metagenomes revealed that these sequences are present in multiple other soils and most similar to soils sharing geographic proximity. Core sequences that shared similar patterns of abundances in varying samples were identified with a co-occurrence analysis and observed to be associated with functional modules representing distinct metabolic processes in starch

and sugar metabolism. In soil ecosystems, where high diversity remains to be a key challenge for metagenomic investigations, these core genes and modules represent a subset of critical functions necessary for carbohydrate metabolism, which can be targeted to evaluate important C fluxes in these and other similar soils. The identification of a functional core provides insight into the key functions and phyla related to the production and decomposition of biomass within the soil. Our analyses identified a genomic core that is broadly representative of cumulative soil functions, even in contrasting global soils. From this work we also generated a strategy to improve reference databases for soil microbiomes (Choi et al. 2017).

Objective 4: Investigate microbial functional responses to soil warming.

We conducted a lab incubation study to investigate the differential response of aggregate classes from corn or diversified prairie soils exposed to warming, carbon inputs and moisture. Preliminary results suggest that microorganisms associated with microaggregates are more responsive to cellulose additions than large macroaggregates, confirming results from our published field experiments (Bach et al, 2016). These results are particularly exciting as they challenge the ecosystem paradigm that most carbon cycling is occurring in large macroaggregates, while microaggregates are associated with long-term soil carbon storage and are less active in the carbon cycle (Bach et al. 2018). Instead, our microbial data, and field and lab results together suggest that microaggregates harbor rich and diverse bacterial communities that are sensitive to relatively labile carbon inputs. Although on a mass basis microaggregates are a small proportion of the whole soil, from a surface area perspective they provide a significant microbial habitat that may be critical to soil carbon cycling and storage due to their distinct microbial communities.

Objective 5: Incorporate our data and computational approaches as services within the DOE KBase (Systems Biology Knowledgebase) to be used with community datasets and with scale-out capability.

Metagenomes have been obtained for soil aggregate samples across three cropping systems at COBS field site (n=52, total of 1.6 billion reads). These datasets have been combined and assembled successfully to provide an initial reference. We created reference soil database from manually created genomes in NCBI RefSeq to use in co-occurrence and comparative metagenomic analyses. Data and analysis for determining core genes in multiple metagenomes has been completed and made available on https://github.com/germs-lab/carbon-core-soil-paper and through KBase integration on https://github.com/kbase/cap.

A significant number of novel tools needed to be integrated into the KBase framework, expanding the KBase capabilities as required by this project. A number of new software packages were installed in the current KBase framework, making them available to all KBase developers. All tools were installed using the KBase conventions and their installation passed the required KBase tests.

To enable a KBase service and create an automated processing pipeline for this project all tools have been packaged into individual docker images. The following docker images have been created to support this workflow:

- CAP for in house python tools – tools for determining read coverage from NGS data
- BOWTIE2 -- mapping tools for next generation sequencing reads
- Samtools – a family of tools for the manipulation of DNA sequence read data
- Bedtools – a suite of tools for comparison of DNA sequence read sets

To facilitate the use of the tools in KBase pipelines, the analysis steps have been transformed into a KBase workflow with 8 base tasks, 5 of which are data parallel. The workflow has been tested and deployed successfully, passing all required KBase tests. The workflow can now be executed by any KBase developer.

Publications

Upton RN, Bach EM, Hofmockel KS 2019 Spatio-temporal microbial community dynamics within soil aggregates Soil Biology and Biochemistry, May 1;132:58-68. https://doi.org/10.1016/j.soilbio.2019.01.016

Choi J, Bach EM, Lee J, Flater J, Dooley S, Howe A, Hofmockel KS. 2018 Spatial structuring of cellulase gene abundance and activity in soil. Frontiers in Environmental Science 6:107. https://doi.org/10.3389/fenvs.2018.00107

Upton RN, Bach EM, Hofmockel KS 2018. Belowground response of prairie restoration and resiliency to drought. Agriculture, Ecosystems and the Environment 266: 122-132. https://doi.org/10.1016/j.agee.2018.07.021

Bach EM, Narvaez-Rivera G, Murray K, Bauer J, Hofmockel KS, 2018. The dynamic life of arbuscular mycorrhizal fungal symbionts Ecology. doi:10.1002/ecy.2096

Bach EM, Williams RJ, Hargreaves SK, Yang F, Hofmockel KS, 2018 Greatest soil microbial diversity found in micro-habitats. Soil Biology and Biochemistry. 118:217-26. https://doi.org/10.1016/j.soilbio.2017.12.018

Choi J, Yang F, Stepanauskas R, Cardenas E, Garoutte A, Williams R, Flater J, Tiedje JM, Hofmockel KS, Gelder B, Howe A. 2017 Strategies to improve reference databases for soil microbiomes. The ISME journal. Apr;11(4):829. https://doi.org/10.1038/ismej.2016.168

Howe A, Williams RJ, Yang F, Meyer F, Hofmockel KS, 2016 Characterization of core carbon cycling genes in fertilized prairie soils. Plos One.

Bach E, Hofmockel KS, 2016. A time for every season: Soil aggregate turnover stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy. GCB Bioenergy doi: 10.1111/gcbb.12267. 8(3):588-99. https://doi.org/10.1371/journal.pone.0166578

Bach EM and Hofmockel KS 2015. Coupled Carbon and Nitrogen inputs increase microbial biomass and activity in prairie bioenergy systems. Ecosystems 1-15. doi: 10.1007/s10021-014-9835-8

Bach EM, Hofmockel KS 2014 Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biology & Biochemistry 69: 54-62. https://doi.org/10.1016/j.soilbio.2013.10.033

Williams RJ, Howe AC, Hofmockel KS 2014. Detecting microbial co-occurrence patterns within and between ecosystems. Frontiers in Microbiology 5. https://doi.org/10.3389/fmicb.2014.00358

Presentations

2019 RN Upton, EM Bach, KS Hofmockel. Conversion from row-crop agriculture to restored native Iowan prairies benefits the microbial community and increases drought resiliency. Invited Talk – Ecological Society of America, Special Section: The Role of Soil Microorganisms for Improving the Efficacy of Ecological Restoration, August 15th, 2019, Louisville, Kentucky.

2018 EM Bach, G Rivera-Narveaz, K Murray, JT Bauer, KS Hofmockel, Arbuscular mycorrhizal fungal investment in vesicles and hyphae in perennial prairie plants. Annual Meeting of the Ecological Society of America, Aug. 5-10, New Orleans, LA

2018 RN Upton, and KS Hofmockel. Ecosystem and aggregate scale drivers of soil microbial diversity and function. Invited talk at Montana State University, November 8th, 2018, Bozeman, MT.

2018 J. Choi, “Exploring microbe in the soil, RefSoil: a genome soil reference database”, Colloquium seminar at Department of Computer Science and MS Program of Bioinformatics and Computational Biology at Saint Louis University. St. Louise, MO.

2018 J. Choi, “The dirt on soil microbiomes?”, High Throughput Field-Based Phenotyping Technologies for the Genomes to Fields Initiative, Ames IA

2017 RN Upton, EM Bach, KS Hofmockel. Increased resiliency and activity of microbial mediated carbon cycling enzymes in diversified bioenergy cropping systems. American Geophysical Union, December 11-15, 2017, New Orleans, LA.

2017 RN Upton, and KS Hofmockel A scaling approach to determine the drivers of complex soil microbial communities. Center for Biofilm Engineering Invited talk at Montana State University, March 9, 2017, Bozeman, MT.

2017 KS Hofmockel Microbial ecology of soil carbon cycling in biofuel ecosystems, Microbiology and Molecular Genetics, October 9, Michigan State University.

2017 KS Hofmockel Plant-Microbe-Mineral Interactions, EMSL 20th Anniversary Celebration August 4, Richland, WA.

2017 RN Upton, KS Hofmockel, ME Jarchow, Leguminous plants drive fungal community structure in Iowan prairies Ecological Society of America, August 10, Portland, OR.

2017 RN Upton, KS Hofmockel Soil fungi response to seasonal and annual variance in three agroecosystems. Soil Ecology Society Conference, Fort Collins, CO, June 7.

2017 J Choi, EM Bach, J Flater, S Dooley, J Lee, KS Hofmockel, AC Howe, Microbial drivers of cellulose decomposition in agricultural soils: Linking genes to carbon metabolism. Argonne Soil Metagenomics Meeting, Nov. 1-3, Lemont, IL

2016 J Choi, F Yang, R Stepanauskas, . Cardenas, A Garoutte, R Williams, J Flater, JM Tiedje, KS Hofmockel, B Gelder, A Howe, RefSoil: A reference database of soil microbial genomes, Samuel Roberts Noble Foundation, Ardmore, OK

2016 KS Hofmockel Plant-soil-microbe interactions regulating soil C storage, AGU, SanFrancisco, CA, December 14 J. Choi, F. Yang, R. Stepanauskas, E. Cardenas, A. Garoutte, R. Williams, J. Flater, J. M. Tiedje, K. Hofmockel, B. Gelder, A. Howe. “Exploring microbes in the soil: from biodiversity to carbon cycliing”, Pacific Northwest National Laboratory, Richland, WA. (2016).

2016 KS Hofmockel Molecular understanding of cellulose cycling in soil, École Centrale Lyon Environmental Microbial Genomics Group, Université de Lyon, France, December 5.

2016 KS Hofmockel From atoms to ecosystems: A multi-scale approach to carbon cycling science, SLAC National Acceleration Laboratory, Menlo Park, CA, August 25.

2016 KS Hofmockel Microbial drivers of cellulose cycling in biofuel crop soils, JGI User meeting, San Francisco, CA, March 23.

2016 KS Hofmockel Scaling microbial ecology to soil biogeochemistry. Stanford School of Earth, Energy & Environment, Stanford, CA, April 20.

2015 KS Hofmockel Microbial cycling of carbon in biofuel agroecosystems. Environmental Molecular Sciences Laboratory, PNNL, Richland, WA October 27.

2015 KS Hofmockel Multi-scale Approach to Terrestrial Carbon Cycling Science. Environmental Molecular Sciences Laboratory, PNNL, Richland, WA May 6.

2015 KS Hofmockel. Metagenomic Approaches for Soil Carbon Cycling. Pittcon, New Orleans, LA, March 12.

2015 EM Bach, RJ Williams, KS Hofmockel, Soil fungal community composition and ecosystem functioning in restored tallgrass prairies managed for bioenergy. Midwest Great Lakes Regional Chapter of the Society for Ecological Restoration, March 27-29, Chicago, IL

2015 R Williams, KS Hofmockel, A Howe. Elucidating microbial drivers of decomposition and the carbon cycle through a co-occurrence framework. Prague, Czech Rebpublic, November 29.

2014 RJ Williams, Adina Howe, KS Hofmockel Mining Soil Aggregate Metagenomes for Ecologically Relevant Extracellular Enzymes. Argonne Soil Metagenomics Meeting, Oct 2, Chicago IL.

2014 EM Bach and KS Hofmockel, Soil structure affects seasonal microbial activity in restored grassland agroecosystems. Soil’s Role in Restoring Ecosystem Services, March 6-9, Sacramento, CA

2014 KS Hofmockel, RJ Williams, AC Howe. Carbon availability structures microbial community composition and function in soil aggregate fractions. American Geophysical Union, San Francisco CA, December 16.

2014 KS Hofmockel. Ecosystem C-N Cycling CSU Crop & Soil Science Department, Fort Collins, CO, September 4.

2014 KS Hofmockel, RJ Williams, EM Bach, AC Howe. Microbial community composition and function in soil aggregates from a fertilized prairie. Ecological Society of America, 99th Annual Meeting Sacramento, CA Aug 10-15.

2014 A Howe. Is bigger better – advice as metagenomes grow. International Society of Microbial Ecology General Meeting 15 (Invited roundtable workshop organizer) Seoul, Korea. August 27.

2014 RJ Williams, A Howe, KS Hofmockel. Mining Soil Aggregate Metagenomes for Ecologically Relevant Extracellular Enzymes. Argonne Soil Metagenomics Meeting, October 2, Chicago IL.

2013 Bach EM, Hofmockel, KS Ecosystem functioning in restored tallgrass prairie affected by fertilizer addition. 5th World Conference on Ecological Restoration. Oct. 7-11. Madison, WI.

2013 KS Hofmockel, A Chuang Howe, R Williams, F Yang, E Bach, S Hargreaves, K Keegan, F Meyers. Linking microbial metagenomes to biogeochemical cycles in biofuel cropping systems. Ecological Society of America, 98th Annual Meeting Minneapolis, MN.

2013 RJ Williams, KS Hofmockel, F Yang, SK Hargreaves, EM Bach, AC Howe, K Keegan, F Meyer Soil structure and agricultural management drives niche differentiation among soil microbial communities Ecological Society of America, 98th Annual Meeting Minneapolis, MN.

2013 EM Bach, KS Hofmockel. Biological, physical, and temporal constraints on extracellular enzyme activity in prairie and corn bioenergy systems. Ecological Society of America, 98th Annual Meeting Minneapolis, MN.

Posters

J. Choi, F. Yang, R. Stepanauskas, E. Cardenas, A. Garoutte, R. Williams, J. Flater, J. M. Tiedje, K. Hofmockel, B. Gelder, A. Howe. "RefSoil: A reference database of soil microbial genomes", International Society for Microbial Ecology (ISME), Montreal, Canada (2016).

2015 A Wolf, KS Hofmockel, Agro-ecosystem type and soil aggregate size impact soil carbon dynamics. Ecology of Soil Microorganisms. Prague, Czech Republic, November 29.

2015 KS Hofmockel, RJ Williams, A Howe. Key microbial decomposition pathways in soils from bioenergy agroecosystems. Genomic Science Contractors Grantees Meeting XIII XIII/USDA-DOE Plant Feedstock Genomics for Bioenergy Meeting, Tysons, VA February 23.

2014 A Wolf, A Howe, KS Hofmockel. Microbial cellulose utilization at the aggregate scale. Argonne Soil Metagenomic Meeting, Chicago, IL Oct 2.

2013 RJ Williams, KS Hofmockel, F Yang, S Hargreaves, E Bach, AC Howe, K Keegen, F Meyer. Comparison of Soil Microbial Communities Under Different Bioenergy Crops Suggests Sustainable Options. Genomic Science Contractors-Grantees Meeting XI USDA-DOE Plant Feedstock Genomics for Bioenergy Awardee Meeting, Bethesda, MD 25 February.

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