U.S. Department of Energy: Final Technical Report

Title of Project:	Documenting the function of non-cultivated microorganisms in terrestrial ecosystems
Grant Number:	DE-SC0010558
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ABSTRACT:

The terrestrial biosphere contains a large fraction of global carbon and nearly 70% of the organic carbon in these systems is found in soils. Global changes in atmospheric CO_2, temperature, precipitation, and ecosystem N inputs, are expected to impact primary production and carbon inputs to soils, but it remains difficult to predict the response of soil processes to anthropogenic change. Models that predict soil C-cycling as a function of ecosystem properties do not explain well variation in soil processes. Our difficulty in predicting the response of soil processes to environmental change suggests a need for a greater understanding of the biotic mechanisms that govern the soil C-cycle. Changes in microbial community structure and function have been proposed to impact soil C-cycling. However, our ability to predict the impacts of these changes on terrestrial ecosystems is constrained by our limited understanding of mechanisms that drive microbial processes in soil systems.

We have applied a newly developed approach of Microbial Food Web Mapping to chart the carbon assimilation dynamics of microbial taxa in soils. This method made it possible to track different classes of ^{13}C-labeled substrates into the DNA of nearly every member of the soil community simultaneously over time. Microbial Food Web Mapping was used to identify and characterize microbial taxa that fill key roles in the soil carbon cycle. We mapped the assimilation of carbon into thousands of discrete soil microorganisms as a function of soil carbon content and pH. In so doing we characterized assimilatory carbon metabolism, an essential component of soil C-cycling, for a vast array of uncultivated soil microorganisms. Further, we characterized the spatial and temporal dynamics of these microorganisms across a series of sites representing variation in soil characteristics. This project revealed fundamental aspects of soil C-cycling and provided ecological and metabolic insights on diverse uncultivated soil organisms that are widespread and play major roles in the global C-cycle.

The genetic capacity of microbial communities can be studied through ‘omic approaches but it remains difficult to make direct links between the genetic capacity of microorganisms and their

function in the soil C-cycle. Microbial Food Web Mapping makes it possible to link gene sequences to soil C-cycle processes as they occur in soil. This approach allows us to characterize the activity of non-cultivated microorganisms in a range of terrestrial systems. This data will be used to build a base of information about the role of non-cultivated organisms in critical C-cycle processes in terrestrial ecosystems and will provide insight on the manner in which soil communities metabolize soil organic matter. The results generated by this project will improve our ability to examine the impacts of management decisions, soil history, and environmental change on the behavior of microbial communities in terrestrial ecosystems, revealing the ecological mechanisms by which microbes regulate both C mineralization and carbon retention in soils, and improving our ability to predict changes in terrestrial ecosystem processes in the face of accelerating global change.

PROJECT REPORT:

The major objectives of this project were to further develop and apply DNA stable isotope probing (DNA-SIP) approaches for characterizing the activities of non-cultivated microorganisms in soil microbial food webs. A series of approaches for analyzing DNA-SIP data were developed and validated and then these tools were applied to soils that differ in management history. We sought to characterize the ecological characteristics of microbes that perform major carbon transformations in soils. We have shown that life history traits explain significant variation in microbial processes and are more predictive of microbial activity than genomic potential or phylogeny. We also demonstrate how DNA-SIP can be applied to reveal intricate details of food web structure in soils.

Development of HTS-SIP for analysis of DNA-SIP data

We have shown that the combination of high throughput sequencing with stable isotope probing (HTS-SIP) is a powerful method for mapping *in situ* metabolic processes to thousands of microbial taxa. However, accurate mapping of metabolic processes to taxa is complex. Multiple HTS-SIP data analysis methods have been developed, including high-resolution stable isotope probing (HR-SIP), multi-window high-resolution stable isotope probing (MW-HR-SIP), quantitative stable isotope probing (q-SIP), and ΔBD. Currently, the computational tools to perform these analyses are either not publically available or lack documentation, testing, and developer support. To address this shortfall, we developed the *HTSSIP* R package, a toolset for conducting HTS-SIP analyses in a straightforward and easily reproducible manner.

Validation of DNA-SIP analytical methods

There are several different methods for analyzing DNA-SIP data and despite the power of SIP experiments, it remains difficult to comprehensively evaluate method accuracy across a wide range of experimental parameters. We developed a toolset (SIPSim) that simulates DNA-SIP data, and we use this toolset to systematically evaluate different methods for analyzing DNA-SIP data. Specifically, we employed SIPSim to evaluate the effects that key experimental parameters (*e.g.* level of isotopic enrichment, number of labeled taxa, relative abundance of labeled taxa, community richness, community evenness, and beta-diversity) have on the specificity, sensitivity, and balanced accuracy (defined as the product of specificity and sensitivity) of DNA-

SIP analyses. Furthermore, SIPSim can predict analytical accuracy and power as a function of experimental design and community characteristics, and thus should be of great use in the design and interpretation of DNA-SIP experiments.

Characterization of life history traits associated with soil carbon cycling

We explored microbial contributions to decomposition using a sophisticated approach to DNA Stable Isotope Probing (SIP). Our experiment evaluated the dynamics and ecological characteristics of functionally defined microbial groups that metabolize labile and structural C in soils. We added to soil a complex amendment representing plant derived organic matter substituted with either ^{13}C-xylose or ^{13}C-cellulose to represent labile and structural C pools derived from abundant components of plant biomass. We found evidence for ^{13}C-incorporation into DNA from ^{13}C-xylose and ^{13}C-cellulose in 49 and 63 operational taxonomic units (OTUs), respectively. The types of microorganisms that assimilated ^{13}C in the ^{13}C-xylose treatment changed over time being predominantly *Firmicutes* at day 1 followed by *Bacteroidetes* at day 3 and then *Actinobacteria* at day 7. These ^{13}C-labeling dynamics suggest labile C traveled through different trophic levels. In contrast, microorganisms generally metabolized cellulose-C after 14 days and did not change to the same extent in phylogenetic composition over time. Microorganisms that metabolized cellulose-C belonged to poorly characterized but cosmopolitan soil lineages including *Verrucomicrobia*, *Chloroflexi* and *Planctomycetes*.

Metagenomic-SIP reveals microbial traits that govern the cellulose economy of soil

Microbial decomposition of cellulose is an extra-cellular process that creates an exploitable resource by opportunists. We hypothesized that fitness tradeoffs between competent cellulose-degraders and opportunists structure the ecological and physiological traits of cellulolytic soil consortia. Using high-resolution stable isotope probing with ^{13}C-cellulose and deep shotgun metagenomic sequencing, we contrasted genomic features of cellulolytic and non-cellulolytic taxa enriched in ^{13}C from agricultural soil. The most highly ^{13}C-enriched taxa were cellulolytic *Cellvibrio,* which exhibited a strategy of self-sufficiency (prototrophy), rapid growth and competitive exclusion via the production of bacteriocins abundant in the accompanying metaproteome. Cellulolytic taxa were likely to possess adaptations for surface colonization, revealing a common strategy among root-colonizing and cellulose-degrading members of *Proteobacteria*. The community of bacteria and fungi observed were generally associated with agricultural soils, while several endoglucanase genes were associated more broadly with plant matter degradation and traits of specific taxonomic groups. These results highlight characteristics of cellulose-degrading consortia which exemplify the selection pressures shaping populations of soil decomposers.

Characterization of fungi that mediate cellulose degradation

Fungi are essential to soil carbon cycling due to their propensity for decomposing organic polymers such as cellulose. We performed high throughput sequencing enabled stable isotope probing (HTS-SIP) with ^{13}C-cellulose to characterize the dynamics of fungi and bacteria during cellulose degradation in an agricultural soil. A total of 1900 fungal taxa were observed and 190 of these assimilated ^{13}C-cellulose during a 30-day incubation. A majority of ^{13}C-labeled fungi belonged to Ascomycota, Basidiomycota, and Mucoromycota. However, most ^{13}C-labeled fungi could not be annotated at the species (71%, n =134), or genus (49%, n = 93) level. Mucoromycota were ^{13}C-labeled early, and by day 3 the most abundant ^{13}C-labeled organism

belonged to Mortierella. In contrast, ^{13}C-labeled Ascomycota increased in diversity through day 14 and their relative abundance comprised more than 40% of fungal ITS sequences by day 30. These results show that: *i*) the majority of fungal taxa that assimilated ^{13}C from ^{13}C-cellulose are uncultivated and poorly characterized, *ii*) the beta-diversity of ^{13}C-labeled fungi changed dramatically over time during decomposition, *iii*) a relatively small number of the ^{13}C-labeled taxa were highly abundant, and increased in relative abundance over time, and *iv*) fungi responded to cellulose more rapidly and in greater numbers than did bacteria.

Characterizing microbial food web structure across different management regimes

Physical disturbance of soil causes loss of soil carbon. It remains unclear whether changes in microbial community structure and function that accompany the physical disturbance of soils contribute to the loss of soil carbon stocks. We employed high resolution DNA stable isotope probing (HR-SIP) to evaluate the temporal dynamics of microbial metabolism associated with the degradation of dissolved (^{13}C-xylose) and particulate (^{13}C-cellulose) forms of carbon in soils from a long-term tillage experiment. In addition, we used high throughput sequencing of 16S rRNA gene amplicons to assess seasonal variation in taxon relative abundance in relation to tillage history and pattern of isotope incorporation in HR-SIP experiments. Bacterial communities vary significantly with tillage as expected (PERMANOVA, $R^2 = 0.14$, $p = 0.001$). No-till soils also had significantly higher rates of soil respiration and ^{13}C-xylose mineralization, but not ^{13}C-cellulose mineralization relative to tilled soil. The bacteria that incorporated ^{13}C xylose initially (days 1 and 3) differed in tilled vs. no-till soils, though similar taxa were ultimately enriched in both soil types over time. In contrast, the bacteria that incorporated ^{13}C cellulose remained similar between tilled and no-till soils throughout the experiment. The taxa participating in carbon transformations differed as a function of soil management history, with implications for carbon fate. These results suggest that changes in the structure of the microbial community caused by tillage affects xylose degradation but not cellulose degradation. We hypothesize that this difference is associated with niche partitioning between dissolved and particulate substrates.

Microbial ecological traits that drive carbon cycling are linked to substrate bioaccessibility

We performed a HR-SIP experiment with nine ^{13}C-labeled substrates representing organic matter present during plant biomass degradation (cellulose, xylose, glucose, glycerol, vanillin, palmitic acid, amino acids, lactate, and oxalate). We identified >1200 OTUs that incorporated one or more substrates, and many of these "incorporators" had no closely related isolate. The dynamics of substrate incorporation and CO_2 production both corresponded to substrate bioaccessiblity, and progressed from high to low bioaccessiblity. Taxonomic relatedness only weakly predicted OTU incorporation dynamics, suggesting high functional redundancy. However, we found that incorporation dynamics could be used to organize OTUs into discrete response groups (RGs), and these RGs differed in traits associated with a more copiotrophic or oligotrophic ecological strategy (e.g., *rrn* copy numbers). These findings suggest substrate bioaccessibility and the copiotrophic-oligotrophic niche axis strongly dictate bacterial community structure and C cycling dynamics. To extrapolate beyond our HR-SIP experiment, we mapped RGs to a pyrogenic organic matter (PyOM) and stover amendment experiment of Whitman and colleagues, and we found that RGs had a more consistent response to C amendments than taxonomic groupings. These findings help to resolve the linkages between bacterial community

structure and soil carbon cycling, which is needed for developing more accurate microbially-explicit biogeochemical models.

PRODUCTS DELIVERED:

Journal Articles (In Preperation)

1. Wilhelm, R.C., Pepe-Ranney, C., Weisenhorn, P., Lipton, M., Buckley, D.H. (2018) Competitive exclusion and metabolic dependency structure the cellulose economy in agricultural soil. *In prep*.
2. Koechli, C., Campbell, A. N., Berthrong, S. T., Pepe-Ranney, C., **Buckley, D. H.** (2018) Metabolic states of cellulose degrading bacteria in soil revealed by comparing results from RNA-SIP and DNA-SIP experiments. *In prep.*
3. Berthrong, S. T., **Buckley**, **D. H.,** Drinkwater, L. E. (2018) Long term changes in organic matter content drive microbial community composition and spatial structure in soils. *In Prep.*
4. Koechli, C., Youngblut, N. Pepe-Ranney, C., **Buckley, D. H.** (2018) High resolution DNA stable isotope probing reveals bacterial contributions to carbon cycling vary with respect to land management. *In prep.*
5. Youngblut, N., Koechli, C., **Buckley, D. H.** (2018) Soil microbial food web mapping with high resolution stable isotope probing. *In prep.*

Journal Articles (Published and submitted)

6. Koechli, C., Campbell, A. N., Berthrong, S. T., Pepe-Ranney, C., **Buckley, D. H.** (2018) DNA-SIP reveals the majority of cellulolytic *Fungi* in soils remain uncharacterized. Soil Biology and Biochemistry. *In Review.*
7. Sirois, S. H. and **Buckley, D. H.** (2018) Factors governing extracellular DNA (eDNA) degradation dynamics in soil. *Environmental Microbiology Reports*. *Accepted*.
8. Youngblut, N., Barnett, S. E., **Buckley, D. H.** (2018) HTSSIP: an R package for analysis of high throughput sequencing data from nucleic acid stable isotope probing (SIP) experiments. *PlosOne*. **13**:e0189616. doi.org/10.1371/journal.pone.0189616
9. Youngblut, N., **Buckley, D. H.** (2018) SIPSim: A modeling toolkit to predict accuracy and aid design of DNA-SIP experiments. *Frontiers in Microbiology*. **9**:570. doi.org/10.3389/fmicb.2018.00570
10. Whitman, T., Pepe-Ranney C., Enders, A., Koechli, C., Campbell, A. N., **Buckley, D. H.,** Lehmann, J. (2016) Pyrogenic organic matter, soil organic matter, and plant root interactions determined using three-part partitioning with stable C isotopes and microbial community analysis. *International Society for Microbial Ecology Journal.* **10**:2918-2930. doi: 10.1038/ismej.2016.68
11. Pepe-Ranney, C., Campbell, A. N., Koechli, C., Berthrong, S. T., **Buckley, D. H.** (2016) Unearthing the ecology of soil microorganisms using a high resolution DNA-SIP approach to explore cellulose and xylose metabolism in soil. *Frontiers in Microbiology.* **7**:703. doi: 10.3389/fmicb.2016.00703

12. Youngblut, N. D. and **Buckley, D. H.** (2014) Intra-genomic variation in G+C content and its implications for DNA Stable Isotope Probing (DNA-SIP). *Environmental Microbiology Reports*. doi: 10.1111/1758-2229.12201
13. Pepe-Ranney, C., Koechli, C., Potrafka, R., Andam, C., Eggleston, E., Garcia-Pichel, F., **Buckley, D.H.** (2015) Non-cyanobacterial diazotrophs dominate dinitrogen fixation in biological soil crusts at the early stage of crust formation. *International Society for Microbial Ecology Journal*. 10:287–298. doi:10.1038/ismej.2015.106

Abstracts

1. Koechli, C., Youngblut, N. D., **Buckley, D. H.** (2017) High resolution DNA stable isotope probing of soil indicates changes in microbial community metabolism associated in disturbance due to tillage. Presented at the Principle Investigators Meeting of the DOE Genomic Sciences Program.
2. Youngblut, N. D., Koechli, C., **Buckley, D. H.** (2016) Mapping microbial food web dynamics in soil with high resolution stable isotope probing. Published in the Proceedings of the 16th International Symposium on Microbial Ecology.
3. Koechli, C., Youngblut, N. D., Pepe-Ranney, C., **Buckley, D. H.** (2016) High Resolution DNA Stable Isotope Probing of soil indicates changes in microbial community metabolism associated in disturbance due to tillage. Published in the Proceedings of the 16th International Symposium on Microbial Ecology.
4. Pepe-Ranny, C., Campbell, A. N., **Buckley, D. H.** (2016) DNA-SIP enabled community genomics of cellulose degraders in an agricultural soil. Presented at the Principle Investigators Meeting of the DOE Genomic Sciences Program.
5. Koechli, C., Youngblut, N. D., **Buckley, D. H.** (2016) Use of high resolution DNA stable isotope probing to elucidate the role of bacteria in carbon cycling in soils under differing land management. Presented at the Principle Investigators Meeting of the DOE Genomic Sciences Program.
6. Youngblut, N. D., Koechli, C., **Buckley, D. H.** (2016) Soil microbial food web mapping with high resolution stable isotope probing. Presented at the Principle Investigators Meeting of the DOE Genomic Sciences Program.
7. Youngblut, N. D., Koechli, C. , Barnett, S. , and **Buckley, D. H.** (2017) Mapping microbial food web dynamics in soil with high resolution stable isotope probing. Presented at the Principle Investigators Meeting of the DOE Genomic Sciences Program.
8. Pepe-Ranny, C., Campbell, A. N., **Buckley, D. H.** (2015) Targeting unknowns just underfoot: Microbial ecology and community genomics of C-cycling in soil informed and enabled with DNA-SIP. Presented at the Fall Meeting of the American Geophysical Union.
9. Campbell, A. N., Pepe-Ranney, C., Berthrong, S. T., Koechli, C., **Buckley, D. H.** (2015) High resolution DNA stable isotope probing reveals that root exudate addition to soil changes the identity of the microbes that degrade cellulose but not the rate of degradation. Presented at the Fall Meeting of the American Geophysical Union.
10. Pepe-Ranny, C., Campbell, A. N., **Buckley, D. H.** (2015) Unearthing the microbial ecology of soil carbon cycling with DNA-SIP. Presented at the Applied and Environmental Microbiology Gordon Research Conference.
11. Koechli, C., Youngblut, N. D., Pepe-Ranney, C., **Buckley, D. H.** (2015) Use of high resolution DNA Stable Isotope Probing to elucidate the role of *Bacteria* in carbon cycling in

soils under differing land management. Published in the Proceedings of the 116th General Meeting of the American Society of Microbiology.

12. Youngblut, N. D., Koechli, C., **Buckley, D. H.** (2015) Soil microbial food web mapping with high resolution stable isotope probing. Published in the Proceedings of the 116th General Meeting of the American Society of Microbiology.
13. Pepe-Ranney, C., Koechli, C., Potrafka, R., Andam, C., Eggleston, E., Garcia-Pichel, F., **Buckley, D.H.** (2015) Non-cyanobacterial diazotrophs dominate dinitrogen fixation in biological soil crusts at the early stage of crust formation. Published in the Proceedings of the 116th General Meeting of the American Society of Microbiology.
14. Whitman, T, Pepe-Raney, C., **Buckley, D. H.**, Lehmann, C.J. (2014) Pyrogenic and Fresh Organic Matter Effects on Soil Microbial Communities. Presented at the 2014 Fall Meeting of the American Geophysical Union.
15. Campbell, A.N., Pepe-Ranney, C., Berthrong, S.T., Koechli, C., **Buckley, D. H.** (2014) Tracking the temporal dynamics of cellulose utilization by bacteria and fungi in soil using ^{13}C-stable isotope probing and next generation sequencing. Published in the Proceedings of the 115th General Meeting of the American Society of Microbiology.
16. Pepe-Ranney, C., Campbell, A.N., Koechli, C., **Buckley, D.H.** (2014) Metagenomic analysis of soil microorganisms that assimilate 13C from 13C-cellulose provides evidence for the activity of non-cultivated *Chloroflexi* found widely in soils. Published in the Proceedings of the 115th General Meeting of the American Society of Microbiology.
17. Koechli, C., Campbell, A.N., Pepe-Ranney, C., Berthrong, S.T., Buckley, D.H. (2014) Mapping the flow of ^{13}C-cellulose through RNA and DNA of bacterial communities in an agricultural soil. Published in the Proceedings of the 115th General Meeting of the American Society of Microbiology.

Presentations

1. *Invited Keynote Speaker*, Environmental Chemistry and Microbiology Student Symposium, Pennsylvania State University, PA, April 14, 2018
2. *Invited Speaker*, Department of Ecology, Evolutionary Biology, and Behavior, Michigan State University, MI, February 29, 2018
3. *Invited Keynote Speaker*, Soil Metagenomics Meeting, Argonne National Laboratory, IL, November 2, 2017
4. *Invited Distinguished Speaker*, MBL Semester in Environmental Science Program, Woods Hole, MA, Oct. 20, 2017
5. *Contributed Seminar*, Microbiology Symposium at Cornell, Ithaca, NY, October 9, 2017
6. *Invited Speaker*, Department of Microbiology, UMass, Amherst, MA, September 19, 2017
7. *Invited Speaker*, Multi-Omics for Microbiomes Conference, Pasco, WA, August 3, 2017
8. *Invited Speaker - IdeasLab,* World Economic Forum Annual Meeting, Davos, Switzerland, January 18, 2017
9. *Invited Speaker*, School of Integrative Plant Sciences Annual Research Symposium, Cornell University, Ithaca, NY, October 13, 2015
10. *Invited Distinguished Speaker*, Pacific Northwest National Laboratory Biological Sciences Division, Richland, WA, September 3, 2015
11. *Invited Distinguished Speaker*, Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, April 17, 2015

12. *Invited Keynote Speaker*, USDA-DOE Genomic Science Program Meeting, Tysons Corner, VA, Feb 24, 2015
13. *Invited Speaker*, Catskill Environmental Research and Monitoring conference, Highmount, NY, October 23, 2014
14. *Invited Speaker*, Cross-Scale Biogeochemistry and Climate Seminar Series, Cornell University, Ithaca, NY, March 14, 2014