Title of Project:	Genes, isotopes, and ecosystem biogeochemistry: dissecting methane flux at the leading edge of global change
Institution:	University of Arizona
Date Received:	February 22, 2016
Principal Investigators:	Scott Saleska and Virginia Rich, Co-I's Gene Tyson, Jeff Chanton, Patrick Crill, Changshen Li, Steve Frolking

Overview:

This project integrates across three fields (microbiology, biogeochemistry, and modeling) to understand the mechanisms of methane cycling in thawing permafrost. We have made substantial progress in each area, and in cross-cutting interdisciplinary synthesis.

Large releases of CH_4 from thawing permafrost to the atmosphere, a strong positive feedback to global warming, are plausible but little is known about the controls on such release. Our project ("*IsoGenie*") addresses the key question: What is the interplay of microbial communities and soil organic matter composition in the decomposition of organic C to CH_4 across a permafrost thaw gradient?

Project Activities and Results:

1) The following are the **major activities undertaken** during this project:

a) Measurement of isotopologues of CH_4 at Stordalen Mire (S. Saleska):

We used a quantum cascade laser (QCL) spectrometer (Aerodyne Research Inc.) that measures CH_4 isotopologues to acquire a full three years of observations of isotopic composition of methane fluxes (2011-2013) at Stordalen Mire in Abisko, Sweden. (see Fig 1). In the final year of the project, we successfully upgraded the system to include D isotopes of CH_4 (ie. the full complement is now measured: ^{12}C-CH_4, ^{13}C-CH_4 and CH_3D).

b) Biogeochemical modeling using DNDC development/applications (Changsheng Li, UNH)

The DNDC biogeochemical model was tested against previous datasets as well as the new isotopic datasets collected at Stordalen mire. These new data included the measurements described above: CH_4 fluxes/isotope (^{12}C-CH_4 and ^{13}C-CH_4) at the palsa, *Sphagnum* and *Eriophorum* autochmaber sites from 2011, 2012 and 2013.

c) Microbial community characterization (Tyson & Rich)

During the last year of the project, microbial community characterization efforts included methodological advancements in molecular biology and informatics approaches, characterization of novel uncultivated organisms, and deeper analyses of communities in

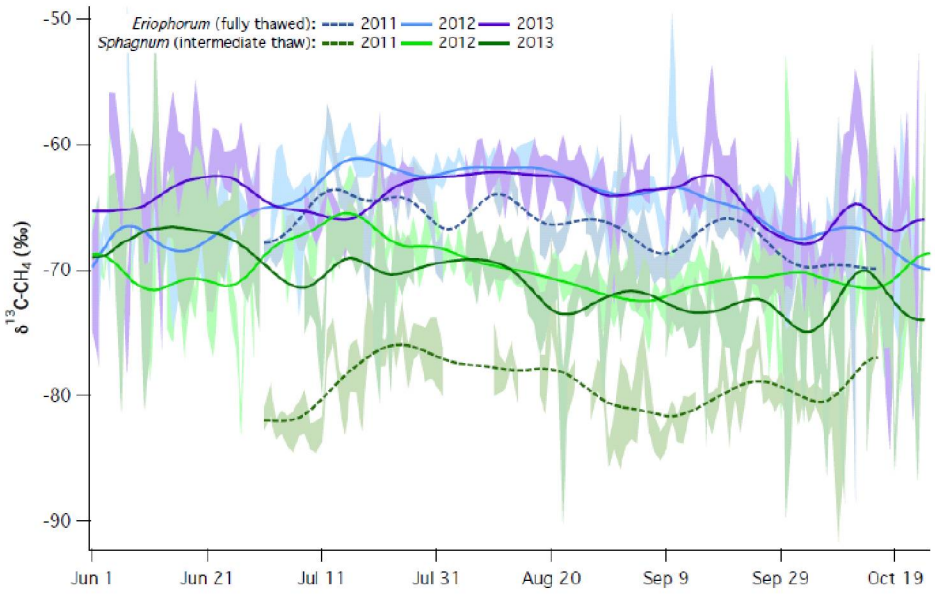

Figure 1. δ^{13}C-CH_4 of source CH_4 measured at Stordalen mire in autochambers in partly thawed sites *dominated by Sphagnum spp.* (greens) and in fully thawed *Eriophorum* (blues) for 2011-2013.

specific habitats of interest across the thaw gradient. We sampled communities in the 2013 growing season as a "bonus" year to ensure continuity of community monitoring in tandem with ongoing geochemical measurements, and for the first time sampled an expanded higher-resolution thaw gradient.

2) **Significant results**

a) Measurement of isotopologues of CH_4 at Stordalen mire (S. Saleska):

Measurements at Stordalen constitute a multi-year data set (2011-2013) quantifying δ^{13}C-CH_4 patterns across a permafrost thaw sequence (Figure 1). These results identify distinct annual and inter-annual patterns in δ^{13}C-CH_4 associated with the wetland communities' characteristic of different thaw stages (intermediate thaw *Sphagnum* bog and fully thawed *Eriophorum* fen). At the *Eriophorum* site δ^{13}C-CH_4 shows an annual pattern suggestive of more hydrogenotrophic production early and late in the season, with predominantly acetoclastic production during late summer peaks in plant productivity. In contrast δ^{13}C signatures where *Sphagnum* is present suggest that hydrogenotrophy dominates CH_4 production across the growing season. At the *Sphagnum* site large (~10‰) differences in δ^{13}C-CH_4 between years, with lower ^{13}C in the warm and sunny 2011 season, also highlight current gaps in our understanding of the relationship between climate conditions and the microbial production and consumption processes that influence the isotopic composition of emitted CH_4. The addition of δ D measurements starting in 2013 (data analysis ongoing) will provide new insights into the relative role of CH_4 oxidation and shifts in production pathways on observed CH_4 dynamics.

b. Biogeochemical modeling with DNDC:

DNDC captured the magnitude and seasonality of CH_4 fluxes observed at the plots in Stordalen (2013 shown here for example; Figure 2). Modeling the C isotopes still remains a challenge because both the field data and model settings require better interpretations. There are quite a number of C isotope-related parameters, which define the fractionation rates as well as the initial (or boundary) conditions and that vary in large ranges. We lack several of the values for these parameters directly for our sites therefore we used an interative fitting method to test these parameters based on comparison between measured and modeled $\delta^{13}C$ of the emitted CH_4 gas. The tests resulted in a group of parameters as follows:

PDB = 0.0112372;// Standard d13C
alf_CO2_CH4 = 1.095;//alfa from CO2 to CH4:1.080 [Conrad 2005: 1.045-1.082]
alf_DOC_CH4 = 1.026;//alfa from DOC to CH4:1.040 [Conrad 2005: 1.000-1.032]
alf_CH4ep = 1.016;//alfa for CH4 transport by plant
alf_CH4eb = 1.0;//alfa for CH4 transport by ebbulition
alf_CH4ed = 1.001;//alfa for CH4 transport by diffusion
alf_CH4_CO2 = 1.025;//alfa from CH4 to CO2
del13C_CO2 = -0.026;//initial d13C of peat_CO2
del13C_DOC = -0.026;//initial d13C of peat_DOC .

with the above-listed parameter values, DNDC could repeat the observed $\delta^{13}C$ data for *Eriophorum* during the growing season in 2011, 2012 and 2013; for *Sphagnum* during the growing season in 2012 and 2013 (Fig 2 shows 2013 modeling results). The discrepancies between model and measurements during the non-growing season (when CH_4 production rates are low) remain unknown. In addition, the measured $\delta^{13}C$ values for *Sphagnum* in 2011 were much lower than that of 2012 or 2013 (Figures 1). We will need more observations to constrain the isotope simulations, for example, using measured hydrogen (H_2) flux/pool in the soils.

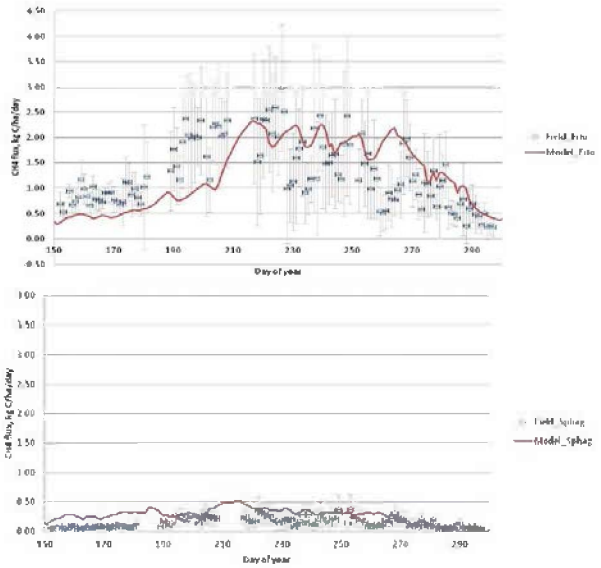

Figure 2. Comparison of modeled with measured CH4 fluxes at the a) *Eriophorum* and b) *Sphagnum* plots in Stordalen mire for 2013.

c. Microbial community characterization (Tyson & Rich)

(i) The concordance of characterization using pooled single-copy phylogenetic marker genes from shallow metagenomes vs. 16S rRNA amplicon sequencing was found to be strong, although still produced a significant variation in the data (Figure 3).

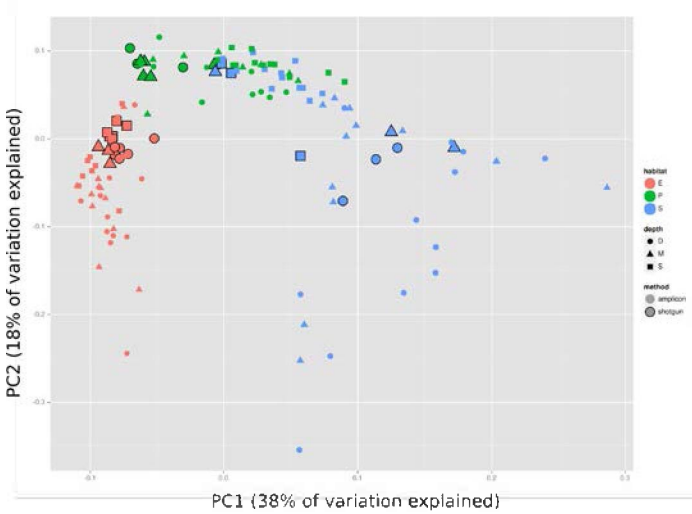

Figure 3: Principal component analysis of microbial community composition across samples from Stordalen Mire as assessed by 16S rRNA amplicon sequencing (via 454 'pyrotags') versus conserved single-copy phylogenetic marker genes from shallow metagenomes (generated using the same DNA extracts).

(ii) Mining of the shallow metagenomes for the methanogen functional marker gene *mcrA* showed a strong concordance with 16S rRNA-based methanogen characterization, in both relative abundance and taxonomic affiliation (eg Figure 4).

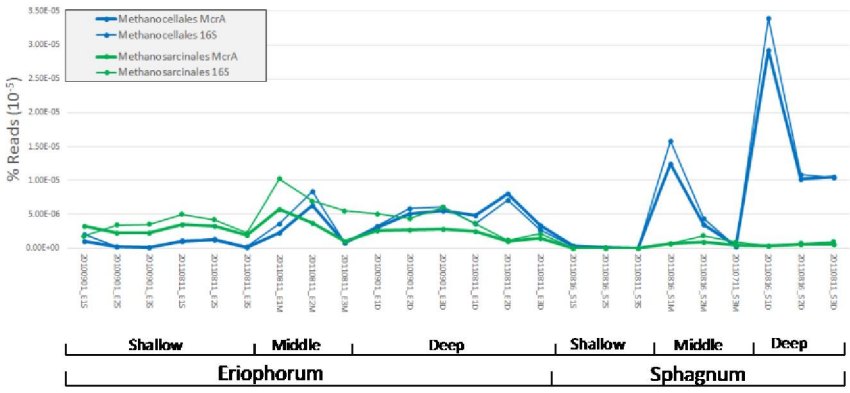

Figure 4. Relative abundance as percentage of total reads (from shallow metagenomes) of *Methanocellales* and *Methanosarcinales* 16S rRNA, across methanogenic habitats and depths in the Mire.

(iii) From 31 deep metagenomes spanning the 3 habitats and both 2010 and 2011, using the GroopM tool (Imelfort et al., 2014), ~130 genomes bins have been generated. Further assembly of these bins have generated 42 near- complete genomes (>80% complete, <10% contamination) (Figure 5). Improvement on draft genomes was achieved using the new tools of M-suite. One demonstrative example is an WPS2 genome from the Palsa habitat: it started post-GroopM binning and assembly as 5 pieces, >100X coverage, 67% GC, but was estimated to only be 50% complete by single gene analysis (CheckM). FinishM was used to improve it using 'explore' and 'visualise' and 'assemble' modes, now it is 96% complete; the original contigs simply did not contain the missing 50%.

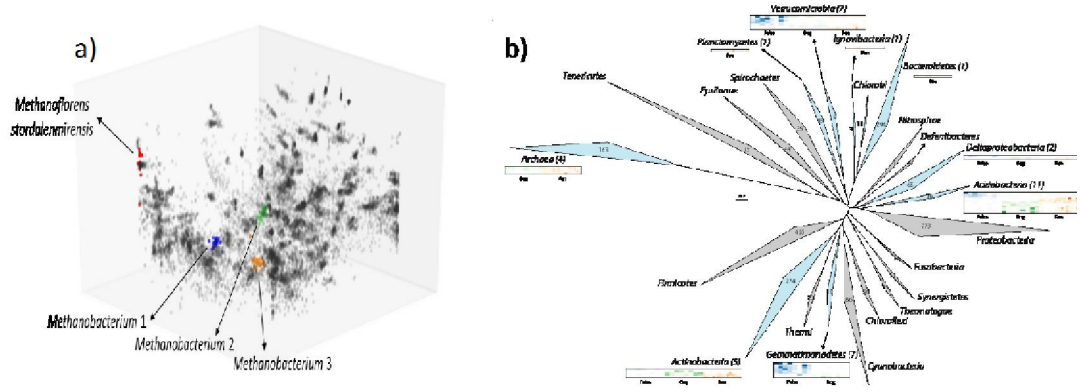

Figure 5. The successful assembly of numerous uncultivated genomes from related deep metagenomes from Stordalen Mire. (a) Metagenomic reads mapped in parameter space by oligonucleotide frequency and relative abundance in related metagenomes, with particular genome bins denoted. (b) The phylogenetic distribution of assembled genomes with pale blue clades indicating placement of assembled genomes, and inset heatmaps denoting relative abundance of those genomes across Mire.

(iv) A protocol has been developed to successfully separate viral particles from the peat/soil matrix across the three canonical thaw habitats at the site, paving the way for viral metagenomics.

(v) A detailed examination of the first "collapsed palsa" stage of permafrost thaw has clearly shown that the resident microbiota in the permafrost layer do not become more like those in the overlying active layer upon thaw, but diverge significantly to an entirely distinct community (Figure 6), suggesting strong response to thaw with likely impacts on transformation of the previously-frozen organic matter.

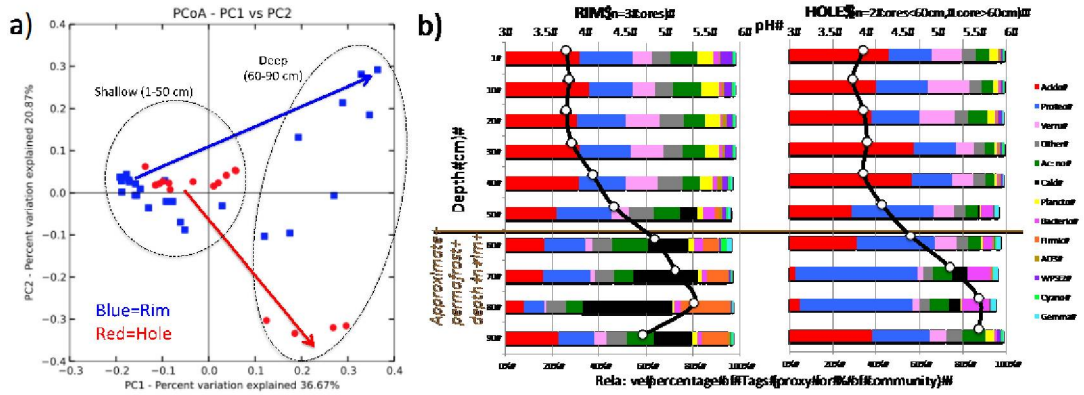

Figure 6. Microbial community during the first "collapsed palsa" stage of permafrost thaw, with depth. (a) Principal component analysis of microbial community composition in intact "rim" samples versus collapse "hole" samples shows similar active layer communities, despite radically different hydrology, but highly divergent communities at depth. (b) An examination of the thirteen most abundant phyla (by relative percentage of iTags) shows the details of the community transition at the permafrost boundary (which also corresponds to transition in pH). This shift is associated with a change from high Acidobacteria levels in the active layer to Caldiserica and Firmicutes in the permafrost layers (rim) or Proteobactiera and Bacteroidetes in those layers post-thaw.

(v) With methods in place for maximizing metaproteome characterization from these challenging peat-matrix samples, we are now able to compare proteomes across the thaw gradient. Microbial carbon metabolism is clearly shifting, and (somewhat surprisingly) methanogenesis is the strongly dominant proteome signal (comprising the majority of the top 10 most abundant proteins by spectral count) in all habitats where methane production is occurring, despite methanogens not being the most abundant cells.

Products Delivered: List any publications or manuscripts currently in preparation

McCalley, C.K., Woodcroft BJ, Hodgkins SB, Wehr, R.A., Kim EH, Mondav, R.; Crill PM, Chanton J, Rich VI, Tyson GW, Saleska SR, 2014. Methane dynamics regulated by microbial community response to permafrost thaw, *Nature*, **514**: 478-481

Hodgkins, S.B. M.M. Tfaily, C. K. McCalley, T.A. Logan, P.M. Crill, S.R. ***Saleska,*** V.I. Rich, and J.P. Chanton. 2014. Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production. *Proc. Nat. Acad. Sci.* doi: 10.1073/pnas.1314641111

Mondav R, Woodcroft BJ, Kim EH, McCalley CK, Hodgkins SB, Crill PM, Chanton J, Hurst GB, Verberkmoes NC, ***Saleska*** SR, Hugenholtz P, Rich VI, Tyson GW. 2014. Discovery of a novel methanogen prevalent in thawing permafrost. *Nature Communications.* **5**: 3212. doi: 10.1038/ncomms4212.

Imelfort M, Parks D, Woodcroft BJ, Dennis P, Hugenholtz P, Tyson GW. GroopM: an automated tool for the recovery of population genomes from related metagenomes. PeerJ. 2014;2:e603

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