4/1/19 FINAL Report for Award #: DE-SC0010580

Title of Project: Pathways to Carbon Liberation: A Systems Approach to Understanding Carbon Transformations and Losses from Thawing Permafrost
Lead Institution: University of Arizona
Sponsoring program office: Office of Science, Biological & Environmental Research Division
Program Manager: Joe Graber / Dawn Adin
Project Period: 09/01/2013 - 08/31/2018
Co-lead PIs: Scott Saleska, Co-lead PI: Virginia Rich
Co-Investigators:

- **Gene W. Tyson, University of Queensland, Australia (Molecular microbial ecology)**
- **Matthew Sullivan, University of Arizona (Phage ecology & evolution)**
- **Jeffrey Chanton, Florida State University (Biogeochemistry)**
- **Patrick Crill, University of Stockholm, Sweden (Biogeochemistry)**
- **Steven Frolking, University of New Hampshire (Modeling)**
- **Changsheng Li, University of New Hampshire (Modeling)**
- **Ruth Varner, University of New Hampshire (Aquatic Biogeochemistry)**
- **Greg Hurst, Oak Ridge National Laboratory (Metaproteomics)**
- **Nathan Verberkmoes, New England BioLabs (Metaproteomics)**

Abstract:

Our ***objective*** *in this project was to discover how microbial communities mediate the fate of carbon in thawing permafrost under climate change.* We proposed a systems approach integrating ***(a)*** **molecular microbial and viral ecology**, ***(b)*** **molecular organic chemistry and stable and radiocarbon isotopes, and *(c)* state-of-the-art modeling, along a chronosequence of permafrost thaw in subarctic Sweden.**

The fate of carbon (C) in thawing permafrost is an outstanding challenge of modern biogeochemistry and climate change. Permafrost C pools are large (~1700 PgC), and C dynamics of thawing permafrost complex: old C decomposes as it is liberated from thawing permafrost as CO_2 or CH_4 , with a significant fraction cycling through lake sediments, even as new C accumulates due to thaw-initiated ecological succession. Our work is allowing better prediction of the net effect of these processes.

Microbes mediate C dynamics in thawing permafrost, but a mechanistic understanding of how to scale microbial population dynamics, genomic potential, and expression to ecosystem-scale processes has been missing. A key question was: What is the interplay of microbial communities and organic matter chemical structure in the decomposition/preservation of organic C across a thaw gradient? And intriguingly, what (if any) is the role of phage (viruses that infect prokaryotic cells) in mediating these processes? Viruses appear to play a large role in driving oceanic function, but these phenomena are virtually unstudied in terrestrial systems. **This endeavor linking microbial and viral dynamics, organic geochemistry and trace gas**

production will improve models of C cycling in permafrost systems, and clarify the fate of C under future climates.

Technical Approach – We conducted a study at Stordalen Mire, Sweden, along a permafrost thaw chronosequence encompassing permafrost palsas and their initial collapse stage, thawing bog sites (dominated by *Sphagnum* moss), fully-thawed and inundated fen sites (dominated by *Eriophorum spp* or *Carex* spp.), and lakes.

Our project used cutting edge technologies in both biogeochemistry and molecular microbial ecology to advance systems biology research on microbial carbon cycling through: **(a)** systems-level mapping of chemical states and ages of organic matter (via FT-ICR-MS and $_{14}$C analysis) along thaw gradients to associated microbial communities, biochemical potentials, and activities (via meta-genomics, -transcriptomics, –proteomics, and viral genomics), and to CO_2 and CH_4 fluxes; **(b)** experimental incubations using Quantum Dot Probing, to test key hypotheses, arising from (a), about particular microbially-driven biochemical degradation pathways; **(c)** bioinformatics designed to simultaneously enhance the **DOE Knowledgebase,** and **(d)** integrated ecosystem C-cycle modeling testable by soil organic chemical and microbial data.

Results from this project spanned 4 categories: (i) integrative across disciplines, and disciplinarily-focused in (ii) microbiology, (iii) biogeochemistry, and (iv) modeling. In integrated understanding of the system, we answered the questions posed above, mapping resident microbes to carbon transformations to reveal a carbon-processing shift with permafrost thaw, and discovering abundant, diverse and habitat-specific viruses that appear to be impacting carbon cycling, while also relating microbial communities to modeled processes. In microbiology-specific papers, we dug into viromes, microbial community networks, and informatic methods, while also introducing a new permafrost-associated phylum and its ecogenomic context.

Relevance to DOE FOA - With a focus on discovering how microbial communities mediate the fate of old and new carbon in permafrost systems with climate change, our work directly responds to the call for *"–omics driven basic research on the contribution of... microbial communities to C cycling processes in terrestrial ecosystems."* We bridge all three of the focal areas of the call, including (i) "*Systems biology studies"*, and development of –omics approaches *(ii) "to investigate microbial community functional processes",* and (iii) "*for imaging and analysis of microbially-mediated carbon cycling."*

Summary:

This project integrates across three fields (microbiology, biogeochemistry, and modeling) to understand the mechanisms of methane cycling in thawing permafrost. Permafrost contains roughly a half of the soil carbon on the planet, and roughly twice as much carbon as is currently in the atmosphere. As this carbon thaws, its potential release to the atmosphere would be a powerful accelerant to climate change. It can be emitted as carbon dioxide, or the more potent greenhouse gas methane (approximately 33 times as

strong as carbon dioxide over a hundred years). We have studied how microbes, plants, soil organic matter, and these carbon gas emissions change as permafrost thaws “naturally” (without us warming it) in an Arctic wetland called Stordalen Mire. To do this, we’ve used cutting-edge methods in microbiology and biogeochemistry, allowing much higher resolution (temporal, phylogenetic, and structural) measurements, which we have worked with several model platforms to distill into predictive relevance for charting how these systems will respond over time. We have made substantial progress in each disciplinary area, and in cross-cutting interdisciplinary syntheses overall, as manifest in peer-reviewed publications and in presentations to the scientific community. Our specific findings include: (i) recovery of metagenome-assembled genomes for 60% of the cells in this thawing ecosystem, their linkage to stages of carbon processing such as cellulose degradation, and analysis of how community processing changes with permafrost thaw; (ii) recovery of viral genomes from the site, which were so novel that they doubled the number of viral genera known in the world, and which could be linked to carbon cycling at the site in several ways; (iii) detailed analyses of the soil organic matter to examine how chemical structures and energetic potential shifts with permafrost thaw, in the process upgrading the ecological importance of several previously-underappreciated chemical transformations in these bog and fen habitats; and (iv) linking these observations and discoveries to process models for the site to improve their accuracy.

Introduction:

A fundamental challenge of modern biology is to understand how information encoded in the genes of organisms translates into physiological and biogeochemical processes manifested at ecosystem to global scales (*DOE, 2008*). A parallel challenge of earth sciences is to understand how earth systems regulate climate change (*IPCC, 2007*). These grand challenges intersect in the global carbon (C) cycle, which is both a key driver of climate and mediated by biological processes such as microbial degradation and transformation of soil organic matter (SOM) into the greenhouse gases carbon dioxide (CO_2) and methane (CH_4).

A key aspect of these challenges is understanding conjoined C cycle and climate implications of the predicted dramatic loss of northern permafrost (*Slater and Lawrence, 2013)*. Large releases of C (including as CH_4) from thawing permafrost (*Denman et al., 2007*) are plausible (*Schuur et al., 2011*), but uncertain due to the need to better understand the ‘pathways to C liberation’ during permafrost thaw. *The key research questions we addressed in this project were:*

Question 1: **How do microbial and viral communities mediate the dynamics of organic-C accumulation, transformation, and loss with permafrost thaw?**

This question was and continues to be important because permafrost C pools are large (~1700 PgC) (*Tarnocai et al., 2009*) and their loss would be an important feedback to climate change. However, potential losses are difficult to predict and complex. Thaw causes old C in frozen peat to be mobilized, and either decomposed directly or hydrologically transported to lake sediments. Superimposed on old-C dynamics are new C additions, becausethaw initiates ecological succession in both vegetation and associated microbial communities (*Schuur et al., 2009; Backstrand et al., 2010**).

Question 2: **How do these communities control the fraction of C liberated as CH_4 versus CO_2?**

This question is important because the partitioning of liberated C between CH_4 and CO_2 will ultimately determine the magnitude of microbially mediated feedbacks to climate from thawing permafrost, yet our understanding of controls on CH_4 emission, in particular, is poor. Resolving this challenge – and, as important, understanding future trajectories – likely depends on understanding wetlands, the largest natural source of CH_4 to the atmosphere (Denman et al., 2007).

To address these questions, we proposed to build on our previous investigation of permafrost CH_4 dynamics ("Project 1"), through a systems approach integrating ***(a)* molecular microbial and viral ecology**, ***(b)* stable and radiocarbon isotope and organic-geochemistry of SOM, dissolved organic matter (DOC), CH_4 and CO_2, and *(c)* state-of-the-art modeling,** along a chronosequence of permafrost thaw at a leading edge of climate change – a subarctic peatland system in Sweden where climate change- induced loss of permafrost is already accelerating.

Our ability to pursue these questions via these approaches were substantially enhanced by recent technical advances in high-resolution characterization of organic matter chemistry, and high-throughput nucleic-acid sequencing and microbial labeling techniques. These methods are now permiting a uniquely detailed combined approach that is revealing biogeochemical consequences of microbial community dynamics in a way that is improving our understanding of the fate of permafrost C on a changing planet.

Methods, Assumptions and Procedures:

Our project used cutting edge technologies in both biogeochemistry and molecular microbial ecology to advance systems biology research on microbial carbon cycling through: **(a)** systems-level mapping of chemical states and ages of organic matter (via FT-ICR-MS and ^{14}C analysis) along thaw gradients to associated microbial communities, biochemical potentials, and activities (via meta-genomics, -transcriptomics, –proteomics, and viral genomics), and to CO_2 and CH_4 fluxes; **(b)** experimental incubations using Quantum Dot Probing (or, alternatively if QDP prove infeasible, stable-isotope probing), to test key hypotheses, arising from (a), about particular microbially-driven biochemical degradation pathways; **(c)** bioinformatics designed to simultaneously enhance the DOE Knowledgebase, and **(d)** integrated ecosystem C-cycle modeling testable by soil organic chemical and microbial data. Details about the methods that were successful and their implementation are available in the publications listed, and linked, below in the Results and Discussion section.

Results and Discussion:

The results and discussion for this project are mostly reported in publications of the projects, as linked herein. These are divided into the 4 categories of projects described in the Abstract: integrative (i.e. interdisciplinary), microbial-focused, biogeochemistry-focused, and modeling-focused. Please note that due to the large team size of this project and its ambitious multi-disciplinary scope, these publications typically cite additional funding sources, including from other DOE-BER awards.

1. Integrative-focused

Results integrating microbial and biogeochemical perspectives included:

(1) An analysis using metagenome-assembled genomes (many of them representing previously undescribed organisms) to identify microbial community-structured pathways of carbon-processing and methane emission in thawing permafrost soils **(Woodcroft & Singleton et al. 2018)**.
(2) A novel investigation **(Emerson et al. 2018)** into whether and how viruses infecting soil microbes might importantly structure biogeochemistry of such systems. This work identified intriguing evidence from viral genomes suggesting that viruses do, in fact, play an important role in carbon cycling in permafrost soils.
(3) An investigation **(Singleton et al. 2018)** into how methanotrophs mediate methane emission from thawing permafrost.
(4) a combined analysis **(Wilson et al. 2019)** of microbial communities and geochemical reaction networks that showed that the most important reactions in the system were acetoclasty, hydrogenotrophy, CO2 production, and homoacetogenesis, with little evidence for anaerobic CH4 oxidation.

2. Microbial-focused

Martinez et al. (2019) reported on the identification, within our field sites, of novel dominant Stordalen Mire Caldiserica (SMC) MAGs The SMC clade rose to community dominance within permafrost, with a peak metagenome-based relative abundance of ~60%. This work was indicating a global distribution of this clade, predominantly in anaerobic, carbon-rich and cold environments.

In addition, two papers focused on developing methods and on preliminary analyses of soil-microbe associated viruses (**Trubl et al. 2016, 2018**), and one on an analysis of microbial community composition and distribution across the habitats of the mire (**Mondav et al., 2017**).

3. Biogeochemistry-focused

One of the most intriguing biogeochemical findings of the project was that hydrogenation of organic matter was an important terminal electron sink during anaerobic decomposition (**Wilson et al., 2017**). This mechanism is important because it sustains CO2 production and suppresses methanogenesis, thus it controls climate feedbacks by altering CO2:CH4 production ratios.

In **Hodgkins et al (2016),** we reported that presence of dense Sphagnum moss, a feature that is dominant in the intermediate thaw stages, appeared to be the main driver of variation in Dissolved Organic Matter (DOM) elemental composition and optical properties at Stordalen. DOM from sites with Sphagnum had greater aromaticity, higher average molecular weights, and greater O/C, consistent with a higher abundance of phenolic compounds that likely inhibit decomposition. These compounds are released by Sphagnum and may accumulate. These results indicate that Sphagnum acts as an inhibitor of rapid decomposition and CH4 release in thawing subarctic peatlands, consistent with lower rates of CO2 and CH4 production previously observed at these sites.

4. Modeling-focused

To improve predictions of CH_4 transformations, we originally proposed to use three different models: **(a) Wetland-DNDC** (with a focus on predicting CH4 vs. CO2 production); **(b) the Holocene Peat Model (HPM**, with a focus on dynamics of long-term peat accumulation and loss, including tracking the fate of old and new carbon); and **(c) the Land Model 3 (LM3)**, a component of the GFDL global climate model (CM3.0). We made substantial progress with DNDC and HPM (summarized below), but the work with LM3 (and unfunded collaboration) could not be completed.

We modified the original DNDC model to incorporate acetate and stable carbon isotopic dynamics associated with CH_4 cycling, as reported in Deng et al. (2017). DNDC thus explicitly simulates acetate dynamics, and the relative importance of acetotrophic and hydrogenotrophic methanogenesis (AM and HM), and predicts the C isotopic signature ($\delta^{13}C$) in soil C pools and emitted gases. The modified DNDC was tested against observed CH_4 fluxes, CH_4 production pathways, and $\delta^{13}C$ in emitted CH_4 ($\delta^{13}C\text{-}CH_4$). A sensitivity analysis indicated that α_{AM}, α_{HM}, and α_{MO} were the most important factors in determining the $\delta^{13}C\text{-}CH_4$. The model framework simulating stable C isotopic dynamics sets a robust basis for better constraining biogeochemical processes of CH_4 transformations in process-based models.

With HPM (Frolking et al. 2014), we elucidated the relationship **between net carbon balance and the apparent accumulation rate inferred from cores, including at the Stordalen research site.**

Published/ in review Integrative results:

Wilson RM, Neumann RB, Crossen K, Raab N, Hodgkins SB, Saleska SR, Bolduc B, Woodcroft B, Tyson G, J.P. Chanton JP, Rich VI. 2019. Microbial Community Analyses Inform Geochemical Reaction Network Models for Predicting Pathways of Greenhouse Gas Production. **Frontiers in Earth Science**. 7:59. DOI:10.3389/feart.2019.00059.

Rich VI. Viewpoint: Climate change microbiology - problems and perspectives. Fellow invited contributors: Hutchins DA, Jansson JK, Remais J, Singh BK, Trivedi P. 2019. In press at **Nature Reviews Microbiology**.

Woodcroft* BJ, Singleton* CM, Boyd JA, Evans PN, Emerson JB, Zayed AAF, Hoelzle RD, Lamberton TO, McCalley CK, Hodgkins SB, Wilson RM, Purvine SO, Nicora CD, Li C, Frolking S, Chanton JP, Crill PM, Saleska SR, Rich VI, Tyson GW. 2018. Genome-centric view of carbon processing in thawing permafrost. **Nature**. 560: 49–54. DOI 10.1038/s41586-018-0338-1. Nature microbiology blog https://naturemicrobiologycommunity.nature.com/channels/346-behind-the-paper/posts/36570-genomic-centric-view-of-carbon-processing-in-thawing-permafrost.

Emerson JB, Roux S, Brum JR, Bolduc B, Woodcroft BJ, Jang HB, Singleton CM, Solden LM, Naas AE, Boyd JA, Hodgkins SB, Wilson RM, Trubl G, Li C, Frolking S, Pope PB, Wrighton KC, Crill PM, Chanton JP, Saleska SR, Tyson GW, Rich VI, Sullivan MB. 2018. Host-linked soil viral ecology along a permafrost thaw gradient. **Nature Microbiology**. 3: 870–880. DOI 10.1038/s41564-018-0190-y, Open access. Nature microbiology blog https://naturemicrobiologycommunity.nature.com/channels/346-behind-the-paper/posts/36561-soil-viruses-unlocking-the-secret-garden.

Singleton CM, McCalley CK, Woodcroft BJ, Boyd JA, Evans PN, Hodgkins SB, Chanton JP, Frolking S, Crill PM, Saleska SR, Rich VI, Tyson GW. 2018. Methanotrophy across a natural permafrost thaw environment. **ISMEJ**. 12: 2544–2558. DOI 10.1038/s41396-018-0065-5, Open access.

Fahnestock MF, Bryce JG, McCalley CK, Montesdeoca M, Bai S, Li Y, Driscoll CT, Crill PM, Rich VI, Varner RK. Mercury reallocation in thawing subarctic peatlands. Submitted to PNAS.

Published Microbiology results:

Martinez MA, Woodcroft BJ, Ignacio Espinoza JC, Zayed AA, Singleton CM, Boyd JA, Li Y-F, Purvine S, Maughan H, Hodgkins SB, Anderson D, Sederholm M, Temperton B, Bolduc B, IsoGenie Project Coordinators, Saleska SR, Tyson GW, Rich VI. 2019. Discovery and ecogenomic context of a global Caldiserica-related phylum active in thawing permafrost, Candidatus Cryosericota phylum nov, Ca. Cryosericia class nov, Ca. Cryosericales ord. nov., Ca. Cryosericaceae fam. nov., comprising the four species Cryosericum septentrionale gen. nov. sp. nov., Ca. C. hinesii sp. nov., Ca. C. odellii sp. nov., Ca. C. terrychapinii sp. nov.. **Systematic and Applied Microbiology**, as part of the special issue “Classification of Uncultured Bacteria and Archaea”, Editors: Ramon Mosello-Mora and Konstantinos Konstantinidis. 42: 54-66.

Trubl G, Jang HB, Roux S, Emerson JB, Solonenko N, Vik DR, Solden L, Ellenbogen J, Runyon AT, Bolduc B, Woodcroft BJ, Saleska SR, Tyson GW, Wrighton KC, Sullivan MB, Rich VI. 2018. Soil viruses are underexplored players in ecosystem carbon processing. **mSystems**, 3(5), e00076-18.

Mondav R, McCalley CM, Hodgkins SB, Frolking S, Saleska SR, Rich VI, Chanton JP, Crill PM. 2017. Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. **Environmental Microbiology**. 19: 3201–3218.

Trubl G, Solonenko N, Chittick L, Solonenko SA, Rich VI†, Sullivan MB†. 2016. Optimization of viral resuspension methods for carbon-rich soils along a permafrost thaw gradient. **Peer J**. 4:e1999. († co-corresponding authors)

Trubl G, Roux S, Solonenko N, Li Y-F, Bolduc B, Rodríguez-Ramos J, Eloe-Fadrosh E, Rich† VI, Sullivan† MB. Towards optimized viral metagenomes from challenging soils. Submitted to PeerJ. († co-corresponding authors).

Published Biogeochemistry results:

Hodgkins SB, Richardson C, Dommain R, Wang H, Glaser P, Verbeke B, Winkler B, Missilmani M, Flanagan N, Ho M, Hoyt A, Harvey C, Cobb A, Rich VI, Vining S, Hough M, De La Cruz F, Toufaily J, Hamdan R, Cooper WT, Chanton J. 2018. Tropical peatland carbon storage linked to global latitudinal trends in peat recalcitrance. **Nature Communications**. 9: 3640. DOI: 10.1038/s41467-018-06050-2.

- Recipient of the 2018 "Nobel Peat Prize”: https://twitter.com/MACecohydrology/status/1082767317900120064

Wilson* RM, Tfaily MM*, Rich VI, Keller JK, Bridgham SD, Medvedeff C, Meredith L, Hanson PJ, Hines M, Pfeifer-Meister L, Saleska SR, Crill P, Cooper WT, Chanton JP, Kostka JE. 2017. Hydrogenation of Organic Matter as a Terminal Electron Sink Sustains High CO_2:CH_4 Production Ratios Regulating Climate Feedbacks in Boreal Peatlands and Thawing Permafrost. **Organic Geochemistry.** 112: 22–32.

Hodgkins SB, Tfaily MM, McCalley CK, Rich VI, Saleska SR, Crill PM, Chanton JP, Cooper WT. 2016. Elemental composition and optical properties reveal changes in dissolved organic matter along a permafrost thaw chronosequence in a subarctic peatland. **Geochimica et Cosmochimica Acta.** 187: 123–140.

Published Modeling results:

Deng J, McCalley C, Frolking S, Chanton J, Crill P, Varner R, Tyson G, Rich V, Saleska S, Hines M, Li C. 2017. Adding Stable Carbon Isotopes Improves Model Representation of the Role of Microbial Communities in Peatland Methane Cycling. **Journal of Advances in Modeling Earth Systems.** 9: 1412–1430. DOI: 10.1002/2016MS000817

Frolking, Steve, Julie Talbot, Zack M. Subin. 2014. Exploring the relationship between peatland net carbon balance and apparent carbon accumulation rate at century to millennial time scales. **The Holocene** 24(9):1167-1173. DOI: 10.1177/0959683614538078.

Conclusion

In this project we have been charting how to scale from genes to ecosystems at the ‘leading edge of climate change’ in a thawing permafrost system. We have documented a shift in isofluxes at high temporal resolution, a shift in bulk and dissolved organic matter at high molecular resolution, and a shift in microbiota at genome-scale resolution. Collectively, this information has revealed that dissolved organic matter becomes more labile as permafrost thaws, and in tandem the microbial carbon processing subtly shifts to simpler carbon degradation, acetogenesis, fermentation, and methane cycling. In addition, we have evidence that the 2-hydrogen transform dominates in these systems, with important implications for microbial redox, and a potential explanation for the high carbon dioxide to methane emissions ratio observed in many peatlands, including the first stage of permafrost thaw at ours. We have improved the Wetland-DNDC model to represent the isotopic effects of differential methane production and consumption pathways, and tuned it to our site’s long-term data. We also developed a protocol for separating and genetically characterizing viral particles from the site, which together with informatic recovery of viral sequences from the bulk soil metagenomes, have provided the first insights into viral ecology at this site. In a combined outcome of this project and the subsequent one, we doubled known viral genera globally by recovering viral populations from this site. Likewise, in a combined, lengthy outcome of this project and the subsequent one, we recovered ~1500 microbial metagenome-assembled genomes, representing 60% of the cells at the site at the genus level. In tandem with the large-molecule metabolomics, and characterization of microbial gene expression via metatranscriptomics and metaproteomics, these viral and microbial population genomes allow us to chart community-scale carbon cycling at our site, and with modeling we begin to extend the insights gained in space to other sites and in time into the future.